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RESEARCH ARTICLE

Novel 1-acyl-4-substituted semicarbazide derivatives of primaquine – synthesis, cytostatic, antiviral and antioxidative studies

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Abstract

A series of novel 1,4-substituted semicarbazides **5a–g** with a primaquine moiety bridged by a carbonyl group at position 1 and a cycloalkyl, aryl, benzyloxy or hydroxy substituent at position **4** were prepared and biologically evaluated. The synthetic pathways applied for preparation of the title compounds involved benzotriazole as synthetic auxiliary. Primaquine semicarbazides **5a–g** and their synthetic precursors benzotriazolecarbonyl semicarbazides **4** were evaluated for cytostatic, antiviral and antioxidative activities. All compounds of the series **5** showed high selectivity towards MCF-7 cells (breast carcinoma) with IC_{50} values in the low micromolar range and the most active was benzyl derivative **5c** (IC_{50} 1 ± 0.2 μM). The benzhydryl derivative **5e** showed significant cytostatic activities towards all the tested cell lines (IC_{50} 4–18 μM). The same compound was the strongest lipoxygenase inhibitor as well (51%). The highest antioxidant activity was demonstrated for the hydroxy derivative **5g** and benzotriazolecarbonyl semicarbazides **4b,c** (61.2–68.5%). No antiviral activity was observed against a wide variety of DNA and RNA viruses.

Keywords: Primaquine, semicarbazide, benzotriazole, antitumoural activity, antioxidative activity, antiviral evaluation

Introduction

Hydroxyurea, hydroxysemicarbazide, semicarbazide, semicarbazone and thiosemicarbazone functionalities have been identified as essential pharmacophores for antitumoural, antiviral, antibacterial, antimalarial, anticonvulsant, herbicidal and fungicidal activities[1–6]. Several reports show potent activity of Schiff bases of hydroxysemicarbazides against L1210 murine leukaemia cells[7,8]. The 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) is a metal chelator that potently inhibits ribonucleotide reductase[9,10]. Semicarbazide-based derivatives of peptides (azapeptides) are known as inhibitors of human dipeptidyl peptidase I, the enzyme that plays an important role in the immune system[11], while goserelin, an azapeptide analogue of the luteinising-hormone releasing hormone, is used for the treatment of prostate cancer. Hydroxyurea is a useful drug in therapy of leukaemia and other malignant tumour diseases and its derivative zileuton is an orally active inhibitor of 5-lipoxygenase which is used for the maintenance treatment of asthma[12]. Hydroxyurea is an inhibitor of ribonucleotide reductase, the enzyme described as a target for the chemotherapy of both malignant diseases and malaria[13,14]. Mahajan et al.described 7-chloroquinolyl thioureas as potential antimalarial and anticancer agents[15]. Several research groups found dual antimalarial and anticancer activity of both quinoline and trioxane derivatives as well[16–18]. Based on these findings, we have synthesized a series of compounds containing the antimalarial drug primaquine (PQ) substituted by an *N*-4-cycloalkyl, aryl,

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Abbreviations

AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride;
Btc, 1-benzotriazolecarbonyl;
BtH, benzotriazole;
DPPH, 1,l-diphenyl-2-picrylhydrazyl;
LOX, lipoxygenase;
LP, lipid peroxidation;
NDGA, nordihydroguaiaretic acid;
PQ, primaquine;
TEA, triethylamine

benzyloxy or hydroxy semicarbazide functionality. The newly designed compounds are closely related to biurets, hydroxyureas, hydroxamic acids or azapeptides. Here their synthesis, full chemical characterization, cytostatic, antiviral and antioxidative evaluations are reported. This paper is a continuation of our previous papers dealing with PQ ureas and NSAID semicarbazide derivatives as potential antitumor agents[19-22].

Material and methods

Chemistry

General experimental details

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) in ppm were referred to TMS. Elemental analyses were performed on CHNS LECO analyzer and mass spectra were taken on a HPLC-MS/MS (HPLC, Agilent Technologies 1200 Series; MS, Agilent Technologies 6410 Triple Quad). Solvent systems cyclohexane/ethyl acetate (EtOAc)/methanol (3:1:0.5), cyclohexane/EtOAc (1:1), petrol ether/EtOAc/methanol (3:1:0.75), dichloromethane/methanol (9.5:0.5) and (8.5:1.5) and pre-coated Merck silica gel 60 F_{254} plates were used for thin-layer chromatography (TLC). Spots were visualized by short-wave UV light and iodine vapour. Column chromatography was performed on silica gel (0.063–0.200 mm), with the same eluents used in TLC. Benzotriazole (BtH), triphosgene, cyclopentylamine, cyclohexylamine, benzylamine, 2-phenylethanamine, benzhydrylamine, *O*-benzylhydroxylamine hydrochloride, triethylamine (TEA), hydrazine hydrate and 10% Pd/C were purchased from Aldrich. The PQ base was prepared from primaquine diphosphate (Aldrich) prior to use. The PQ solution was protected from light during the whole procedure. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), Trolox and nordihydroguaiaretic acid (NDGA) were purchased from Aldrich Chemical Co. Soybean lipoxygenase (LOX) and linoleic acid sodium salt were obtained from Sigma Chemical Co.

1-Benzotriazole carboxylic acid chloride (1)

Compound **1** was prepared from BtH and triphosgene, according to the previously published procedure[23,24].

1-(N-Cycloalkyl/arylcarbamoyl)benzotriazoles (2a–f)

The title compounds were synthesized from chloride **1** and corresponding amine, following our previously published procedure[22,25]. The following derivatives were prepared: 1-(*N*-cyclopentylcarbamoyl)benzotriazole (**2a**), 1-(*N*-cyclohexylcarbamoyl)benzotriazole(**2b**),1-(*N*-benzylcarbamoyl)benzotriazole (**2c**), 1-(*N*-[2-phenylethyl] carbamoyl)benzotriazole (**2d**), 1-(*N*-benzhydrylcarbamoyl)benzotriazole (**2e**) and 1-(*N*-benzyloxycarbamoyl) benzotriazole (**2f**).

General procedure for preparation of 4-cycloalkyl or aryl semicarbazides (3a–f)

The title compounds were synthesized from 1-(*N*-alkyl/arylcarbamoyl)benzotriazoles (**2a–f**) and hydrazine hydrate, according to our slightly modified procedure (molar ration of reactants 1:3, dioxane, 18 h, r.t.)[22,25]. The following derivatives were prepared: 4-cyclopentylsemicarbazide (**3a**), 4-cyclohexylsemicarbazide (**3b**), 4-benzylsemicarbazide (**3c**), 4-(2-phenylethyl)semicarbazide (**3d**), 4-benzhydrylsemicarbazide (**3e**) and 4-benzyloxysemicarbazide (**3f**). These compounds are commercially available or published elsewhere.

General procedure for preparation of 1-(1-benzotriazolecarbonyl)-4-cycloalkyl/aryl semicarbazides (4a–f)

To a solution of BtcCl (**1**) (0.908 g, 5.0 mmol) in anhydrous toluene (15 mL), a solution of semicarbazide **3a–f** (5.0 mmol) and TEA (0.697 mL, 5 mmol) in anhydrous dioxane (20 mL) was added drop-wise (ice bath). The reaction mixture was stirred 1 h at room temperature or under an ice-bath and used in the next reaction step without further work-up (**4a** and **4b**) or evaporated under reduced pressure (**4c**–**f**). The residue was dissolved in 30 mL EtOAc/water mixture (1:1). The organic layer was extracted with water (3 × 40 mL), dried over anhydrous sodium sulphate and evaporated under reduced pressure. Compound **4e** precipitated from EtOAc/water mixture and the crude product was filtered off.

1-(1-Benzotriazolecarbonyl)-4-cyclopentylsemicarbazide (4a)

From the reaction of 0.716 g (5.0 mmol) **3a**, 0.908 g (5.0 mmol) **1** and 0.697 mL (5 mmol) TEA under an ice bath for 1 h the crude product **4a** was obtained and used in further reaction step without purification. Its structure was confirmed indirectly.

1-(1-Benzotriazolecarbonyl)-4-cyclohexylsemicarbazide (4b)

From the reaction of 0.786 g (5.0 mmol) **3b**, 0.908 g (5.0 mmol) **1** and 0.697 mL (5 mmol) TEA under an ice bath

for 1 h the crude product **4b** was obtained and used in further reaction step without purification. Its structure was confirmed indirectly.

1-(1-Benzotriazolecarbonyl)-4-benzylsemicarbazide (4c)

From the reaction of 0.826 g (5.0 mmol) **3c**, 0.908 g (5.0 mmol) **1** and 0.697 mL (5 mmol) TEA at room temperature for 1 h and purification of the product from ether/petrol ether, 0.884 g (57%) of **4c** was obtained; mp 146°C–147°C; IR (KBr, ν/cm^{-1}) 3310, 1753, 1659, 1576, 1495, 1450, 1380, 1290, 1254, 1029, 747, 698, 615; ^{1}H NMR (300 MHz, DMSO) δ 10.92 (1s, 1H, 1′), 8.40 (1s, 1H, 2′), 8.23, 8.20 (2d, 2H, 3, 6, J = 8.27 Hz), 7.75, 7.58 (2t, 2H, 4, 5, J = 7.40 Hz), 7.35–7.21 (m, 6H, arom., 4′), 4.28 (d, 2H, 5′, J = 6.09 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 158.41 (3′), 150.15 (1), 145.65, 132.05 (2, 7), 140.93 (6′), 130.58, 126.99, 126.18 (4, 5, 9′), 128.56, 127.42 (7′, 8′, 10′, 11′), 120.29 (6), 113.98 (3), 43.10 (5′); elemental analysis: calcd for $C_{15}H_{14}N_6O_2$: C, 58.06; H, 4.55; N, 27.08. Found: C, 58.40; H, 4.65; N, 27.36.

1-(1-Benzotriazolecarbonyl)-4-(2-phenylethyl) semicarbazide (4d)

From the reaction of 0.896 g (5.0 mmol) **3d**, 0.908 g (5.0 mmol) **1** and 0.697 mL (5 mmol) TEA at room temperature for 1 h and purification of the product from ether/petrol ether, 0.778 g (48%) of **4d** was obtained; mp 135°C–136°C; IR (KBr, ν/cm^{-1}) 3299, 1766, 1745, 1650, 1488, 1449, 1380, 1288, 1236, 1030, 750, 700; ^{1}H NMR (300 MHz, DMSO) δ 10.83 (1s, 1H, 1′), 8.30 (1s, 1H, 2′), 8.23, 8.19 (2d, 2H, 3, 6, J = 8.39 Hz), 7.75, 7.58 (2t, 2H, 4, 5, J = 7.59 Hz), 7.30–7.16 (m, arom., 5H), 6.81 (s, 1H, 4′), 3.27 (q, 2H, 5′, J = 6.75 Hz), 2.73 (t, 2H, 6′, J = 7.59 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 158.20 (3′), 150.12 (1), 145.65, 132.02 (2, 7), 140.05 (7′), 130.60, 126.47, 126.20 (4, 5, 10′), 129.12, 128.78 (8′, 9′, 11′, 12′), 120.31 (6), 113.96 (3), 41.51, 36.42 (5′, 6′); elemental analysis: calcd for $C_{16}H_{16}N_6O_2$: C, 59.25; H, 4.97; N, 25.91. Found: C, 59.60; H, 5.03; N, 25.66.

1-(1-Benzotriazolecarbonyl)-4-benzhydrylsemicarbazide (4e)

From the reaction of 1.206 g (5.0 mmol) **3e**, 0.908 g (5.0 mmol) **1** and 0.697 mL (5 mmol) TEA at room temperature for 1 h and purification of the product from ether/petrol ether 1.468 g (76%) of **4e** was obtained; mp 164°C–166°C; IR (KBr, ν/cm^{-1}) 3322, 1771, 1663, 1570, 1495, 1446, 1376, 1367, 1286, 1231, 1018, 793, 750, 703, 630; ^{1}H NMR (300 MHz, DMSO) δ 10.88 (1s, 1H, 1′), 8.30 (1s, 1H, 2′), 8.23, 8.20 (2d, 2H, 3, 6, J = 8.43 Hz), 7.75, 7.57 (2t, 2H, 4, 5, J = 7.66 Hz), 7.66 (d, 1H, 4′), 7.37–7.25 (m, arom., 10H), 6.05 (d, 1H, 5′, J = 8.43 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 157.56 (3′), 150.19 (1), 145.66, 132.00 (2, 7), 143.41 (6′, 12′), 130.64, 126.22 (4, 5), 128.74, 127.80 (7′, 8′, 10′, 11′, 13′, 14′, 16′, 17′), 127.33 (9′, 15′), 120.32 (6), 113.92 (3), 57.22 (5′); elemental analysis: calcd for $C_{21}H_{18}N_6O_2$: C, 65.27; H, 4.70; N, 21.75. Found: C, 65.40; H, 4.99; N, 21.55.

1-(1-Benzotriazolecarbonyl)-4-benzyloxysemicarbazide (4f)

From the reaction of 0.906 g (5.0 mmol) **3f**, 0.908 g (5.0 mmol) **1** and 0.697 mL (5 mmol) TEA at room temperature for 1 h and purification of the product from ether/petrol ether, 1.191 g (73%) of **4f** was obtained; mp 90°C–93°C (decomp.) mp[22] 90°C–93°C.

General procedure for preparation of primaquine semicarbazide derivatives (5a–f)

Method A: To a solution of **4a–f** (5.0 mmol) in toluene (15 mL), a solution of PQ (1.297 g, 5.0 mmol) and TEA (2.09 mL, 15.0 mmol) in dioxane (20 mL) was added drop-wise. The reaction mixture was stirred on ice bath for 2 (**5a**) or 8 h (**5b**) and evaporated under reduced pressure. The obtained residue was triturated and stirred with a dioxane/water and EtOAc/water mixtures and finally with water.

Method B: Solution of compounds **4a–f** (4 mmol), PQ (1.037 g, 4.0 mmol) and TEA (1.12 mL, 8.0 mmol) in dioxane (30 mL) was stirred at room temperature for 3 h and evaporated under reduced pressure.

Method C: Solution of compounds **4a–f** (4 mmol), PQ (1.037 g, 4.0 mmol) and TEA (1.67 mL, 12.0 mmol) in dioxane (30 mL) was stirred at room temperature for 3 h and evaporated under reduced pressure. The obtained residue was dissolved in EtOAc (30 mL) and extracted with 0.1% sodium hydroxide solution (3 × 30 mL), washed with water, dried over anhydrous sodium sulphate and evaporated under reduced pressure.

N^1-cyclopentyl-N^2-[4-(6-methoxyquinolin-8-ylamino)pentyl] hydrazine-1,2-dicarboxamide (5a)

Method A: From the reaction of 1.442 g (5.0 mmol) **4a**, 1.297 g (5.0 mmol) PQ and 2.09 mL (15 mmol) TEA on ice bath for 2 h and purification of the product by column chromatography (eluent dichloromethane/MeOH 9.5:0.5 and 9:1) and trituration with ether, 0.279 g (13%) of **5a** was obtained; mp 190°C–194°C; IR (KBr, ν/cm^{-1}) 3306, 3223, 3088, 2939, 2868, 1661, 1618, 1551, 1520, 1455, 1422, 1388, 1326, 1218, 1199, 1158, 1051, 1031, 971, 937, 899, 820, 790, 681, 625; MS/MS m/z 429.5 (M + H)$^+$; ^{1}H NMR (300 MHz, DMSO) δ 8.54 (d, 1H, 11, J = 2.76 Hz), 8.08 (d, 1H, 13, J = 7.81 Hz), 7.46–7.42 (m, 3H, 1′, 2′, 12), 6.48 (s, 1H, 17), 6.36 (t, 1H, 2, J = 4.84 Hz), 6.27 (s, 1H, 15), 6.11, 6.08 (2d, 2H, 8, 4′, J = 8.96 Hz, 8.04 Hz), 3.90–3.83 (m, 1H, 5′), 3.83 (s, 3H, 18), 3.63–3.61 (m, 1H, 6), 3.04 (q, 2H, 3), 1.76–1.20 (m, 15H, 4, 5, 7, 6′–9′); ^{13}C NMR (75.5 MHz, DMSO) δ 159.47 (1), 159.18 (16), 158.64 (3′), 145.11 (9), 144.70 (11), 135.26 (13), 134.99 (10), 130.05 (14), 122.56 (12), 96.54 (17), 92.05 (15), 55.45 (18), 51.38 (5′), 47.52 (6), 39.54 (3), 33.84 (5), 33.07 (6′, 9′), 27.15 (4), 23.67 (7′, 8′), 20.67 (7); Elemental analysis: calcd for $C_{22}H_{32}N_6O_3$: C, 61.66; H, 7.53; N, 19.61. Found: C, 61.80; H, 7.64; N, 19.75.

N^1-cyclohexyl-N^2-[4-(6-methoxyquinolin-8-ylamino)pentyl] hydrazine-1,2-dicarboxamide (5b)

Method A: From the reaction of 1.512 g (5.0 mmol) **4b**, 1.297 g (5.0 mmol) PQ and 2.09 mL (15 mmol) TEA on ice bath for 8 h and re-crystallization from methanol/water, 1.394 g (63%) of **5b** was obtained; mp 163°C–168°C; IR (KBr, ν/cm^{-1}) 3312, 3228, 3091, 2932, 2855, 1664, 1618,

1548, 1521, 1456, 1423, 1389, 1336, 1285, 1239, 1220, 1201, 1158, 1133, 1052, 1031, 822, 791, 680, 625, 532; ^{1}H NMR (300 MHz, DMSO) δ 8.53 (d, 1H, 11, J = 4.04 Hz), 8.07 (d, 1H, 13, J = 8.07 Hz), 7.47–7.40 (2s, q, 3H, 1′, 2′, 12), 6.47 (s, 1H, 17), 6.35 (t, 1H, 2, J = 4.84 Hz), 6.26 (s, 1H, 15), 6.11, 5.96 (2d, 2H, 8, 4′, J = 8.07 Hz), 3.82 (s, 3H, 18), 3.65–3.57 (m, 1H, 6), 3.40 (m, 1H, 5′), 3.03 (q, 2H, 3), 1.72–1.04 (m, 17H, 4, 5, 7, 6′-10′); ^{13}C NMR (75.5 MHz, DMSO) δ 159.47 (1), 159.15 (16), 158.27 (3′), 145.10 (9), 144.69 (11), 135.27 (13), 134.98 (10), 130.05 (14), 122.56 (12), 96.55 (17), 92.06 (15), 55.45 (18), 48.41 (5′), 47.52 (6), 39.53 (3), 33.84 (5), 33.44 (6′, 10′), 27.14 (4), 25.64 (8′), 25.08 (7′, 9′), 20.66 (7); MS/MS m/z 443.3 $(M + H)^+$; elemental analysis: calcd for $C_{23}H_{34}N_6O_3$: C, 62.42; H, 7.74; N, 18.99. Found: C, 62.21; H, 7.55; N, 18.60.

N^1-benzyl-N^2-[4-(6-methoxyquinolin-8-ylamino)pentyl] hydrazine-1,2-dicarboxamide (5c)

Method B: From the reaction of 1.241 g (4.0 mmol) **4c**, 1.037 g (4.0 mmol) PQ and 1.12 mL (8 mmol) TEA at room temperature for 3 h and purification of the product by trituration with ether/EtOAc/petrol ether and ether/petrol ether, 1.153 g (64%) of **5c** was obtained; mp 200°C–203°C; IR (KBr, ν/cm^{-1}) 3306, 3223, 3089, 2933, 1664, 1616, 1572, 1555, 1519, 1455, 1423, 1387, 1322, 1219, 1201, 1158, 1051, 1030, 820, 790, 735, 700, 626; ^{1}H NMR (300 MHz, DMSO) δ 8.54 (d, 1H, 11, J = 3.87 Hz), 8.08 (d, 1H, 13, J = 8.12 Hz), 7.66 (1s, 1H, 1′), 7.61 (1s, 1H, 2′), 7.42 (q, 1H, 12, J = 4.06 Hz), 7.26–7.14 (m, arom., 5H, 7′–11′), 6.90 (t, 1H, 4′, J = 5.42 Hz), 6.47 (s, 1H, 17), 6.42 (t, 1H, 2, J = 4.84 Hz), 6.26 (s, 1H, 15), 6.12 (d, 1H, 8, J = 8.70 Hz), 4.21 (d, 2H, 5′, J = 5.80 Hz), 3.82 (s, 3H, 18), 3.66–3.57 (m, 1H, 6), 3.05 (q, 2H, 3), 1.65–1.46 (m, 4H, 4, 5), 1.20 (d, 3H, 7, J = 6.00 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 159.48 (1), 159.25 (16), 159.08 (3′), 145.12 (9), 144.71 (11), 141.09 (6′), 135.27 (13), 135.00 (10), 130.06 (14), 128.48, 127.40 (7′, 8′,10′, 11′), 126.85 (9′), 122.58 (12), 96.54 (17), 92.05 (15), 55.46 (18), 47.54 (6), 43.00 (5′), 39.65 (3), 33.85 (5), 27.20 (4), 20.69 (7); MS/MS m/z 451.3 $(M + H)^+$; elemental analysis: calcd for $C_{24}H_{30}N_6O_3$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.63; H, 6.77; N, 18.78.

N^1-[4-(6-methoxyquinolin-8-ylamino)pentyl]-N^2-phenylethylhydrazine-1,2-dicarboxamide (5d)

Method B: From the reaction of 1.297 g (4 mmol) **4d**, 1.037 g (4.0 mmol) PQ and 1.12 mL (8 mmol) TEA at room temperature for 3 h and purification of the product by trituration with ether/EtOAc, 1.264 g (68%) of **5d** was obtained; mp 174°C–176°C; IR (KBr, ν/cm^{-1}) 3302, 3226, 3088, 2933, 1662, 1616, 1575, 1554, 1520, 1455, 1423, 1387, 1326, 1219, 1200, 1157, 1131, 1051, 1131, 820, 790, 750, 700, 624; ^{1}H NMR (300 MHz, DMSO) δ 8.50 (d, 1H, 11, J = 3.79 Hz), 8.04 (d, 1H, 13, J = 7.81 Hz), 7.54 (1s, 1H, 1′), 7.47 (1s, 1H, 2′), 7.39 (q, 1H, 12, J = 4.24 Hz), 7.26–7.11 (m, arom., 5H, 8′–12′), 6.44 (s, 1H, 17), 6.33–6.28 (2t, 2H, 2, 4′), 6.24 (s, 1H, 15), 6.09 (d, 1H, 8, J = 8.70 Hz), 3.80 (s, 3H, 18), 3.62–3.57 (m, 1H, 6), 3.20 (q, 2H, 5′, J = 6.47 Hz), 3.02 (q, 2H, 3), 2.66 (t, 2H, 6′, J = 7.14 Hz), 1.63–1.43 (m, 4H, 4, 5), 1.18 (d, 3H, 7, J = 6.03 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 159.49, 159.10, 159.02 (1, 16, 3′), 145.14 (9), 144.71 (11), 140.11 (7′), 135.26 (13), 135.02 (10), 130.06 (14), 129.09, 128.73 (8′, 9′,11′, 12′), 126.40 (10′), 122.55 (12), 96.57 (17), 92.11 (15), 55.46 (18), 47.56 (6), 41.28 (5′), 39.64 (3), 36.48 (6′), 33.89 (5), 27.15 (4), 20.70 (7); MS/MS m/z 465.3 $(M + H)^+$; elemental analysis: calcd for $C_{25}H_{32}N_6O_3$: C, 64.63; H, 6.94; N, 18.09. Found: C, 64.88; H, 7.11; N, 18.48.

N^1-benzhydryl-N^2-[4-(6-methoxyquinolin-8-ylamino)pentyl] hydrazine-1,2-dicarboxamide (5e)

Method C: From the reaction of 1.546 g (4 mmol) **4e**, 1.037 g (4.0 mmol) PQ and 1.67 mL (12.0 mmol) TEA at room temperature for 3 h and purification of the product by column chromatography (eluent dichloromethane/MeOH 9.5:0.5) and re-crystallized from ether/petrol ether, 1.369 g (65%) of **5e** was obtained; mp 90°C (decomp.); IR (KBr, ν/cm^{-1}) 3311, 2960, 2933, 2869, 1662, 1616, 1575, 1555, 1519, 1454, 1423, 1387, 1220, 1202, 1158, 1052, 1030, 822, 791, 747, 700, 625; ^{1}H NMR (300 MHz, DMSO) δ 8.51 (d, 1H, 11, J = 3.13 Hz), 8.05 (d, 1H, 13, J = 8.13 Hz), 7.61 (s, 1H, 1′), 7.54 (s, 1H, 2′), 7.39 (q, 1H, 12, J = 4.17 Hz, 3.75 Hz), 7.29–7.17 (m, arom., 10H, 7′–11′, 13′–17′), 6.98 (d, 1H, 4′, J = 8.34 Hz), 6.45 (s, 1H, 17), 6.35 (t, 1H, 2), 6.24 (s, 1H, 15), 6.09 (d, 1H, 8, J = 8.55 Hz), 5.91 (d, 1H, 5′, J = 8.34 Hz), 3.80 (s, 3H, 18), 3.63–3.55 (m, 1H, 6), 3.02 (q, 2H, 3), 1.62–1.41 (m, 4H, 4, 5), 1.17 (d, 3H, 7, J = 5.84 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 159.51 (1), 159.12 (16), 158.29 (3′), 145.14 (9), 144.71 (11), 143.57 (6′, 12′), 135.26 (13), 135.03 (10), 130.07 (14), 128.74, 127.60 (7′, 8′,10′, 11′, 13′, 14′, 16′, 17′), 127.26 (9′, 15′), 122.56 (12), 96.57 (17), 92.13 (15), 57.21(5′), 55.47 (18), 47.56 (6), 39.68 (3), 33.89 (5), 27.19 (4), 20.69 (7); MS/MS m/z 527.3 $(M + H)^+$; elemental analysis: calcd for $C_{30}H_{34}N_6O_3$: C, 68.42; H, 6.51; N, 15.96. Found: C, 68.80; H, 6.44; N, 16.13.

N^1-benzyloxy-N^2-[4-(6-methoxyquinolin-8-ylamino)pentyl] hydrazine-1,2-dicarboxamide (5f)

Method C: From the reaction of 1.305 g (4 mmol) **4f**, 1.037 g (4.0 mmol) PQ and 1.67 mL (12.0 mmol) TEA at room temperature for 3 h and purification of the product by column chromatography (eluent dichloromethane/MeOH 9.5:0.5) and trituration with ether, 0.802 g (43%) of **5f** was obtained; mp 102°C–104°C; IR (KBr, ν/cm^{-1}) 3300, 3216, 3089, 2934, 1670, 1617, 1573, 1520, 1455, 1423, 1387, 1328, 1219, 1201, 1157, 1051, 1030, 820, 790, 738, 697, 625; ^{1}H NMR (300 MHz, DMSO) δ 9.48, 8.42, 7.50 (3s, 3H, 1′, 2′, 4′), 8.55 (dd, 1H, 11, J = 3.92 Hz), 8.08 (d, 1H, 13, J = 8.19 Hz), 7.45–7.31 (m, arom., 6H, 7′–11′, 12), 6.48 (d, 1H, 17, J = 2.26 Hz), 6.28 (d, 1H, 15, J = 2.14 Hz), 6.18 (t, 1H, 2, J = 5.70 Hz), 6.13 (d, 1H, 8, J = 8.78 Hz), 4.77 (s, 2H, 5′), 3.83 (s, 3H, 18), 3.68–3.59 (m, 1H, 6), 3.04 (q, 2H, 3), 1.66–1.43 (m, 4H, 4, 5), 1.22 (d, 3H, 7, J = 6.17 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 160.16 (1), 159.47 (16), 158.98 (3′), 145.11 (9), 144.72 (11), 136.95 (6′), 135.27 (13), 135.00 (10), 130.05 (14), 129.23, 128.59 (7′, 8′,10′, 11′), 128.42 (9′), 122.58 (12), 96.58 (17), 92.08 (15), 77.85 (5′),

55.46 (18), 47.51 (6), 39.71 (3), 33.87 (5), 27.24 (4), 20.70 (7); MS/MS *m/z* 467.3 $(M + H)^+$; elemental analysis: calcd for $C_{24}H_{30}N_6O_4$: C, 61.79; H, 6.48; N, 18.01. Found: C, 61.83; H, 6.40; N, 17.86.

N^1-hydroxy-N^2-[4-(6-methoxyquinolin-8-ylamino)pentyl] hydrazine-1,2-dicarboxamide (5g)

A suspension of **5e** (0.467 g, 1.0 mmol) in methanol (15 mL) was hydrogenated for 1.5 h in the presence of 10% Pd/C (100 mg). The catalyst was filtered off and the solvent was evaporated under reduced pressure. The crude product was re-crystallized from ether/petrol ether. Yield: 0.226 g (60%). mp 112°C; IR (KBr, ν/cm^{-1}) 3262, 2929, 2857, 1654, 1616, 1576, 1558, 1520, 1456, 1423, 1388, 1221, 1202, 1159, 1052, 1031, 822, 791, 625; ^{1}H NMR (300 MHz, DMSO) δ 8.70, 8.55, 8.21, 7.42 (4s, 4H, 1′, 2′, 4′, OH), 8.54 (d, 1H, 11), 8.08 (dd, 1H, 13, J = 8.36 Hz), 7.43–7.41 (q, 1H, 12), 6.48 (d, 1H, 17, J = 2.33 Hz), 6.27 (d, 1H, 15, J = 2.33 Hz), 6.17 (t, 1H, 2, J = 5.82 Hz), 6.14–6.11 (m, 1H, 8), 3.83 (s, 3H, 18), 3.66–3.58 (m, 1H, 6), 3.04 (q, 2H, 3), 1.64–1.46 (m, 4H, 4, 5), 1.21 (d, 3H, 7, J = 6.21 Hz); ^{13}C NMR (75.5 MHz, DMSO) δ 160.6 (1), 158.92 (16), 158.51 (3′), 144.52 (9), 144.15 (11), 134.76 (13), 134.38 (10), 129.50 (14), 122.02 (12), 96.05 (17), 91.54 (15), 54.91 (18), 46.96 (6), 39.08 (3), 33.30 (5), 26.67 (4), 20.12 (7); MS/MS *m/z* 377.3 $(M + H)^+$; elemental analysis: calcd for $C_{17}H_{24}N_6O_4$: C, 54.24; H, 6.43; N, 22.33. Found: C, 54.41; H, 6.55; N, 21.98.

Biological evaluation

Cytostatic activity assays

The HCT 116 (colon carcinoma), MCF-7 (breast carcinoma), H 460 (lung carcinoma) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% foetal bovine serum (FBS), 2 mmol L^{-1} L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} 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[26,27]. Briefly, the cells were inoculated onto standard 96-well microtiter plates on day 0. Test agents were then added in five consecutive 10-fold dilutions (10^{-8} to 10^{-4} mol L^{-1}) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. The solvent (DMSO) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentrations (maximal concentration of DMSO was 0.25%). After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay which detects dehydrogenase activity in viable cells. The absorbance was measured on a microplate reader at 570 nm. Each test point was performed in quadruplicate in at least two individual experiments. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from dose–response curves using linear regression analysis by fitting the test concentrations that give percentage of growth (PG) values above and below the reference value (i.e. 50%). Each result is a mean value from three separate experiments.

The cytostatic activity of the test compounds against L1210, HeLa and CEM cells was determined with cells grown in suspension (L1210, CEM) or monolayers (HeLa) in 200 μL-wells of 96-well microtiter plates (initial cell number: 5–7.5 × 10^4 cells/well). After 48 (L1210) or 72 (CEM, HeLa) h, the tumour cell number was determined by the use of a Coulter counter.

Antiviral activity assays

Antiviral activity against vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, herpes simplex virus type 1 (KOS) and type 2 (G), thymidine kinase-deficient herpes simplex virus type 1 (TK$^-$ KOS ACV^r), vaccinia virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, Punta Toro virus, influenza virus A (H1N1; H3N2) and B, feline corona virus (FIPV) and feline herpes virus, HIV-1 and HIV-2 was determined essentially, as described previously[28]. After a 2 h incubation period, residual virus was removed and the infected cells were further incubated with the medium containing different concentrations of the test compounds. After incubation for 3 days at 37°C, virus-induced cytopathogenicity was monitored microscopically. Antiviral activity was expressed as the concentration required to reduce virus-induced cytopathogenicity by 50% (EC_{50}).

Antioxidant activity assays

Determination of the reducing activity of the stable radical DPPH

To an ethanolic solution of DPPH (0.05 mM) in absolute ethanol an equal volume of the compounds (final concentration 100 μM) dissolved in DMSO was added. The mixture was shaken vigorously and allowed to stand for 20 or 60 min. Absorbance at 517 nm was determined spectrophotometrically, and the percentage of activity was calculated. Each experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean (Table 1).

Inhibition of linoleic acid lipid peroxidation

The water soluble azo compound AAPH is used as a free radical initiator for *in vitro* studies of free radical production. Production of conjugated diene hydroperoxide by oxidation of linoleic acid sodium salt in an aqueous solution is monitored at 234 nm. This assay can be used to follow oxidative changes and to understand the contribution of each tested compound. An amount of 10 μL of the 16 mM linoleic acid sodium salt solution was 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 aliquots (10 μL) in the assay without antioxidant, and lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37°C was monitored by recording the increase in

Table 1. Interaction with DPPH, *in vitro* inhibition of LP, soybean LOX and theoretically calculated Clog *p* values.

Compd.	DPPH[a] (%) 20 min	DPPH[a] (%) 60 min	LP[a] (%)	LOX inhibition[a] (%)	Clog *p* [b]
4b	61.2	69.2	na	na	1.92
4c	66.6	77.4	na	na	2.25
5a	3.7	3.5	1.0	na	3.45
5b	12.0	15.7	2.4	29.4	4.01
5c	12.6	16.7	2.0	35.4	3.71
5d	2.4	1.3	2.0	45.2	4.08
5e	10.0	13.2	3.0	51.0	5.05
5f	8.0	15.0	1.2	9.6	3.51
5g	68.5	72.0	2.0	14.1	1.03
NDGA	84	91	–	81	–
Trolox	–	–	67	–	–

[a] Concentration of the tested compound: 100 μM;
[b] Clog *p* values were calculated by the ClogP programme of Biobyte Corp[35];
na– no activity.

absorption at 234 nm caused by conjugated diene hydroperoxides. All tests were undertaken on three replicates, and the results were averaged.

Soybean LOX inhibition study in vitro

The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 mL) and 0.2 mL of the enzyme solution (1 part of enzyme/9 parts of saline $\times 10^{-4}$, *w*/*v* in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor.

Results and discussion

Chemistry

A series of novel 1,4-substituted semicarbazides **5a–g** with PQ moiety bridged by carbonyl group at position 1 and cycloalkyl, aryl, benzyloxy or hydroxy substituent at position 4 were prepared and biologically evaluated. The title compounds contain a region of six or seven electronegative atoms bound to primary amino group of PQ. Therefore, they are closely related to biurets, hydroxyureas, hydroxamic acids or azapeptides.

Preparation of compounds **5a–f** involved BtH as synthetic auxiliary. The BtH moiety in 1-(*N*-cycloalkyl/arylcarbamoyl)benzotriazoles (1-benzotriazole carboxylic acid amides) **2a–f** and 1-(1-benzotriazolecarbonyl)-4-cycloalkyl/aryl semicarbazides **4a–f** activated the molecules for nucleophilic substitution with hydrazine hydrate or PQ, allowing the preparation of semicarbazides **3a–f** and PQ-semicarbazide derivatives **5a–f**, respectively. The BtH was introduced in the intermediate products 2 and 4 by means of 1-benzotriazole carboxylic acid chloride (BtcCl, 1), which was first described by our research group and now is commercially available[23,24]. The TEA was used as a catalyst and/or HCl acceptor in several synthetic steps. The synthetic pathway is shown in Figure 1: the starting chloride **1** in reaction with corresponding amine (cyclopentylamine, cyclohexylamine, benzylamine, 2-phenylethanamine, benzhydrylamine, O-benzylhydroxylamine) gave 1-carbamoylbenzotriazoles **2a–f**. In the next reaction step, the BtH was replaced by hydrazine hydrate to give 4-substituted semicarbazides **3a–f**. Benzotriazolecarbonyl semicarbazides **4a–f** were prepared by the reaction of products **3** with chloride **1**. In the final reaction step, benzotriazole ring of **4a–f** was substituted with PQ affording PQ-semicarbazide derivatives **5a–f**. Compound **5g** with a free hydroxyl group at position 4 of semicarbazide backbone was prepared by hydrogenolysis of benzyloxysemicarbazide **5f**.

Structures of the synthesized compounds are supported by infrared, mass, ^{1}H and ^{13}C NMR spectra and confirmed by elemental analysis. The chemical shifts are consistent with the proposed structures. The presence of the PQ residue was confirmed in ^{1}H (^{13}C) spectra of **5a–g**: hydrogen atoms next to pyridine nitrogen occurred between 8.50 and 8.55 (144.15 and 144.72), OCH_3 group appeared as a singlet between 3.80 and 3.83 (54.91 and 55.47), C-6 appeared as a multiplet between 3.54 and 3.68 (46.96 and 47.56), C-7 occurred as a doublet between 1.17 and 1.22 (20.12 and 20.70), while NH group at position 8 appeared as a doublet between 5.96 and 6.13 ppm. Semicarbazide NH groups 1′ and 2′ of compounds **5** showed as two close singlets at 7.47–7.61 ppm, while those of compounds **4** were shifted to 10.83–10.88 and 8.30–8.40, respectively. The NH groups at positions 2 and 4′ were approximately at 6.90 and 6.35 ppm. Signals of two carbonyl C-atoms in the PQ semicarbazide spectra were very close and appeared between 158.27 and 160.6 ppm, while C-1 carbonyl in benzotriazolecarbonyl semicarbazides **4** was shifted to the lower parts per million values (150.12–150.19). Atom enumeration of compounds **4** and **5** is shown in Figure 2. The IR spectra of **5a–f** showed absorption bands at 3410–3262 (NH groups), 1674–1650, 1576–1548 and 1521–1519 cm^{-1} (semicarbazide carbonyls), while carbonyl group bound to BtH in compound **4** was absorbed at 1771–1738 cm^{-1}.

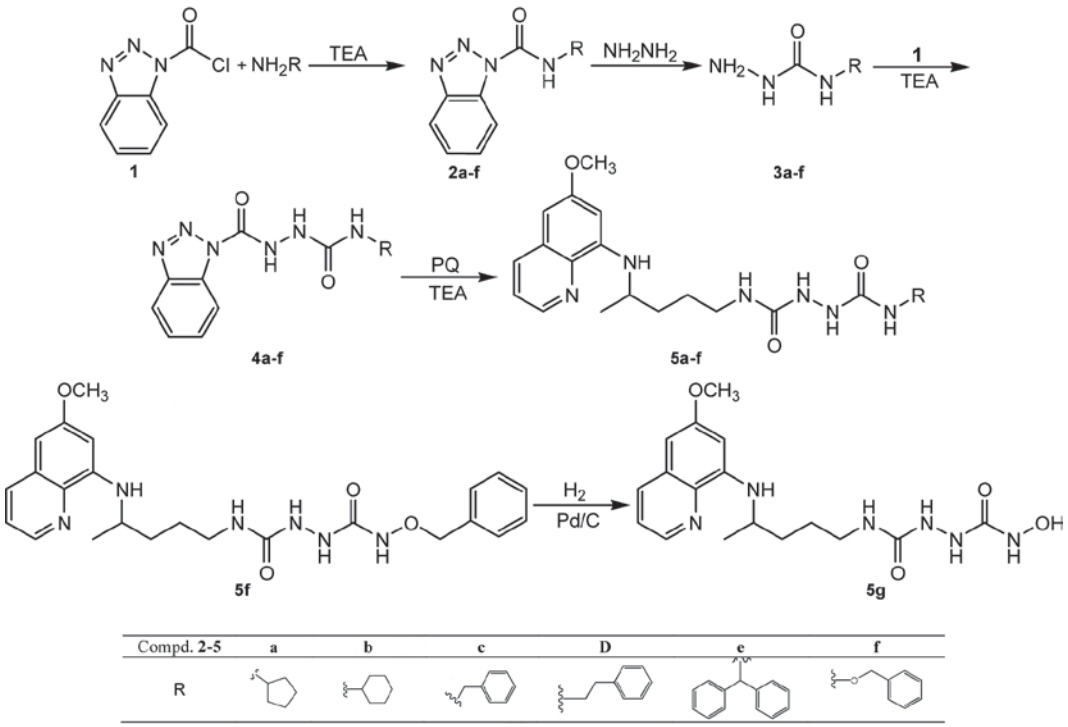


Figure 1. Synthesis of PQ semicarbazide derivatives 5a–g.

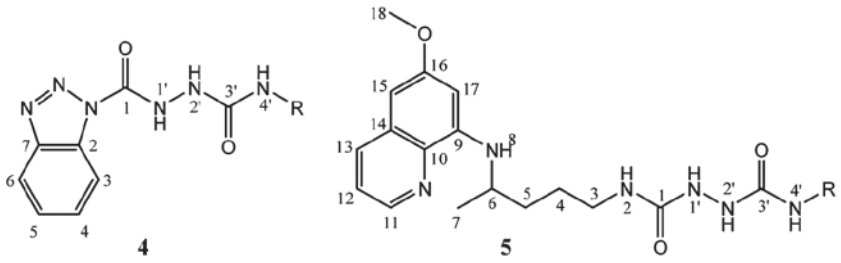


Figure 2. Atom enumeration of compounds 4 and 5.

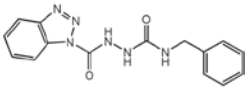
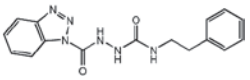
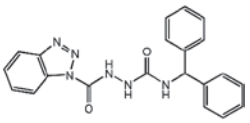
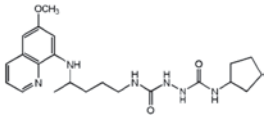
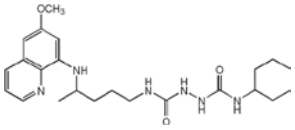
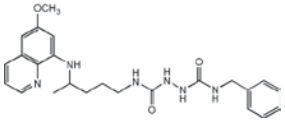
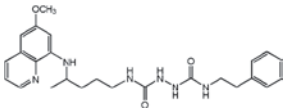
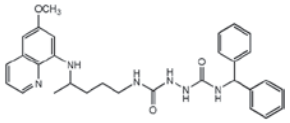
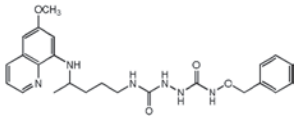
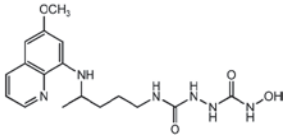
Biological evaluation

Cytostatic activity

The PQ-semicarbazide derivatives **5a–g** and their synthetic precursors benzotriazolecarbonyl semicarbazides **4** were evaluated for their cytostatic activities against a variety of tumour cell lines, which are derived from several cancer types. The following cell lines were used: human HCT 116, MCF-7, H 460, CEM, HeLa and murine L1210. All compounds of the series **5** showed high selectivity towards MCF-7 cells (breast carcinoma) with IC_{50} values in the low micromolar range (Table 2). Benzyl derivative **5c** was the most active compound (IC_{50} 1 ± 0.2 μM), while benzhydryl derivative **5e** showed significant cytostatic activities towards all the tested cell lines (IC_{50} 4–17 μM) and the highest activity against MCF-7 cells. These data are in agreement with our previous results obtained for NSAID semicarbazide derivatives[22], where the presence of a bulky lipophilic group, preferably a benzhydryl group, at the *N*-4 position of the semicarbazide backbone was strictly required, while the NSAID terminal end of these molecules was less important to display anti-proliferative potency. On the other hand, analogue compounds of the series **4** with the same semicarbazide moiety, containing a BtH ring instead of PQ or NSAID residues were practically inactive, which indicates the importance of the PQ/NSAID part of the molecule.

Most interesting, however, is the high sensitivity of MCF-7 tumour cells to almost all the compounds. This phenomenon was already noted in the authors' previous work with urea derivatives of PQ [20]. Some other authors reported selectivity of quinidine, PQ, *N*-dipeptidylprimaquines and imidazolin-4-one PQ derivatives towards MCF-7 cell line as well[16,29]. Selective growth inhibitory activity towards breast cancer cells could be explained by selective induction of cytochrome P450 (CYP-1) enzymes specifically in the MCF-7 line, known as CYP-1 inducible cell line[30]. These enzymes catalyse the initial step in either detoxification or bio-activation of environmental toxins and xenobiotics. The transcriptional regulation of the CYP1A1 gene by polycyclic aromatic hydrocarbons

Table 2. Inhibitory effects of PQ semicarbazide derivatives **5a-g** and their synthetic precursors **4** on the proliferation of malignant tumour cell lines.

		IC_{50}[a] (μM)					
Compd.	Structural formula	HCT 116	MCF-7	H 460	L1210	CEM	HeLa
4b		> 100	> 100	> 100	> 250	> 250	> 250
4c		> 100	> 100	> 100	≥ 250	> 250	> 250
4d		> 100	79 ± 16	> 100	–	–	–
5a		> 100	11 ± 8	> 100	> 250	> 250	> 250
5b		> 100	16 ± 12	> 100	144 ± 86	≥ 250	150 ± 74
5c		> 100	1 ± 0.2	> 100	> 250	> 250	> 250
5d		> 100	4 ± 0.3	> 100	> 250	> 250	> 250
5e		17 ± 1	4 ± 0.6	17 ± 0.9	18 ± 2	11 ± 1	16 ± 1
5f		16 ± 2	1.5 ± 1.3	32 ± 4	21 ± 1	21 ± 5	12 ± 11
5g		≥ 100	3 ± 0.2	> 100	173 ± 45	179 ± 34	202 ± 67
PQ20		20 ± 6	28 ± 10	30 ± 7	nt	nt	nt

[a]IC_{50}; the concentration that causes 50% growth inhibition.
[b]nt; not tested.

(PAH) and halogenated aromatic hydrocarbons (HAH) is mediated via ligand-dependent activation of the aryl hydrocarbon receptor (AhR), which translocates to the nucleus upon activation, dimerizes to the aryl hydrocarbon receptor nuclear translocator (arnt) protein and binds to the xenobiotic response element (XRE) in the regulatory region of the CYP1A1 gene[31]. Probably, the observed selectivity towards breast cancer cells could be related to the PQ moiety, although PQ does not conform to the structural features of AhR ligands (planar hydrophobic aromatic compounds) and, accordingly, does not bind with high specificity to the AhR. It has been shown that several non-planar compounds with little aromaticity, such as benzimidazole compounds (e.g. some antiparasitic drugs) also induce the CYP1A1 mRNA expression, but the mechanism of its induction has not been well understood[32]. Moreover, it was demonstrated that PQ is a potent inducer of CYP1A1 in the rat hepatoma H4IIE cells[31]. It uses the AhR for the transcriptional activation of the CYP1A1 gene and causes an inhibition of CYP1A1 enzyme degradation. A similar concept of sensitivity of MCF-7 cells towards

benzothiazoles mediated by AhR has been shown concerning the discovery of a class of 2-(4-aminophenyl) benzothiazoles, potential antitumour agents that showed remarkably similar, highly selective profiles of antitumour activity[33]. The intriguing specificity of PQ semicarbazides towards MCF-7 cells should be further studied in more detail and fostered towards the discovery of novel AhR-dependent toxicity/antitumour activity.

Antiviral activity

Compounds **4b, 4c** and **5a–g** were evaluated against a broad variety of viral infections, including herpes simplex virus type 1 (HSV-1) and HSV-2, vaccinia virus and vesicular stomatitis virus in HEL cell cultures, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures, vesicular stomatitis virus, Coxsackie B4 virus and respiratory syncytial virus in HeLa cell cultures, feline coronavirus (FIPV) and feline herpes virus in CRFK cell cultures, influenza A (H1N1 and H3N2) and B in MDCK cell cultures and human immunodeficiency virus type 1 (HIV-1/III_B) and HIV-2(ROD) in CEM cell cultures. No specific antiviral effects were noted for any of the compounds evaluated against any of the tested viruses (data not shown).

Antioxidant activity

In the present investigation, antioxidant activity of the PQ derivatives **4b, 4c** and **5a–g** were studied and compared with the well-known antioxidant agents, such as NDGA and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). Two different antioxidant assays were used: (1) interaction with the stable free DPPH radical and (2) interaction with the water-soluble azo compound AAPH, used as a source of peroxyl radicals[34]. The DPPH interaction of the tested compounds was examined at 100 μM concentration after 20 and 60 min. The results are shown in Table 1. Derivative **5g** with hydroxy group at position **4** was the only PQ semicarbazide that showed significant interaction (68.5%), followed by 1-(1-benzotriazolecarbonyl)-4-aryl semicarbazides **4b** and **4c** (61.2% and 66.6%, respectively). A slight increase (69.2–77.4%) in antioxidant activity was observed after 60 min. Low lipophilicity values (1.03–2.25) are correlated with higher DPPH interaction. The antioxidant ability of the other compounds of the series **5** was very low (2.4–12.6%).

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Under our experimental conditions, no inhibition of lipid peroxidation (LP) by the tested compounds was observed.

The LOXs play a significant role in membrane LP by forming hydroperoxides in the lipid bilayer from the biotransformation of arachidonic acid catalyzed by LOX. The LOX inhibitors have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, certain types of cancer and cardiovascular diseases. In this context, it was decided to evaluate the newly prepared derivatives for their ability to inhibit soybean LOX[36]. It has been shown that inhibition of plant LOX activity by NSAIDs is qualitatively similar to their inhibition of the rat mast cell LOX and may be used as a simple qualitative screen for such activity. Compound **5e**, followed by **5d**, was found to be the most potent among the PQ semicarbazides **5**, while the tested compound **4** showed no activity. The fact that the most active compounds have high Clog *p* values (5.05 and 4.08, respectively) support the findings that lipophilicity is an important physicochemical property for LOX inhibitors.

Conclusions

The PQ semicarbazides **5**, described here, showed significant selective cytostatic activity against the proliferation of MCF-7 cells with IC_{50} values between 1 and 16 μM (five compounds in the range 1–4 μM). The most active compound was the benzyl derivative **5c**. Benzhydryl derivative **5e** showed cytostatic activities towards all the tested cell lines (IC_{50} 4–18 μM). The same compound was the strongest LOX inhibitor as well. It is worth to note that in the series of NSAID semicarbazide derivatives previously described by our group[22], the most active compounds were also the benzhydryl derivatives. On the other hand, the highest antioxidant activity was found for the hydroxy derivative **5g** and the benzotriazolecarbonyl semicarbazides **4b, 4c**. The prepared PQ semicarbazide derivatives represent useful lead compounds in development of specific agents against breast cancer. They have potential as antimalarial agents as well, but their antimalarial evaluation still remains to be performed.

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Declaration of interest

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