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Urea and carbamate derivatives of primaquine: Synthesis, cytostatic and antioxidant activities

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ABSTRACT

The novel urea primaquine derivatives **3** were prepared by aminolysis of primaquine benzotriazolide **2** with several hydroxyamines and ethylendiamine, while carbamates **4** were synthesized from the same precursor **2** and alcohols. All compounds are fully chemically characterized and evaluated for their cytostatic and antioxidant activities. The most prominent antiproliferative activity was obtained by compounds **3c**, **3d**, **3g**, and **5b** (IC_{50} = 9–40 μM). 1-(5-Hydroxypentyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (**3c**) showed extreme selectivity toward SW 620 colon cancer cells (IC_{50} = 0.2 μM) and a bit less toward lung cancer cells H 460. Hydroxyurea **3h** showed the highest interaction with DPPH. Primaquine twin drug **3g** showed very significant inhibition on LOX soybean (IC_{50} = 62 μM). Almost all the tested derivatives highly inhibited lipid peroxidation, significantly stronger than primaquine phosphate.

1. Introduction

Primaquine (PQ) is a well-known antimalarial drug and interesting molecule for derivatization in the search of potential biologically active agents.[1–5] Recently, we have reported the synthesis of a series of primaquine derivatives bearing urea substituent attached to the primaquine side-chain.[6] These derivatives showed a modest antiproliferative activity on several tumor cell lines, whereby pyridine-substituted derivative of urea exhibited the highest cytostatic effect and some specific activity against human cytomegalovirus (HCMV). The present work is aimed toward developing urea primaquine derivatives with one or more hydroxyl groups in urea region. If the hydroxyl group is directly attached to nitrogen atom, *N*-hydroxyureas are formed. These compounds are effective metal chelators related to hydroxamic acids, and therefore potential antimalarial and cytostatic agents.[7] In this paper we report synthesis and full chemical characterization of new primaquine urea and carbamate derivatives and screening of their antiproliferative activity.

A number of studies have shown the pro-oxidant effects of primaquine in blood.[8–11] In addition, its metabolites have been shown to undergo redox-cycling both in vivo and in vitro. It was found that primaquine treatment causes an oxidative stress in the liver and the kidneys as well as hemotoxicity by generating reactive oxygen species within erythrocytes that overwhelm antioxidant defences. The aerobic toxicity of primaquine is mediated by superoxide anion and H_2O_2 and possibly by redox-active labile metals.[12] Having these facts in mind we have also focused the present study on the oxidant/antioxidant ability of the newly prepared primaquine derivatives.

2. Results and discussion

2.1. Chemistry

A series of new urea primaquine derivatives **3a**–**g** with one or more hydroxyl groups in urea region were prepared. If the hydroxyl group is directly attached to nitrogen atom, *N*-hydroxyurea is formed (**3h**). Scheme 1 outlines the general preparative route. The sequence leading from **1** to **3** is identical with the one described earlier.[6] Benzotriazolide **2** was synthesized by acylation of primaquine with 1-benzotriazole carboxylic acid chloride (**1**).[6,13]

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; BtcCl, benzotriazole carboxylic acid chloride; DMEM, Dulbecco's modified Eagle's medium; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FBS, fetal bovine serum; LO, lipoxygenase; LP, lipid peroxidation; NDGA, nordihydroguaiaretic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; P-Bt, primaquine benzotriazolide.

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0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
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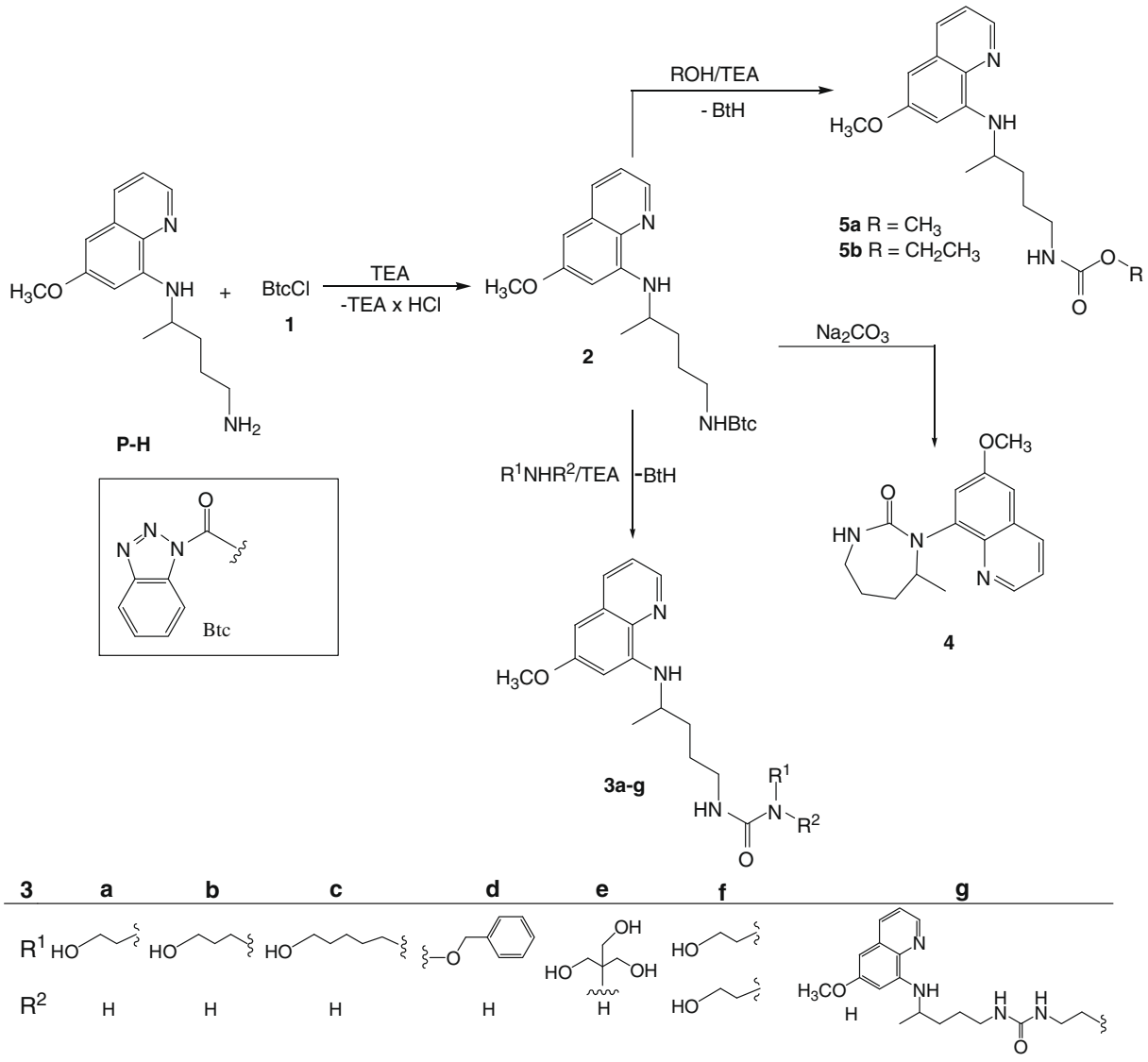


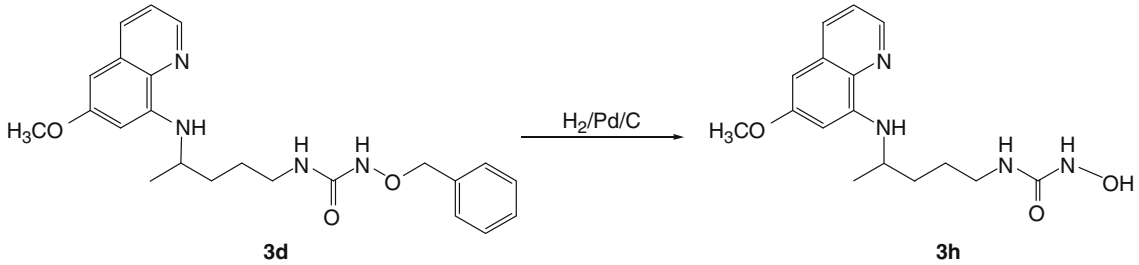
Scheme 1.

Urea primaquine derivatives **3a–g** were prepared by the reaction of benzotriazolide **2** with the corresponding amines. *N*-hydroxyurea **3h** was prepared by hydrogenolysis of 1-*O*-benzylhydroxy-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (**3d**) (Scheme 2). Diazepane derivative **4** was obtained by cyclization of benzotriazolide **2** in alkalic medium. Alcoholysis of compound **2** with methanol and ethanol gave carbamates **5a,b**, while propanol, *i*-propanol, *n*-butanol, *i*-butanol, *tert*-butanol, and triethanolamine failed to react.

Structures of the novel compounds were deduced from the analysis of their IR, ^{1}H, and ^{13}C NMR spectra and confirmed by the elemental analysis. The chemical shifts were consistent with the proposed structures of the novel compounds (Table 1).

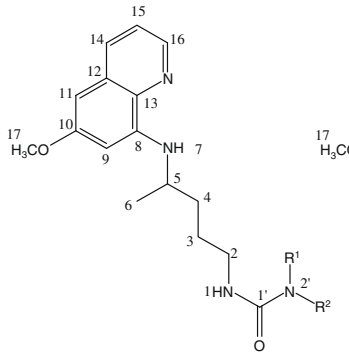
2.2. Cytostatic activity

The results of the cytostatic effect of tested compounds are presented in Table 2 and Figure 1. Diazepane derivative **4** and

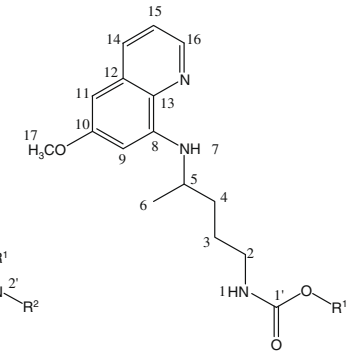


Scheme 2.

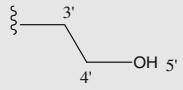
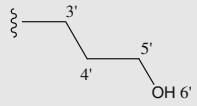
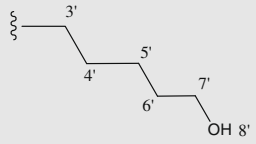
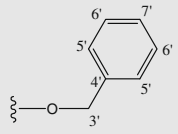
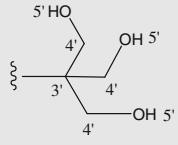
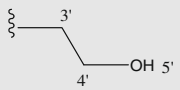
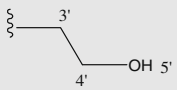
Table 1
^{1}H and ^{13}C NMR spectra of primaquine derivatives **3a**–**h**, **4**, and **5a**,**b**



3a-g

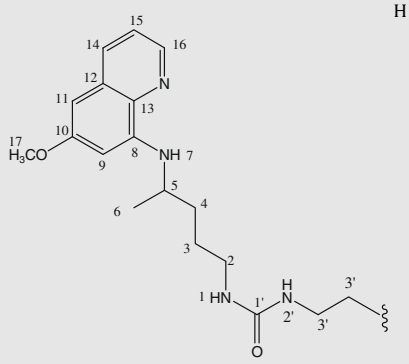
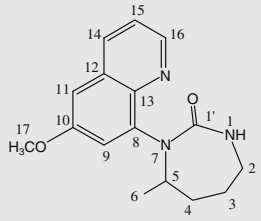
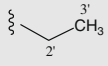


5a,b

Compd	R^1	R^2	δ
3a		H	8.54–8.53 (d, 1H, 16), 8.08–8.07 (d, 1H, 14), 7.44–7.41 (m, 1H, 15), 6.47 (s, 1H, 9), 6.26 (s, 1H, 11), 6.12–6.11 (d, 1H, 7), 5.96–5.94 (t, 1H, 2′), 5.80–5.78 (t, 1H, 1), 4.64–4.63 (t, 1H, 5′), 3.82 (s, 3H, 17), 3.64–3.59 (m, 1H, 5), 3.37–3.33 (m, 2H, 4′), 3.05–2.98 (m, 2H, 2), 2.99–2.98 (m, 2H, 3′), 1.65–1.61 (m, 2H, 3), 1.54–1.41 (m, 2H, 4), 1.21–1.20 (d, 3H, 6) 158.98 (10), 158.20 (1′), 144,61 (8), 144.22 (16), 134.77 (14), 134.49 (13), 129.55 (12), 122.07 (15), 96.07 (9), 91.59 (11), 60.82 (4′), 54.95 (17), 47.02 (5), 42.04 (3′), 39.91–39.07 (2), 33.51 (4), 26.88 (3), 20.20 (6)
3b		H	8.52–8.51 (d, 1H, 16), 8.06–8.04 (d, 1H, 14), 7.41–7.39 (m, 1H, 15), 6.45 (d, 1H, 9), 6.24 (d, 1H, 11), 6.10–6.08 (d, 1H, 7), 5.83–5.82 (t, 1H, 2′), 5.73–5.71 (t, 1H, 1), 4.42–4.40 (t, 1H, 6′), 3.80 (s, 3H, 17), 3,61–3.58 (m, 1H, 5), 3.38–3.35 (q, 2H, 3′), 3.02–2.95 (m, 3H, 2, 5′), 1.62–1.59 (m, 2H, 3), 1.51–1.39 (m, 4H, 4, 4′), 1.20–1.18 (d, 3H, 6) 158.98 (10), 158.25 (1′), 144.61 (8), 144.22 (16), 134.77 (14), 134.49 (13), 129.55 (12), 122.07 (15), 96.08 (9), 91.59 (11), 58.39 (5′), 54.95 (17), 47.03 (5), 39.91–39.08 (2), 36.34 (3′), 33.49 (4), 33.20 (4′), 26.88 (3), 20.20 (6)
3c		H	8.55–8.54 (d, 1H, 16), 8.10–8.07 (d, 1H, 14), 7.46–7.42 (m, 1H, 15), 6.49–6.48 (d, 1H, 9), 6.27 (d, 1H, 11), 6.14–6.11 (d, 1H, 7), 5.80–5.70 (m, 2H, 2′, 1), 4.36–4.32 (t, 1H, 8′), 3.89–3.88 (s, 3H, 17), 3.83–3.58 (m, 1H, 5), 3.38–3.28 (m, 2H, 3′), 3.05–2.90 (m, 3H, 2, 7′), 1.62–1.14 (m, 13H, 4′, 5′, 6′, 3, 4, 6) 159.47 (10), 158.52 (1′), 145.10 (8), 144.72 (16), 135.27 (14), 134.98 (13), 130.05 (12), 122.55 (15), 96.57 (9), 92.07 (11), 61.13 (7′), 55.45 (17), 47.51 (5), 40.82–39.16 (3′, 2), 33.99 (4), 32.75 (6′), 30.42 (4′), 27.40 (3), 23.40 (5′), 20.70 (6)
3d		H	9.02–9.00 (s, 1H, 2′), 8.56–8.53 (d, 1H, 16), 8.10–8.06 (d, 1H, 14), 7.50–7.38 (m, 6H, 15, 5′, 6′, 7′), 6.78–6.75 (s, 1H, 9), 6.45 (s, 1H, 11), 6.21 (s, 1H, 7), 6.13–6.10 (d, 1H, 1), 4.70–6.68 (s, 2H, 3′), 3.33 (s, 3H, 17), 3.62 (m, 1H, 5), 3.02 (q, 2H, 2), 1.46 (m, 4H, 3, 4), 1.31–1.20 (d, 3H, 6) 160.17 (10), 159.47 (1′), 145,09 (8), 144.72 (16), 137.09 (4′), 135.29 (14), 135.00 (13), 130.05 (12), 129.16 (5′), 128.60 (6′), 128.39 (7′), 122.58 (15), 96.57 (9), 92.07 (11), 77.64 (3′), 55.45 (17), 47.48 (5), 40.84–39.15 (2), 33.77 (4), 27.05 (3), 20.65 (6)
3e		H	8.55–8.53 (d, 1H, 16), 8.09–8.06 (d, 1H, 14), 7.45–7.41 (m, 1H, 15), 6.55–6.47 (m, 2H, 9, 11), 6.27–6.26 (d, 1H, 7), 6.14–6.11 (d, 1H, 2′), 5.72–5.69 (s, 1H, 1), 5.12–5.03 (t, 3H, 5′), 3.83 (s, 3H, 17), 3.68–3.41 (m, 7H, 5, 4′), 3.04–2.96 (m, 2H, 2), 1,68–1.40 (m, 4H, 3, 4), 1.22–1.20 (d, 3H, 6) 158.48 (10, 1′), 145.11 (8), 144.73 (16), 135.28 (14), 134.98 (13), 129.97 (12), 122.58 (15), 96.59 (9), 92.10 (11), 61.86 (4′), 60.94 (3′), 55.46 (17), 47.51 (5), 40.89–39.15 (2), 34.03 (4), 27.16 (3), 20.77 (6)
3f			8.54 (d, 1H, 16), 8.10–8.07 (d, 1H, 14), 7.46–7.42 (m, 1H, 15), 6.49–6.48 (s, 1H, 9), 6.33–6.31 (t, 1H, 1), 6.29–6.26 (s, 1H, 11), 6.14–6.11 (d, 1H, 7), 4.84–4.77 (t, 2H, 5′), 3.84 (s, 3H, 17), 3.69–3.63 (m, 1H, 5), 3.49–3.39 (m, 4H, 3′), 3.30–2.24 (t, 2H, 2), 3.09–3.02 (m, 4H, 4′), 1,69–1.60 (m, 2H, 3), 1.55–1.44 (m, 2H, 4), 1.23–1.21 (d, 3H, 6) 159.45 (10), 158.70 (1′), 145,05 (8), 144.72 (16), 135.33–135.27 (14), 134.99 (13), 139.05 (12), 122.57 (15), 96.56 (9), 92.06 (11), 60.49 (4′), 55.42 (17), 50.56 (3′), 47.52 (5), 40.82–39.14 (2), 33.89 (4), 27.16 (3), 20.64 (6)

(*continued on next page*)

Table 1 (*continued*)

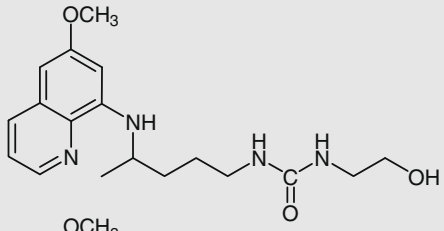
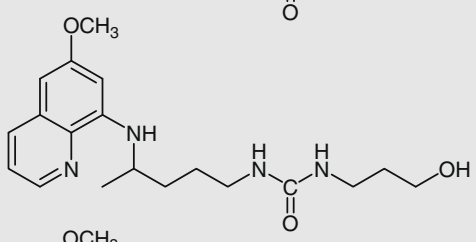
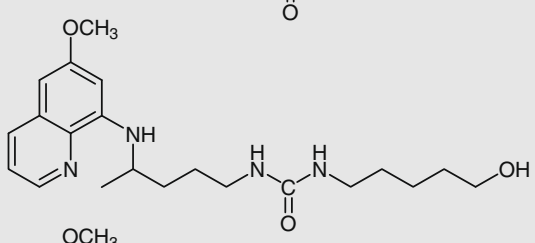
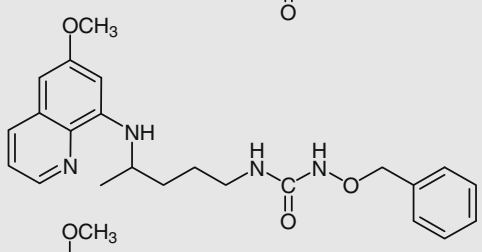
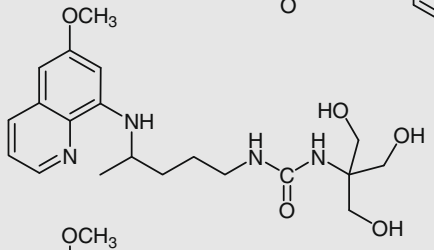
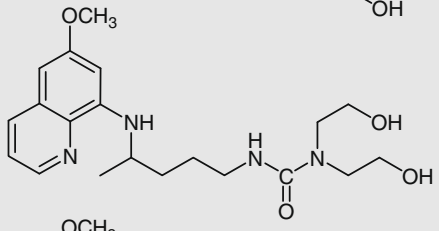
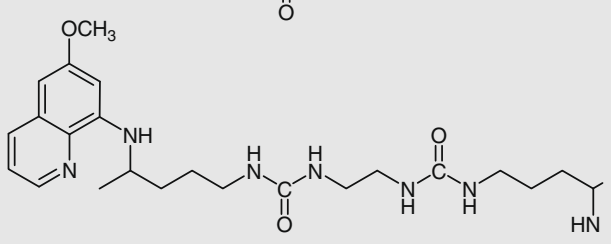
Compd	R^1	R^2	δ
3g		H	8.53 (d, 1H, 16), 8.08–8.06 (d, 1H, 14), 7.43–7.41 (m, 1H, 15), 6.47–6.46 (s, 1H, 9), 6.25 (s, 1H, 11), 6.12–6.10 (d, 1H, 7), 5.93–5.91 (t, 1H, 2′), 5.80 (s, 1H, 1), 3.81 (s, 3H, 17), 3.62–3.60 (m, 1H, 5), 3.33–3.30 (s, 2H, 3′), 2.99–2.97 (m, 2H, 2), 1.63–1.60 (m, 2H, 3), 1.52–1.41 (m, 2H, 4), 1.20–1.19 (d, 3H, 6) 158.97 (10), 158.13 (1′), 144.60 (8), 144.21 (16), 134.76 (14), 134.49 (13), 129.55 (12), 122.06 (15), 96.07 (9), 91.57 (11), 54.94 (17), 47.02 (5), 40.04 (3′), 39.92–39.08 (2), 33.50 (4), 26.83 (3), 20.18 (6)
3h		H	8.57–8.53 (d, 1H, 16), 8.49 (s, 1H, 2′), 8.23 (s, 1H, 1), 8.09–8.06 (d, 1H, 14), 7.45–7.40 (m, 1H, 15), 6.72 (s, 1H, 3′), 6.48–6.47 (s, 1H, 9), 6.27 (s, 1H, 11), 6.12–6.11 (d, 1H, 7), 3.85–3.83 (s, 3H, 17), 3.64–3.61 (m, 1H, 5), 3.07–3.03 (m, 2H, 2), 1.60–1.46 (m, 4H, 3, 4), 1.24–1.20 (d, 3H, 6) 161.94 (10), 159.47 (1′), 145,11 (8), 144.72 (16), 135.27 (14), 134.98 (13), 130.05 (12), 122.57 (15), 96.57 (9), 92.08 (11), 55.46 (17), 47.50 (5), 40.83–39.16 (2), 33.93 (4), 27.25 (3), 20.68 (6)
4			8.53–8.51 (d, 1H, 16), 8.08–8.05 (d, 1H, 14), 7.44–7.40 (m, 1H, 15), 6.47–6.46 (s, 1H, 9), 6.25–6.24 (t, 1H, 11), 5.78–5.74 (s, 1H, 1), 3.85 (s, 3H, 17), 3.50 (m, 1H, 5), 3.02–2.96 (q, 2H, 2), 1.65–1.40 (m, 4H, 3, 4), 1.23–1.17 (d, 3H, 6) 159.47 (10), 158.51 (1′), 145,10 (8), 144.70 (16), 135.35 (14), 134.98 (13), 130.04 (12), 122.63 (15), 96.56 (9), 92.07 (11), 55.45 (17), 47.56 (5), 41.38–38.89 (2), 33.98 (4), 27.38 (3), 20.68 (6)
5a		–	8.54–8.52 (d, 1H, 16), 8.09–8.06 (d, 1H, 14), 7.44–7.40 (m, 1H, 15), 7.13–7.10 (t, 1H, 1), 6.47 (s, 1H, 9), 6.26–6.25 (s, 1H, 11), 6.13–6.10 (d, 1H, 7), 3.82 (s, 3H, 17), 3.66–3.54 (m, 1H, 5), 3.50 (s, 3H, 2′), 3.02–2.96 (q, 2H, 2), 1.65–1.43 (m, 4H, 3, 4), 1.21–1.19 (d, 3H, 6) 158.47 (10), 158.20 (1′), 145,11 (8), 144.70 (16), 135.26 (14), 135.00 (13), 130.05 (12), 122.57 (15), 96.56 (9), 92.08 (11), 55.45 (17), 51.57 (2′), 47.46 (5), 40.83–39.16 (2), 33.75 (4), 26.76 (3), 20.67 (6)
5b		–	8.53–8.52 (d, 1H, 16), 8.09–8.06 (d, 1H, 14), 7.44–7.40 (m, 1H, 15), 7.09–7.06 (t, 1H, 1), 6.47 (s, 1H, 9), 6.26–6.25 (s, 1H, 11), 6.13–6.10 (d, 1H, 7), 3.98–3.90 (q, 2H, 2′), 3.82 (s, 3H, 17), 3.65–3.57 (m, 1H, 5), 3.01–2.95 (q, 2H, 2), 1.68–1.40 (m, 4H, 3, 4), 1.27–1.19 (d, 3H, 3′), 1.15–1.10 (d, 3H, 6) 159.47 (10), 158.20 (1′), 145,10 (8), 144.70 (16), 135.27 (14), 134.99 (13), 130.05 (12), 122.57 (15), 96.56 (9), 92.07 (11), 59.86 (2′), 55.45 (17), 47.45 (5), 40.82–39.15 (2), 33.75 (4), 26.75 (3), 20.67 (6), 15.13 (3′)

carbamate **5a** did not show any inhibitory effect on the tumor cell lines' proliferation. Similarly, compounds **3a**, **3b**, **3e**, and **3f** were inactive, except certain inhibitory activity toward MCF-7 breast cancer cell line that was sensitive to all these compounds, at least at the highest concentration (10^{-4} M). The most prominent antiproliferative activity was obtained by compounds **3c**, **3d**, **3g**, and **5b** (IC_{50} = 9–40 μM). Surprisingly, hydroxypentyl-substituted **3c** showed extreme selectivity toward SW 620 colon cancer cells (IC_{50} = 0.2 μM) and a bit less toward lung cancer cells H 460. We showed previously that the pyridine primaquine urea derivative showed also more pronounced activity toward SW 620 cells, compared to other solid tumor-derived cells.[6]

Comparison of the here-presented derivatives with the parent compound primaquine indicated that the antiproliferative activity of urea derivatives is comparable to the primaquine activity, but superior to a standard cytostatic drug hydroxyurea. Rossi et al. stated that primaquine exerts proliferative effects on MCF-7 cell lines in the concentration range of 1–6 mg/L. Still, at the concentration comparable to ours (50 mg/L, corresponding to approx. 100 μM) it induced about 20% of inhibition after 24 h. This could correspond to our results, since we measured the viability of cells after 72 h, whereby complete inhibition was observed.[14]

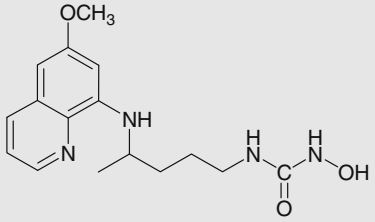
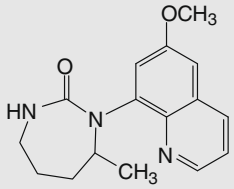
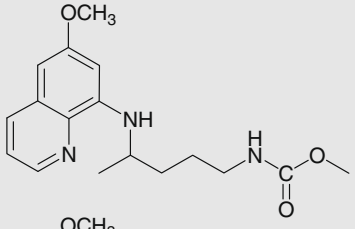
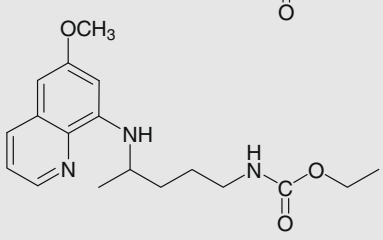
N-Hydroxyurea **3h** with hydroxyl group directly attached to urea nitrogen did not show significant antiproliferative activity as we expected. Diethanol amine derivative **3f** was similarly inactive as triethanol amine derivative **3e**, but both compounds showed certain selectivity toward MCF-7 cell line. Concerning structure activity relationship several points could be concluded: (i) comparing the length of alkyl chain attached to nitrogen atom of urea, it seems that longer chain has better antiproliferative activity, whereby hydroxypentyl urea shows a very differential effect, being selectively cytotoxic to SW 620 cells; (ii) compounds **3e** and **3f** with two or three

Table 2
Inhibitory effects of primaquine derivatives on the growth of malignant tumor cell lines

Compd		Tumor cell growth [IC_{50}[a] (μM)]				
		MOLT-4	HCT 116	SW 620	MCF-7	H 460
3a		>100	>100	>100	25 ± 22	>100
3b		>100	>100	>100	31 ± 30	>100
3c		61 ± 40	29 ± 3	0.2 ± 0.01	44 ± 39	2 ± 0.5
3d		12 ± 0.7	14 ± 0.3	20 ± 2	9 ± 6	21 ± 2
3e		>100	>100	>100	24 ± 5	>100
3f		>100	>100	>100	36 ± 17	>100
3g		10 ± 0.3	15 ± 7	17 ± 15	31 ± 20	18 ± 14

(*continued on next page*)

Table 2 (*continued*)

Compd	Tumor cell growth [IC_{50}[a] (μM)]				
	MOLT-4	HCT 116	SW 620	MCF-7	H 460
3h 	>100	57 ± 2	35 ± 17	44 ± 36	>100
4 	⩾100	>100	>100	>100	>100
5a 	>100	>100	>100	>100	>100
5b 	34 ± 4	28 ± 4	45 ± 9	26 ± 5	38 ± 13
Primaquine	N.T.[b]	20 ± 6	⩾100	28 ± 10	30 ± 7
Hydroxyurea	>100	223 ± 120	>100	>100	>100

[a] IC_{50}: the concentration that causes 50% growth inhibition.
[b] N.T.: not tested.

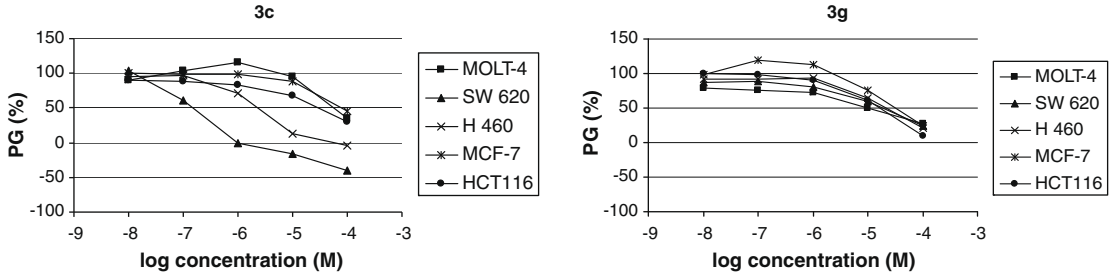


Figure 1. Dose–response profiles for **3c** and **3g** tested on various human cell lines in vitro. The cells were exposed to the compounds at different concentrations, and the percentage of growth (PG) was calculated. Each point represents a mean value of four replicates in three individual experiments.

hydroxyl groups attached to the terminal nitrogen urea atom, showed comparable selectivity toward MCF-7 as compound **3a** bearing only one hydroxyl group at the same distance; the same selectivity was noticed with analogous diethylamine urea that was previously tested;[6] and (iii) the ethyl carbamate **5b** has substantially stronger activity than its methyl analogue **5a**.

Better activity, but less selectivity was achieved by **3g**, which is actually a twin drug, composed of two primaquine molecules connected by ethylenediamide spacer. Comparable activity showed *O*-benzyl derivative **3d** with lipophilic benzene ring. This compound was more active than 1-benzyl-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea, similar compound lacking only oxygen atom, prepared and tested in our previous paper.[6] The fact that compound **3d** is equally active as pyridine derivative described in the same paper suggests that electrophilic atom (oxygen or nitrogen) in lipophilic surrounding potentiates cytostatic activity.

2.3. Antioxidant activity

Several methods are used for the estimation of efficiency of synthetic antioxidants. In this paper the attention is focused on the DPPH assay, which is one of the best-known, frequently employed and accurate methods. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical because of its spare electron delocalization over

Table 3
Interaction with DPPH, in vitro inhibition of soybean lipoxygenase (LOX) and inhibition of lipid peroxidation (LP)

Compd	DPPH 20 min[a] (%)	DPPH 60 min[a] (%)	DPPH 20 min[b] (%)	DPPH 60 min[b] (%)	LOX IC_{50} (μM)	LP inhibition[b] (%)
2	18.4	20.0	16.7	73.8	260	n.a.[c]
3a	3.7	10.6	60.7	51.2	315	81
3b	12.2	27.7	28.6	33.3	230	56
3c	n.a.	n.a.	34.5	46.4	310	63
3d	n.a.	n.a.	11.4	38.1	230	82
3e	24.4	22.3	28.6	40.5	n.a.	86
3f	20.7	7.4	n.a.	11.9	86	85
3g	34.2	27.6	35.7	40.5	62	99
3h	2.2	60.6	88.1	84.5	83	69
3i	15.8	21.3	26.2	19.0	220	99
3j	28.9	16.3	27.4	25.0	n.a.	99
3k	3.9	28.8	42.9	42.9	320	96
4	30.5	40.0	48.8	57.1	72	88
5a	24.4	13.8	75	48.8	n.a.	83
5b	10.8	8.5	n.a.	31.0	n.a.	87
Primaquine	17.2	19.0	18.1	21.9	74	45
Trolox						63
NDGA[d]			81	83		
Caffeic acid					600	

Concentrations of the tested compounds: [a]5×10^{-5} M, [b]1×10^{-4} M.
[c]n.a.—no activity; [d]NDGA—nordihydroguaiaretic acid.

the whole molecule. The reducing abilities of the new primaquine derivatives were determined by the use of the stable radical DPPH at 0.05 and 0.1 mM after 20 and 60 min (Table 3). This interaction indicates their radical scavenging ability in an iron-free system and expresses the reducing activity of compounds.[15] The reducing abilities ranged from 2.2% to 88.1%. Slight differences were observed within the compounds with the time and the concentration. In general, it seems that compounds interact with DPPH in a concentration dependent manner. Compound **3h**, without a methylene spacer between urea nitrogen atom and hydroxyl group, showed the highest interaction value, followed by **2**, **4**, and **3a**. These compounds showed a higher interaction than the parent compound primaquine. Compounds **3c, 3d, 3i**, and **3f** did not present any significant interaction. The small changes in the carbon chain size (2C/3C) did not influence the reducing ability. The presence of 5C chain or of benzyloxy group leads to the loss of the interaction. Between the two **5a–b** carbamates the methyl derivative is more active.

Further on, inhibitory activity on lipid peroxidation of all prepared compounds was screened. Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies in vitro. The water soluble azo compound 2,2′-(2-amidinopropane) dihydrochloride (AAPH) has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals. In our studies AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Under the reported experimental conditions all the tested compounds presented high inhibition of lipid peroxidation, except benzotriazolide **2** (Table 3). The LP inhibition of the tested primaquine derivatives was much higher than the inhibition of primaquine phosphate: 11 of 14 derivatives highly inhibited LP (81–99%), while primaquine showed the inhibition of 45%. These results strongly suggest that the introduction of the urea or carbamate moiety is responsible for improved LP inhibition.

Leukotrienes play an important role as mediators of a variety of inflammatory and allergic reactions and are derived from the biotransformation of arachidonic acid catalyzed by lipoxygenase (LOX). Lipoxygenases play a role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer.[16] Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases. Twin primaquine derivative **3g** was the most potent LOX inhibitor, followed by diazepane derivative **4**, hydroxyurea **3h**, and urea **3f** with IC_{50} = 62, 72, 83, and 86 μM, respectively (Table 3). It is important to note that two most potent compounds from the tested series are the most lipophilic derivatives bearing bulky substituents. However, the results of LOX inhibition are comparable with the result of primaquine phosphate.

3. Conclusions

The most prominent antiproliferative activity was obtained by hydroxypentyl derivative **3c**, *O*-benzyl derivative **3d**, compound bearing two primaquine moieties **3g** and carbamate derivative **5b**, but the activities were comparable to the one obtained by primaquine. Still, the most valuable observation is the extreme selectivity toward SW 620 colon cancer cells (IC_{50} = 0.2 μM) and a bit less toward lung cancer cells H 460 exerted by 1-(5-hydroxypentyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (**3c**). This selectivity should be additionally evaluated and its exact mechanism elucidated. On the other hand, all new derivatives showed significant antioxidant activities. Hydroxyurea **3h** strongly interacted with DPPH. Compound **3g** showed very significant inhibition on LOX soybean. All the tested urea and carbamate derivatives highly inhibited lipid peroxidation, significantly stronger than primaquine phosphate.

4. Experimental

4.1. General methods

Melting points were determined on a Stuart Melting Point Apparatus SMP3 and were uncorrected. IR spectra were recorded on a FTIR Perkin Elmer Paragon 500 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 300 and 75.5 MHz for the ^{1}H and ^{13}C nuclei, respectively. Samples were measured in DMSO-d_6 solutions at 20 °C in 5 mm NMR tubes. Chemical shifts (δ) were referred to TMS. Precoated Merck Silica Gel 60 F_{254} plates and solvent systems cyclohexane/ethyl acetate/methanol (3:1:0.5) or chloroform/methanol (9.5:0.5) were used for thin-layer chromatography. Spots were visualized by short-wave UV light and iodine vapor. Column chromatography was performed on silica gel (0.063–0.200 mm), with chloroform/methanol (9.5:0.5) or cyclohexane/ethyl acetate/methanol (3:1:0.5) as eluents. Primaquine diphosphate, 2-hydroxy-

ethanamine, 3-hydroxypropanamine, 5-hydroxypentanamine, *O*-benzylhydroxylamine hydrochloride, tris(hydroxymethyl)aminomethane, diethanolamine, ethylenediamine, DPPH, AAPH, nordihydroguaiaretic acid (NDGA), sodium linoleate, soybean lipoxygenase, caffeic acid, and trolox were purchased from Sigma–Aldrich. Human cell lines for cytostatic activity were obtained from American Type Culture Collection.

4.2. 1-Benzotriazole carboxylic acid chloride (BtcCl, 1)

BtcCl was prepared from benzotriazole and triphosgene.[13]

4.3. Primaquine benzotriazolide (P-Bt, 2)

Compound **2** was prepared by acylation of primaquine base with chloride **1**.[6] Primaquine base was prepared from primaquine diphosphate, keeping the drug solution light protected.

4.4. Primaquine urea derivatives (3a–g). General procedure

To a solution of 0.500 g (1.24 mmol) of primaquine benzotriazolide **2** and 0.300 g (2.98 mmol) of triethylamine in anhydrous ethanol (30 mL), 1.24 mmol of appropriate amine was added. The reaction mixture was stirred at rt, light protected, and evaporated in vacuum. The obtained residue was dissolved in chloroform (125 mL), extracted three times with NaOH solution (pH up to 9), washed with water, and dried over anhydrous Na_2SO_4. Chloroform was evaporated under reduced pressure to give crude product.

4.4.1. 1-(2-Hydroxyethyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3a)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** and 0.076 g (1.24 mmol) 2-hydroxyethanamine at rt for 4 days and recrystallization from acetone/petrolether, 0.303 g (70.8%) of **3a** was obtained; mp 124–126 °C; IR (KBr): ν_{max} 3390, 3333, 2939, 2860, 1610, 1575, 1516, 1451, 1387, 1221, 1203, 1168, 1053, 1028, 825, 794, 624 cm^{-1}. Anal. ($C_{18}H_{26}N_4O_3$) C, H, N.

4.4.2. 1-(3-Hydroxypropyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3b)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** and 0.093 g (1.24 mmol) 3-hydroxypropanamine at rt for 4 days and recrystallization from acetone/petrolether, 0.312 g (70%) of **3b** was obtained; mp 94–99 °C; IR (KBr): ν_{max} 3346, 2935, 2879, 2860, 1618, 1576, 1518, 1475, 1460, 1450, 1424, 1390, 1371, 1271, 1222, 1205, 1171, 1158, 1073, 1053, 1029, 823, 793, 680, 625 cm^{-1}. Anal. ($C_{19}H_{28}N_4O_3$) C, H, N.

4.4.3. 1-(5-Hydroxypentyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3c)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** and 0.128 g (1.24 mmol) 5-hydroxypentanamine at rt for 4 days and recrystallization from acetone/petrolether and trituration with ether, 0.351 g (73%) of **3c** was obtained; mp 81–88 °C; IR (KBr): ν_{max} 3349, 2933, 2859, 1624, 1579, 1523, 1457, 1425, 1388, 1263, 1237, 1226, 1205, 1170, 1057, 1032, 823, 791 cm^{-1}. Anal. ($C_{21}H_{32}N_4O_3$) C, H, N.

4.4.4. 1-*O*-Benzylhydroxy-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3d)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** and 0.394 g (2.48 mmol) *O*-benzylhydroxylamine hydrochloride at rt for 4 days and purification of the crude product by column chromatography (eluent chloroform/methanol 9.5:0.5), 0.329 g (65%) of **3d** was obtained; oil; IR (KBr): ν_{max} 3386, 3199, 2925, 2863, 1672, 1616, 1577, 1521, 1456, 1386, 1204, 1163, 1051, 1029, 908, 823, 791, 749, 699 cm^{-1}. Anal. ($C_{23}H_{28}N_4O_3$) C, H, N.

4.4.5. 1-(2-Hydroxy-1,1-bis-hydroxymethyl-ethyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3e)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** and 0.150 g (1.24 mmol) tris(hydroxymethyl)aminomethane at rt for 4 days and recrystallization from acetone/petrolether, 0.227 g (45%) of **3e** was obtained; mp 147–149 °C; IR (KBr): ν_{max} 3357, 3208, 2931, 2658, 2300, 1713, 1621, 1590, 1522, 1456, 1426, 1390, 1337, 1281, 1224, 1206, 1164, 1130, 1101, 1048, 1015, 818 cm^{-1}. Anal. ($C_{20}H_{30}N_4O_5$) C, H, N.

4.4.6. 1,1-Bis-(2-hydroxyethyl)-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3f)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** and 0.130 g (1.24 mmol) diethanolamine at rt for 4 days and purification of the crude product by column chromatography (eluent cyclohexane/ethyl acetate/methanol 3:1:0.5), 0.304 g (63%) of **3f** was obtained; oil; IR (KBr): ν_{max} 3369, 2935, 1615, 1577, 1520, 1456, 1424, 1388, 1220, 1203, 1160, 1076, 1051, 971, 873, 833, 791, 755, 679 cm^{-1}. Anal. ($C_{20}H_{30}N_4O_4$) C, H, N.

4.4.7. 1-[4-(6-Methoxy-quinolin-8-ylamino)-pentyl]-3-/2-{3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]-ureido}-ethyl/urea (3g)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2,** 0.037 g (0.62 mmol) ethylenediamine and 0.125 g (1.24 mmol) triethylamine at rt for 2 days and recrystallization from ether, 0.323 g (41.4%) of **3g** was obtained; mp 151–153 °C; IR (KBr): ν_{max} 3342, 3050, 2928, 2859, 2350, 2285, 1623, 1576, 1519, 1453, 1422, 1387, 1337, 1262, 1220, 1202, 1157, 1051, 1029, 969, 900, 821, 791, 677 cm^{-1}. Anal. ($C_{34}H_{46}N_8O_4$) C, H, N.

4.4.8. 1-Hydroxy-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3h)

A suspension of 0.506 g (1.24 mmol) 1-*O*-benzylhydroxy-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (**3d**) and 10% Pd/C (75 mg) in anhydrous methanol (20 mL) was hydrogenated at ambient pressure and rt, light protected, under nitrogen atmosphere. After 3 h the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. After purification of the crude product by column chromatography (eluent chloroform/methanol 95:5), 0.101 g (26%) of **3h** was obtained; mp 144–147 °C; IR (KBr): ν_{max} 3368, 3139, 2920, 2861, 1642, 1560, 1523, 1427, 1386, 1333, 1228, 1206, 1170, 1060, 1038, 981, 904, 846, 829, 821, 790, 777, 749, 678, 644, 624, 564, 534 cm^{-1}. Anal. ($C_{16}H_{22}N_4O_3$) C, H, N.

4.4.9. 1-Cyclopentyl-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3i), 1-cyclohexyl-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3j), 1,1-dicyclohexyl-3-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]urea (3k)

These compounds are prepared according to the previously published method.[6]

4.5. 1-(6-Methoxy-quinolin-8-yl)-7-methyl-[1,3]-diazepan-2-one (4)

To a solution of 0.500 g (1.24 mmol) primaquine benzotriazolide (**2**) in acetone (30 mL), a sodium carbonate solution (w = 10%) (pH up to 10) was added. The reaction mixture was protected from light, stirred for 30 min. at rt and evaporated in vacuum. The obtained residue was dissolved in chloroform (125 mL), extracted three times with the NaOH solution (pH up to 9), washed with water, and dried over anhydrous Na_2SO_4. Chloroform was evaporated under reduced pressure to give product **4**. After purifi-

cation of the crude product by column chromatography (eluent cyclohexane/ethyl acetate/methanol 3:1:0.5), 0.336 g (95%) of **4** was obtained; mp 122–126 °C; IR (KBr): ν_{max} 3376, 2961, 2928, 2857, 1616, 1575, 1520, 1456, 1424, 1388, 1220, 1203, 1159, 1051, 1031, 821, 791 cm^{-1}. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

4.6. Primaquine carbamate derivatives (5a,b). General procedure

To a solution of 0.500 g (1.24 mmol) primaquine benzotriazolide (**2**) in anhydrous methanol or ethanol (30 mL), 0.125 g (1.24 mmol) of triethylamine was added. The reaction mixture was protected from light, refluxed, and evaporated in vacuum. The obtained residue was dissolved in chloroform (125 mL), extracted three times with the NaOH solution (pH up to 9), washed with water, and dried over anhydrous Na_2SO_4. Chloroform was evaporated under reduced pressure to give crude product.

4.6.1. Methyl-*N*-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]-carbamate (5a)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** in anhydrous ethanol for 7 h at 65 °C and purification of the crude product by column chromatography (eluent cyclohexane/ethyl acetate/methanol 3:1:0.5), 0.334 g (85%) of **5a** was obtained; oil; IR (KBr): ν_{max} 3378, 2936, 2867, 1722, 1704, 1694, 1615, 1595, 1578, 1520, 1455, 1423, 1387, 1259, 1220, 1204, 1159, 1051, 1030, 822, 792, 778 cm^{-1}. Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

4.6.2. Ethyl-*N*-[4-(6-methoxy-quinolin-8-ylamino)-pentyl]-carbamate (5b)

From the reaction of 0.500 g (1.24 mmol) primaquine benzotriazolide **2** in anhydrous methanol for 10 h at 80 °C and purification of the crude product by column chromatography (eluent cyclohexane/ethyl acetate/methanol 3:1:0.5), 0.332 g (81%) of **5b** was obtained; oil; IR (KBr): ν_{max} 3377, 2962, 2935, 2868, 1699, 1615, 1595, 1577, 1520, 1455, 1423, 1388, 1336, 1258, 1220, 1204, 1159, 1051, 1031, 822, 792, 679, 625 cm^{-1}. Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

4.7. Cytostatic activity

The experiments were carried out on 5 human cell lines, which are derived from 4 cancer types. MCF-7 (breast carcinoma), SW 620 (colon carcinoma), HCT 116 (colon carcinoma), and H 460 (lung carcinoma) were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM), while MOLT-4 (acute lymphoblastic leukemia) were cultured in suspension in RPMI medium, both supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. The growth inhibition activity was assessed as described previously, according to the slightly modified procedure of the National Cancer Institute, Developmental Therapeutics Program.[6,17]

4.8. Interaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity

To a solution of DPPH (0.05 mM) in absolute ethanol an equal volume of 0.1 or 0.05 mM ethanolic solution of the tested compound was added. After 20 and 60 min the absorbance was recorded at 517 nm and was compared to the appropriate standard NDGA (Table 3). Ethanol was used as a control.[18]

4.9. Soybean lipoxygenase inhibition activity

DMSO solution of the tested compound was incubated with sodium linoleate (0.1 mM) and 0.2 mL of soybean lipoxygenase solution ($1/9 \times 10^4$ *w/v* in saline) at room temperature. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and was compared to the standard inhibitor caffeic acid (Table 3), according to the procedure previously reported.[18]

4.10. Inhibition of linoleic acid lipid peroxidation

Oxidation of linoleic acid to conjugated diene hydroperoxide in an aqueous dispersion is monitored at 234 nm. AAPH was used as a free radical initiator.[19] Ten microliters of the 16 mM linoleic acid dispersion were added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of compounds (10 μL, final concentration 0.1 mM). In the assay with no antioxidant lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation was monitored at 37 °C by recording the increase of absorption at 234 nm caused by conjugated diene hydroperoxides. The results were compared to that of the standard inhibitor trolox.

Acknowledgment

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