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Original article

Novel NSAID 1-acyl-4-cycloalkyl/arylsemicarbazides and 1-acyl-5-benzyloxy/hydroxy carbamoylcarbazides as potential anticancer agents and antioxidants

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ABSTRACT

The novel 1-acyl-4-cycloalkyl/arylsemicarbazides (**5a**–**y**) and 1-acyl-5-benzyloxy/hydroxy carbamoylcarbazides (**8a**–**f**) derived from the nonsteroidal anti-inflammatory drugs ibuprofen, fenoprofen and reduced ketoprofen were prepared, fully chemically characterized and evaluated for their cytostatic, antiviral and antioxidant activities. Compounds **5** and **8** consist of a region rich in electronegative atoms (five to nine nitrogen and oxygen atoms) framed by aryl or cycloalkyl residues on one or both terminal ends. The synthetic pathways applied for the preparation of the title compounds involved a benzotriazole as a synthetic auxiliary in several steps. Three of the tested compounds, namely 4-benzhydryl-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5l**), 4-benzhydryl-1-[2-(3-benzylphenyl) propanoyl]semicarbazide (**5s**), and 4-benzhydryl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5f**) showed pronounced antiproliferative activity in vitro against six cancer cell lines ($IC_{50} = 3–23$ μM). The same compounds highly inhibited soybean lipoxygenase ($IC_{50} = 60$ and 51.5 μM) and lipid peroxidation as well (99, 88 and 74%, respectively). 4-Benzyloxy-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5t**) and 5-benzyloxycarbamoyl-1-[2-(3-benzylphenyl)propanoyl]carbazide (**8c**) exerted complete lipid peroxidation inhibition. Semicarbazides **5w**–**y** and carbazides **8d**–**f** bearing a hydroxamic acid/hydroxyurea moiety showed a modest antiradical activity in DPPH test, while the best radical scavenger was 1-(1-benzotriazolecarbonyl)-4-benzyloxysemicarbazide (**7**). None of the compounds were inhibitory to a broad panel of DNA and RNA viruses in the cell culture at subtoxic concentrations.

1. Introduction

Chronic inflammation is a common and important factor in the pathogenesis of neoplasmas. Inflammation is often accompanied by the excessive formation of reactive oxygen and nitrogen species that are potentially damaging to DNA and cell membranes. In addition, the expression of COX-2 in inflammatory and neoplastic cells has an impact on various carcinogenic pathways [1–4]. NSAIDs usefulness in chemoprevention is well-documented. Numerous experimental, epidemiological, and clinical studies have found that long-term use of NSAIDs is associated with a lower risk of colorectal cancer, adenomatous polyps, and some other types of cancer [5–8]. NSAID chemical modifications were undertaken with the aim of improving the NSAIDs safety profile (increasing the analgetic/anti-inflammatory activity and COX1/COX2 selectivity, reducing the ulcerogenic effect or obtaining dual COX/LOX inhibition) [9,10]. Many efforts were made to get NSAID derivatives with pronounced cytostatic activity as well [11–19]. Thus, we set to prepare a series of new NSAID derivatives rich in nitrogen/oxygen atoms, related to semicarbazides, carbazides, ureas, hydroxyureas and hydroxamic acids, bearing pharmacophores present in numerous antitumor agents [20–23]. Their synthesis, full chemical characterization, and evaluation of their cytostatic, antiviral and antioxidative potential are reported in this paper.

2. Materials and methods

2.1. Synthesis

2.1.1. Materials and general methods

Melting points were determined on a Stuart Melting Point Apparatus SMP3 and were uncorrected. IR spectra were recorded

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on a FTIR Perkin–Elmer Paragon 500 spectrometer. UV–visible double beam spectrophotometer Lambda 20 (Perkin–Elmer) was used for the in vitro tests. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer, operating at 600.13 and 300.13 MHz for the ^{1}H and 150.917 and 75.47 MHz for ^{13}C nuclei. 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. CHN analyses were performed on CHNS LECO analyzer and were within ±0.4%. Solvent systems dichloromethane/methanol (9:1 and 9.5:0.5), cyclohexane/ethyl acetate (1:1), petroleum ether/ethyl acetate/methanol (3:1:0.5 and 1:2:0.1) and precoated silica gel 60 F_{254} plates were used for thin-layer chromatography. Spots were visualized by short-wave UV light, iodine vapor, phosphomolybdic acid or ferric chloride solution. Column chromatography was performed on silica gel (0.063–0.200 mm), with cyclohexane/ethyl acetate/methanol (3:1:0.6) or dichloromethane/methanol (9.5:0.5 or 8.5:1.5) as eluents.

2-(3-Benzylphenyl)propanoic acid was prepared by ketoprofen reduction. 1-Benzotriazole carboxylic acid chloride (BtcCl, **1**) and NSAID (ibuprofen, fenoprofen, and reduced ketoprofen) benzotriazolides (**2a**–**c**) were synthesized according to our previously published procedures [24–26]. For preparation of 1-(*N*-alkyl/arylcarbamoyl)benzotriazoles (1-benzotriazole carboxylic acid amides) **4a**–**g** our synthetic method was applied as well [27]. The following derivatives were prepared: 1-(*N*-cyclopentylcarbamoyl) benzotriazole (**4a**), 1-(*N*-cyclohexylcarbamoyl)benzotriazole (**4b**), 1-(*N*-cyclohexanemethylcarbamoyl)benzotriazole (**4c**), 1-(*N*-benzylcarbamoyl)benzotriazole (**4d**), 1-[*N*-(2-phenylethylcarbamoyl)] benzotriazole (**4e**), 1-(*N*-benzhydrylcarbamoyl)benzotriazole (**4f**) and 1-(*N*-benzyloxycarbamoyl)benzotriazole (**4g**). Benzotriazole (BtH), triphosgene, *O*-benzylhydroxylamine hydrochloride, cyclopentylamine, cyclohexylamine, cyclohexanemethylamine, benzylamine, 2-phenylethanamine, benzhydrylamine, hydrazine hydrate, triethylamine (TEA) and 10% Pd/C were purchased from Aldrich. Ibuprofen, fenoprofen and ketoprofen were gift samples from Pliva, University of Potchefstroom and Belupo. DPPH, AAPH and NDGA were purchased from Aldrich Chemical Co. Soybean LOX and linoleic acid sodium salt were obtained from Sigma Chemical Co. All solvents were of analytical grade purity and dry.

2.1.2. NSAID hydrazides (**3a**–**c**): general procedure

To a solution of hydrazine hydrate (2.43 ml, 50 mmol) in dioxane (10 ml), a solution of NSAID benzotriazolides **2a**–**c** (10 mmol) in dioxane (30 ml) was added dropwise. The reaction mixture was stirred at rt for 1 h and evaporated under reduced pressure. The obtained residue was dissolved in ethyl acetate (30 ml) and extracted with 0.1% sodium hydroxide solution (30 ml × 3), washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure.

2.1.2.1. 2-(4-Isobutylphenyl)propanehydrazide (**3a**).

From 3.074 g of **2a**. The crude product was recrystallized from ether/petroleum ether. Yield 1.96 g (89%); the spectral data were in agreement with literature data [28].

2.1.2.2. 2-(3-Phenoxyphenyl)propanehydrazide (**3b**).

From the reaction of 3.434 g (10 mmol) compound **2b** and purification by column chromatography (mobile phase petrolether/ethyl acetate/methanol 3:1:0.5) 2.307 g (90%) of **3b** was obtained; IR (KBr): ν_{max} 3285, 3040, 2978, 1659, 1631, 1582, 1487, 1445, 1244, 1210, 1164, 963, 928, 757, 692 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.19 (s, 1H), 7.43–6.82 (m, 9H), 4.25 (s, 2H), 3.53 (q, 1H), 1.32 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 172.87, 157.01, 156.92, 144.73, 130.50, 119.04, 130.15, 123.88, 122.86, 118.06, 117.02, 43.58, 18.76. Anal. ($C_{15}H_{16}N_2O_2$) C, H, N.

2.1.2.3. 2-(3-Benzylphenyl)propanehydrazide (**3c**).

From the reaction of 3.554 g (10 mmol) compound **2c** and recrystallization from ether/petroleum ether 2.000 g (78%) of **3c** was obtained; mp 80–82 °C; IR (KBr): ν_{max} 3289, 3191, 3168, 3063, 3023, 2975, 2919, 1637, 1601, 1534, 1486, 1453, 1382, 1261, 1008, 976, 786, 759, 722, 696, 685, 599 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.14 (s, 1H), 7.31–7.03 (m, 9H), 4.17 (s, 2H), 3.90 (s, 2H), 3.48 (q, 1H), 1.33 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.19, 142.66, 141.65, 141.47, 129.14, 128.88, 128.69, 128.15, 127.38, 126.42, 125.44, 43.72, 41.64, 18.88. Anal. ($C_{16}H_{18}N_2O$) C, H, N.

2.1.3. 1-Acyl-4-substituted semicarbazides (**5a**–**y**): general procedure

Method A: A melted mixture of NSAID hydrazide **3a**–**c** (1 mmol), **4a**–**f** (1 mmol) and TEA (0.418 ml, 3 mmol) was stirred at 75–120 °C for 15 min. The residue was cooled to room temperature, dissolved in ethyl acetate (20 ml) and extracted with 0.1% sodium hydroxide solution (20 ml × 3). The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Method B: A melted mixture of NSAID hydrazide **3a**–**c** (1 mmol), **4a**–**f** (1 mmol) and TEA (0.418 ml, 3 mmol) was stirred at 75–120 °C for 15 min. The residue was cooled to room temperature, stirred with acetone/HCl 1:2 solution (30 ml, pH = 1) and the precipitated product **B** was filtered off. Method C: A solution of NSAID hydrazide **3a**–**c** (1 mmol) and (0.268 g, 1 mmol) 1-(*N*-benzyloxycarbamoyl)benzotriazole (**4g**) in dioxane (10 ml) was stirred at 55 °C for 6–8 h. Dioxane was evaporated under reduced pressure, the residue was dissolved in ethyl acetate (20 ml) and extracted with 0.1% sodium hydroxide solution (20 ml × 3). The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Method D: A solution of 1-acyl-4-benzyloxy semicarbazide **5t**–**v** (0.5 mmol) in methanol (10 ml) was hydrogenated for 1.5 h in the presence of Pd/C (50 mg). The catalyst was filtered off and the solvent was evaporated under reduced pressure.

2.1.3.1. 4-Cyclopentyl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5a**).

Method A, 75 °C; from the reaction of 0.220 g (1 mmol) compound **3a** and 0.230 g (1 mmol) **4a** and after recrystallization from ether/petroleum ether 0.212 g (64%) of **5a** was obtained; mp 123–128 °C; IR (KBr): ν_{max} 3355, 3254, 2956, 2871, 1684, 1650, 1557, 1513, 1465, 1315, 1243, 1151, 1079, 1008, 937, 850, 786, 655 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.63, 7.59, (2s, 2H), 7.24 (d, 2H), 7.08 (d, 2H), 6.50 (bs, 1H), 3.84–3.80 (m, 1H), 3.59 (q, 1H), 2.40 (d, 2H), 1.84–1.20 (m, 9H), 1.32 (d, 3H), 0.85 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 175.27, 159.64, 141.54, 141.08, 130.96, 129.18, 53.09, 46.42, 44.76, 34.86, 25.33, 31.76, 24.34, 20.37. Anal. ($C_{19}H_{29}N_3O_2$) C, H, N.

2.1.3.2. 4-Cyclohexyl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5b**).

Method A, 90 °C; from the reaction of 0.220 g (1 mmol) compound **3a** and 0.244 g (1 mmol) **4b** and after recrystallization from ether/petroleum ether 0.276 g (80%) of **5b** was obtained; mp 165–166 °C; IR (KBr): ν_{max} 3307, 3253, 3019, 2986, 2951, 2930, 2852, 1708, 1683, 1641, 1562, 1531, 1506, 1453, 1322, 1253, 1226, 1153, 1079, 1056, 1023, 999, 936, 892, 850, 709, 634, 614 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.67, 7.64 (2d, 2H), 7.23 (d, 2H), 7.08 (d, 2H), 5.71 (d, 1H), 3.59 (q, 1H), 3.39–3.28 (m, 2H), 2.40 (d, 2H), 1.84–1.75 (m, 1H), 1.69–0.95 (m, 10H), 1.32 (d, 3H), 0.85 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.56, 157.60, 139.84, 139.36, 129.27, 127.47, 48.28, 44.70, 43.03, 33.42, 33.38, 24.90, 30.09, 25.65, 22.64, 18.64. Anal. ($C_{20}H_{31}N_3O_2$) C, H, N.

2.1.3.3. 4-Cyclohexanemethyl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5c**).

Method B, 75 °C; from the reaction of 0.220 g (1 mmol) compound **3a** and 0.258 g (1 mmol) **4c** and after recrystallization from ether/petroleum ether 0.338 g (94%) of **5c** was

obtained; mp 172–174 °C; IR (KBr): ν_{max} 3339, 3236, 3122, 2952, 2926, 2849, 1699, 1615, 1565, 1476, 1448, 1368, 1263, 1247, 1175, 1084, 1019, 854, 780, 651, 618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.65, 7.66 (2s, 2H), 7.23 (d, 2H), 7.07 (d, 2H), 5.99 (t, 1H), 3.59 (q, 1H), 2.82 (t, 2H), 2.40 (d, 2H), 1.84–1.75 (m, 1H), 1.66–0.78 (m, 11H), 1.33 (d, 3H), 0.85 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.66, 158.47, 139.79, 139.35, 129.22, 127.50, 45.80, 44.71, 43.06, 38.37, 30.67, 30.65, 25.88, 30.09, 26.56, 22.64, 18.69. Anal. ($C_{21}H_{33}N_3O_2$) C, H, N.

*2.1.3.4. 4-Benzyl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5d**).* Method B, 110 °C; from the reaction of 0.220 g (1 mmol) compound **3a** and 0.252 g (1 mmol) **4d** and after recrystallization from ether/petroleum ether 0.293 g (83%) of **5d** was obtained; mp 173–175 °C; IR (KBr): ν_{max} 3316, 3222, 3123, 3028, 2954, 1654, 1611, 1566, 1469, 1454, 1364, 1236, 1171, 1077, 851, 749, 699, 668 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.70, 7.85 (2s, 2H), 7.32–7.05 (m, 9H), 6.70 (t, 1H), 4.22 (d, 2H), 3.59 (q, 1H), 2.39 (d, 2H), 1.83–1.74 (m, 1H), 1.34 (d, 3H), 0.84 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.83, 158.59, 140.90, 139.78, 139.34, 129.21, 128.58, 127.55, 127.35, 127.00, 44.70, 43.11, 43.07, 30.08, 22.64, 18.81. Anal. ($C_{21}H_{27}N_3O_2$) C, H, N.

*2.1.3.5. 1-[2-(4-Isobutylphenyl)propanoyl]-4-phenylethylsemicarbazide (**5e**).* Method A, 115 °C; from the reaction of 0.220 g (1 mmol) compound **3a** and 0.252 g (1 mmol) **4e** and after recrystallization from ether/petroleum ether 0.268 g (73%) of **5e** was obtained; mp 173–175 °C; IR (KBr): ν_{max} 3566, 3434, 3312, 3026, 2953, 2926, 2869, 1648, 1587, 1557, 1514, 1497, 1455, 1374, 1253, 1168, 1074, 1054, 999, 934, 853, 796, 746, 700, 547 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.66, 7.79 (2s, 2H), 7.38–7.00 (m, 9H), 6.15 (t, 1H), 3.59 (q, 1H), 3.22 (q, 2H), 2.67 (t, 2H), 2.40 (d, 2H), 1.85–1.76 (m, 1H), 1.34 (d, 3H), 0.86 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.72, 158.36, 139.99, 139.79, 139.33, 129.21, 129.10, 128.78, 127.54, 126.48, 44.70, 43.06, 41.32, 36.38, 30.08, 22.64, 18.78. Anal. ($C_{22}H_{29}N_3O_2$) C, H, N.

*2.1.3.6. 4-Benzhydryl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5f**).* Method A, 120 °C; from the reaction of 0.220 g (1 mmol) compound **3a** and 0.328 g (1 mmol) **4f** and after recrystallization from ether/petroleum ether 0.314 g (73%) of **5f** was obtained; mp 151–154 °C; IR (KBr): ν_{max} 3343, 3202, 3028, 2953, 2869, 1647, 1616, 1538, 1495, 1456, 1366, 1268, 1230, 1168, 1077, 1052, 1028, 1004, 942, 848, 746, 700, 669, 631 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.76, 7.84 (2s, 2H), 7.34–7.05 (m, 14H), 6.95 (bs, 1H), 5.91 (d, 1H), 3.58 (q, 1H), 2.39 (d, 2H), 1.83–1.74 (m, 1H), 1.33 (d, 3H), 0.84 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.70, 157.58, 143.51, 143.46, 139.83, 139.26, 129.25, 128.81, 128.79, 127.49, 127.47, 127.44, 127.35, 127.31, 57.14, 44.71, 43.05, 30.06, 22.65, 18.78. Anal. ($C_{27}H_{31}N_3O_2$) C, H, N.

*2.1.3.7. 4-Cyclopentyl-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5g**).* Method A, 90 °C; from the reaction of 0.256 g (1 mmol) compound **3b** and 0.230 g (1 mmol) **4a** and after recrystallization from ether/petroleum ether 0.293 g (80%) of **5g** was obtained; mp 133–135 °C; IR (KBr): ν_{max} 3414, 3281, 3237, 3101, 3025, 2961, 2910, 2869, 1696, 1627, 1638, 1582, 1556, 1532, 1489, 1455, 1443, 1311, 1244, 1213, 1162, 1146, 1131, 1073, 942, 913, 880, 774, 760, 691, 618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.69, 7.63 (2s, 2H), 7.42–6.82 (m, 9H), 5.94 (d, 1H), 3.89–3.78 (m, 2H), 3.63 (q, 1H), 1.79–1.22 (m, 8H), 1.32 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.08, 157.89, 156.99, 156.94, 144.29, 130.49, 119.01, 130.23, 123.87, 122.93, 118.17, 117.12, 51.40, 43.25, 33.13, 24.91, 18.66. Anal. ($C_{21}H_{25}N_3O_3$) C, H, N.

*2.1.3.8. 4-Cyclohexyl-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5h**).* Method A, 90 °C; from the reaction of 0.256 g (1 mmol) compound **3b** and 0.244 g (1 mmol) **4b** and after recrystallization from ether/petroleum ether 0.229 g (60%) of **5h** was obtained; mp 144–146 °C; IR (KBr): ν_{max} 3343, 3215, 2933, 2853, 1695, 1618, 1580, 1555, 1490, 1472, 1448, 1314, 1273, 1235, 1213, 1166, 1074, 1062, 1025, 944, 887, 748, 689, 640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.70, 7.66 (2s, 2H), 7.42–6.83 (m, 9H), 5.84 (d, 1H), 3.63 (q, 1H), 3.39–3.33 (m, 2H), 1.71–1.00 (m, 10H), 1.33 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.08, 157.55, 156.99, 156.94, 144.29, 130.49, 119.01, 130.24, 123.87, 122.95, 118.16, 117.12, 48.33, 43.24, 33.41, 24.91, 25.65, 18.66. Anal. ($C_{22}H_{27}N_3O_3$) C, H, N.

*2.1.3.9. 4-Cyclohexanemethyl-1-[2-(3-phenoxyphenyl)propanoyl] semicarbazide (**5i**).* Method A, 75 °C; from the reaction of 0.256 g (1 mmol) compound **3b** and 0.258 g (1 mmol) **4c** and after recrystallization from ether/petroleum ether 0.198 g (50%) of **5i** was obtained; mp 104–105 °C; IR (KBr): ν_{max} 3339, 3230, 3115, 3026, 2924, 2850, 1701, 1619, 1582, 1568, 1490, 1447, 1373, 1246, 1212, 1166, 1146, 1074, 1024, 1012, 962, 933, 888, 749, 691, 668 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.70, 7.70 (2s, 2H), 7.43–6.84 (m, 9H), 6.09 (t, 1H), 3.63 (q, 1H), 2.84 (t, 2H), 1.62–0.79 (m, 11H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.18, 158.43, 157.03, 156.91, 144.29, 130.19, 118.98, 130.21, 123.85, 122.98, 118.23, 117.14, 45.82, 43.29, 38.38, 30.68, 25.88, 26.56, 18.70. Anal. ($C_{23}H_{29}N_3O_3$) C, H, N.

*2.1.3.10. 4-Benzyl-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5j**).* Method A, 110 °C; from the reaction of 0.256 g (1 mmol) compound **3b** and 0.252 g (1 mmol) **4d** and after recrystallization from ether/petroleum ether 0.233 g (60%) of **5j** was obtained; mp 154–156 °C; IR (KBr): ν_{max} 3323, 3266, 3209, 3109, 3028, 1654, 1621, 1584, 1565, 1490, 1453, 1369, 1315, 1278, 1249, 1235, 1162, 1143, 1071, 935, 876, 751, 694, 644 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.74, 7.89 (2s, 2H), 7.41–6.82 (m, 14H), 6.76 (t, 1H), 4.22 (d, 2H), 3.63 (q, 1H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.35, 158.54, 157.03, 156.90, 144.28, 140.91, 130.49, 128.59, 127.34, 119.00, 130.20, 127.00, 123.84, 123.02, 118.29, 117.14, 43.36, 43.08, 18.77. Anal. ($C_{23}H_{23}N_3O_3$) C, H, N.

*2.1.3.11. 1-[2-(3-Phenoxyphenyl)propanoyl]-4-phenylethylsemicarbazide (**5k**).* Method B, 115 °C; from the reaction of 0.256 g (1 mmol) compound **3b** and 0.252 g (1 mmol) **4e** and after recrystallization from ether/petroleum ether 0.254 g (63%) of **5k** was obtained; mp 116–118 °C; IR (KBr): ν_{max} 3367, 3236, 3026, 2942, 1682, 1642, 1583, 1560, 1542, 1488, 1454, 1379, 1310, 1247, 1211, 1162, 1075, 1012, 944, 916, 866, 796, 747, 698, 637 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.70, 7.82 (2s, 2H), 7.42–6.84 (m, 14H), 6.21 (t, 1H), 3.63 (q, 1H), 3.23 (q, 2H), 2.68 (t, 2H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.23, 158.31, 157.03, 156.90, 144.29, 139.99, 130.49, 129.11, 128.79, 119.00, 130.21, 126.48, 123.85, 123.02, 118.26, 117.14, 43.29, 41.32, 36.38, 18.78. Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

*2.1.3.12. 4-Benzhydryl-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5l**).* Method A, 120 °C; from the reaction of 0.256 g (1 mmol) compound **3b** and 0.328 g (1 mmol) **4f** and after recrystallization from ether/petroleum ether 0.219 g (47%) of **5l** was obtained; mp 128–131 °C; IR (KBr): ν_{max} 3350, 3236, 3031, 2973, 1655, 1613, 1587, 1560, 1488, 1448, 1247, 1210, 1164, 1070, 1052, 1030, 949, 912, 753, 698, 668, 634, 614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.78, 7.85 (2s, 2H), 7.38–6.98 (m, 19H), 6.83 (d, 1H), 5.91 (d, 1H), 3.64 (q, 1H), 1.33 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 172.70, 157.02, 156.50 156,47, 143.70, 143.03, 142.96, 129.97, 118.53, 129.73, 123.35, 122.43, 117.71, 116.65, 128.37, 128.33, 126.97, 126.93, 126.85, 126.81, 56.71, 42.80, 18.24. Anal. ($C_{29}H_{27}N_3O_3$) C, H, N.

*2.1.3.13. 1-[2-(3-Benzylphenyl)propanoyl]-4-cyclopentylsemicarbazide (**5m**).* Method A, 75 °C; from the reaction of 0.254 g (1 mmol) compound **3c** and 0.230 g (1 mmol) **4a** and after recrystallization from ether/petroleum ether 0.259 g (71%) of **5m** was obtained; mp 133–134 °C; IR (KBr): ν_{max} 3279, 3027, 2962, 2872, 1710, 1646, 1555, 1494, 1452, 1372, 1242, 1191, 1152, 1077, 1010, 941, 728, 698,

638 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.67, 7.60 (2s, 2H), 7.31–7.05 (m, 9H), 5.90 (d, 1H), 3.91 (s, 2H), 3.82 (m, 1H), 3.59 (q, 1H), 1.76–1.22 (m, 8H), 1.32 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.40, 157.93, 142.22, 141.61, 141.55, 129.14, 128.86, 128.76, 128.25, 127.48, 126.42, 125.45, 51.38, 43.36, 41.65, 33.13, 23.62, 18.73. Anal. ($C_{22}H_{27}N_3O_2$) C, H, N.

*2.1.3.14. 1-[2-(3-Benzylphenyl)propanoyl]-4-cyclohexylsemicarbazide (**5n**).* Method A, 90 °C; from the reaction of 0.254 g (1 mmol) compound **3c** and 0.244 g (1 mmol) **4b** and after recrystallization from ether/petroleum ether 0.334 g (85%) of **5n** was obtained; mp 143–144 °C; IR (KBr): ν_{max} 3327, 3216, 3027, 2932, 2853, 1658, 1617, 1589, 1477, 1452, 1255, 1232, 1182, 1074, 1065, 943, 891, 769, 751, 701, 639, 618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.68, 7.64 (2s, 2H), 7.30–7.05 (m, 9H), 5.80 (d, 1H), 3.91 (s, 2H), 3.59 (q, 1H), 3.38–3.29 (m, 1H), 1.70–0.97 (m, 10H), 1.32 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.40, 157.60, 142.22, 141.61, 141.55, 129.14, 128.86, 128.77, 128.24, 127.48, 126.42, 125.45, 48.31, 43.36, 41.65, 33.38, 24.89, 25.66, 18.73. Anal. ($C_{23}H_{29}N_3O_2$) C, H, N.

*2.1.3.15. 1-[2-(3-Benzylphenyl)propanoyl]-4-cyclohexanemetylsemicarbazide (**5o**).* Method B, 75 °C; from the reaction of 0.254 g (1 mmol) compound **3c** and 0.258 g (1 mmol) **4c** and after recrystallization from ether/petroleum ether 0.268 g (68%) of **5o** was obtained; mp 134–137 °C; IR (KBr): ν_{max} 3344, 3216, 3027, 2919, 2850, 1663, 1622, 1589, 1474, 1450, 1370, 1273, 1257, 1239, 1184, 1075, 1030, 963, 746, 723, 702, 652, 618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.67, 7.66 (2s, 2H), 7.31–7.05 (m, 9H), 6.04 (t, 1H), 3.91 (s, 2H), 3.59 (q, 1H), 2.82 (t, 2H), 1.66–0.78 (m, 11H), 1.32 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.48, 158.47, 142.21, 141.62, 141.52, 129.13, 128.86, 128.74, 128.24, 127.46, 126.41, 125.47, 45.81, 43.38, 41.65, 38.36, 30.66, 25.88, 26.55, 18.76. Anal. ($C_{24}H_{31}N_3O_2$) C, H, N.

*2.1.3.16. 1-[2-(3-Benzylphenyl)propanoyl]-4-benzylsemicarbazide (**5p**).* Method B, 110 °C; from the reaction of 0.254 g (1 mmol) compound **3c** and 0.252 g (1 mmol) **4d** and after recrystallization from ether/petroleum ether 0.325 g (84%) of **5p** was obtained; mp 150–154 °C; IR (KBr): ν_{max} 3331, 3261, 3211, 3028, 2986, 1707, 1660, 1620, 1563, 1494, 1453, 1431, 1371, 1246, 1174, 1075, 750, 723, 698, 642, 618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.72, 7.86 (2s, 2H), 7.32–7.04 (m, 14H), 6.73 (t, 1H), 4.22 (d, 2H), 3.89 (s, 2H), 3.60 (q, 1H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.67, 158.59, 142.23, 141.63, 141.52, 140.92, 129.15, 128.87, 128.59, 127.35, 128.74, 128.28, 127.46, 127.01, 126.41, 125.55, 43.46, 43.08, 41.64, 18.88. Anal. ($C_{24}H_{25}N_3O_2$) C, H, N.

*2.1.3.17. 1-[2-(3-Benzylphenyl)propanoyl]-4-phenylethylsemicarbazide (**5r**).* Method A, 115 °C; from the reaction of 0.254 g (1 mmol) compound **3c** and 0.252 g (1 mmol) **4e** and after recrystallization from ether/petroleum ether 0.303 g (73%) of **5r** was obtained; mp 118–120 °C; IR (KBr): ν_{max} 3365, 3301, 3239, 3027, 2939, 1686, 1649, 1601, 1555, 1495, 1455, 1379, 1269, 1242, 1154, 1073, 945, 751, 726, 699, 661 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.66, 7.78 (2s, 2H), 7.29–7.04 (m, 14H), 6.17 (t, 1H), 3.90 (s, 2H), 3.58 (q, 1H), 3.21 (q, 2H), 2.65 (t, 2H), 1.32 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.55, 158.37, 142.23, 141.63, 141.52, 140.00, 129.15, 129.11, 128.87, 128.79, 128.75, 128.28, 127.46, 126.48, 126.41, 125.54, 43.41, 41.65, 41.33, 36.39, 18.87. Anal. ($C_{25}H_{27}N_3O_2$) C, H, N.

*2.1.3.18. 4-Benzhydryl-1-[2-(3-benzylphenyl)propanoyl]semicarbazide (**5s**).* Method A, 120 °C; from the reaction of 0.254 g (1 mmol) compound **3c** and 0.328 g (1 mmol) **4f** and after recrystallization from ether/petroleum ether 0.339 g (71%) of **5s** was obtained; mp 166–170 °C; IR (KBr): ν_{max} 3346, 3231, 3028, 1981, 1662, 1611, 1590, 1560, 1494, 1472, 1453, 1372, 1258, 1236, 1078, 1030, 948, 751, 700, 668, 634, 613 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.78, 7.84 (2s, 2H), 7.33–7.00 (m, 19H), 6.99 (d, 1H), 5.91 (d, 1H), 3.89 (s, 2H), 3.60 (q, 1H), 1.33 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.67, 157.56, 143.52, 143.47, 142.13, 141.59, 141.56, 129.14, 128.86, 128.82, 128.80, 127.45, 128.22, 127.34, 127.32, 126.40, 125.47, 57.16, 43.48, 41.63, 18.84. Anal. ($C_{30}H_{29}N_3O_2$) C, H, N.

*2.1.3.19. 4-Benzyloxy-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5t**).* Method C; 6 h; from the reaction of 0.220 g (1 mmol) compound **3a** and after recrystallization from ether/petroleum ether 0.336 g (91%) of **5t** was obtained; mp 87 °C (decomp.); IR (KBr): ν_{max} 3472, 3319, 3240, 3114, 3064, 3031, 2953, 2867, 1714, 1698, 1681, 1638, 1594, 1548, 1514, 1455, 1366, 1349, 1226, 1147, 1082, 994, 933, 910, 850, 802, 751, 698, 620, 592, 545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.73, 9.47, 8.71 (3s, 3H), 7.43–7.32 (m, 5H), 7.26 (d, 2H), 7.08 (d, 2H), 4.74 (s, 2H), 3.62 (q, 1H), 2.40 (d, 2H), 1.87–1.76 (m, 1H), 1.34 (d, 3H), 0.85 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.55, 159.52, 139.77, 139.25, 136.90, 129.17, 129.12, 128.61, 127.62, 128.42, 77.90, 44.71, 43.05, 30.10, 22.65, 18.93. Anal. ($C_{21}H_{27}N_3O_3$) C, H, N.

*2.1.3.20. 4-Benzyloxy-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5u**).* Method C, 7 h; from the reaction of 0.256 g (1 mmol) compound **3b** and after purification by column chromatography (mobile phase dichloromethane/methanol 9.5:0.5) 0.369 g (91%) of **5u** was obtained; oil; IR (KBr): ν_{max} 3237, 3063, 3034, 2979, 2934, 2877, 1668, 1583, 1488, 1455, 1371, 1312, 1244, 1211, 1163, 1073, 942, 914, 753, 695, 614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.74, 9.47, 8.70 (3s, 3H), 7.41–6.83 (m, 14H), 4.75 (s, 2H), 3.66 (q, 1H), 1.35 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 172.59, 158.95, 156.57, 156.38, 143.70, 136.37, 129.98, 128.62, 128.11, 118.48, 129.65, 127.93, 123.32, 122.59, 117.87, 116.65, 77.42, 42.80, 18.37. Anal. ($C_{23}H_{23}N_3O_4$) C, H, N.

*2.1.3.21. 4-Benzyloxy-1-[2-(3-benzylphenyl)propanoyl]semicarbazide (**5v**).* Method C, 8 h; from the reaction of 0.254 g (1 mmol) compound **3c** and after recrystallization from ether/petroleum ether 0.201 g (50%) of **5v** was obtained; mp 104–105 °C; IR (KBr): ν_{max} 3306, 3235, 3029, 2925, 2880, 1691, 1654, 1599, 1520, 1493, 1454, 1382, 1366, 1356, 1273, 1238, 1152, 1074, 1032, 1007, 947, 788, 751, 727, 699, 668, 555, 532, 497 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.72, 9.46, 8.69 (3s, 3H), 7.37–7.02 (m, 14H), 4.72 (s, 2H), 3.88 (s, 2H), 3.59 (q, 1H), 1.31 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.40, 159.52, 142.13, 141.65, 141.47, 136.90, 129.16, 129.13, 128.87, 128.61, 128.70, 128.43, 128.34, 127.44, 126.41, 125.61, 77.90, 43.39, 41.65, 19.00. Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

*2.1.3.22. 4-Hydroxy-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5w**).* Method D; from the reaction of 0.185 g (1 mmol) compound **5t** and after recrystallization from ether/petroleum ether 0.134 g (96%) of **5a** was obtained; mp 123–125 °C; IR (KBr): ν_{max} 3330, 3235, 3027, 2954, 2924, 2869, 1704, 1652, 1556, 1514, 1465, 1383, 1320, 1262, 1141, 1078, 1002, 935, 850, 766, 727, 653, 531 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.65, 8.70, 8.64, 8.41 (4s, 4H), 7.25 (d, 2H), 7.07 (d, 2H), 3.61 (q, 1H), 2.40 (d, 2H), 1.85–1.76 (m, 1H), 1.33 (d, 3H), 0.85 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.45, 160.82, 139.71, 139.33, 129.14, 127.62, 44.71, 43.00, 30.09, 22.65, 19.01. Anal. ($C_{14}H_{21}N_3O_3$) C, H, N.

*2.1.3.23. 4-Hydroxy-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5x**).* Method D; from the reaction of 0.203 g (1 mmol) compound **5u** and after trituration with ether 0.118 g (75%) of **5x** was obtained; mp 149–150 °C (decomp.); IR (KBr): ν_{max} 3330, 3235, 3027, 2954, 2924, 2869, 1704, 1652, 1556, 1514, 1465, 1383, 1320, 1262, 1141, 1078, 1002, 935, 850, 766, 727, 653, 531 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.68, 8.72, 8.65, 8.45 (4s, 4H), 7.42–6.82 (m, 9H), 3.65 (q, 1H), 1.33 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 172.98, 160.78, 157.06, 156.84, 144.29, 130.49, 118.98, 130.13, 123.81, 123.10, 118.36, 117.11, 43.24, 18.95. Anal. ($C_{16}H_{17}N_3O_4$) C, H, N.

*2.1.3.24. 1-[2-(3-Benzylphenyl)propanoyl]-4-hydroxysemicarbazide (**5y**).* Method D; from the reaction of 0.202 g (1 mmol) compound **5v** and after trituration with ether 0.150 g (96%) of **5y** was obtained; mp 123–125 °C; IR (KBr): ν_{max} 3288, 3060, 3028, 2979, 2938, 1687, 1644, 1562, 1516, 1494, 1483, 1451, 1380, 1263, 1159, 1144, 1100, 1077, 1061, 1027, 938, 782, 721, 700, 596, 533 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.66, 8.73, 8.66, 8.43 (4s, 4H), 7.32–7.05 (m, 9H), 3.92 (s, 2H), 3.62 (q, 1H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.29, 160.82, 142.22, 141.66, 141.45, 129.16, 128.87, 128.68, 128.34, 127.38, 126.40, 125.60, 43.34, 41.65, 19.08. Anal. ($C_{17}H_{19}N_3O_3$) C, H, N.

*2.1.3.25. 4-Benzyloxysemicarbazide (**6**).* A solution of 1-(*N*-benzyloxycarbamoyl)benzotriazole (**4g**) (2.683 g, 10 mmol) in 30 ml dioxane was added dropwise to a solution of 0.534 ml (11 mmol) hydrazine hydrate in 10 ml dioxane. The reaction mixture was stirred at rt for 1 h and evaporated under reduced pressure. The analytically pure sample was obtained after column chromatography (dichloromethane/methanol 9.5:0.5) and triturated with ether. Yield 1.486 g (82%); mp 90–92 °C; IR (KBr): ν_{max} 3332, 3287, 3217, 3061, 3030, 2962, 2925, 2875, 1655, 1637, 1517, 1456, 1366, 1325, 1228, 1210, 1192, 1096, 1070, 980, 915, 797, 751, 702, 680, 607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.18 (s, 1H), 7.84 (s, 1H), 7.42–7.29 (m, 5H), 4.71 (s, 2H), 4.01 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 161.67, 137.18, 129.08, 128.63, 128.37, 77.70. Anal. ($C_8H_{11}N_3O_2$) C, H, N.

*2.1.3.26. 1-(1-Benzotriazolecarbonyl)-4-benzyloxysemicarbazide (**7**).* To a solution of 1.357 g (8 mmol) BtcCl (**1**) in anhydrous dioxane (20 ml), a solution of 1.450 g (8 mmol) 4-benzyloxysemicarbazide (**6**) and 1.115 ml (8 mmol) TEA in dioxane (20 ml) was added dropwise. The reaction mixture was stirred at rt for 1 h and evaporated under reduced pressure. The obtained residue was dissolved in 40 ml ethyl acetate/water 1:1 mixture. The organic layer was extracted 3 times with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was triturated with ether. Yield 1.906 g (73%); mp 90–93 °C (decomp.); IR (KBr): ν_{max} 3482, 3376, 3340, 3164, 3005, 2924, 2854, 1738, 1674, 1556, 1487, 1449, 1387, 1287, 1235, 1066, 1033, 905, 754, 744, 704, 680, 652, 609, 580, 539 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.97, 9.87, 9.31, 8.26–8.18 (2d, 2H), 7.77, 7.59 (2t, 2H), 7.49–7.35 (m, 5H), 4.85 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 159.37, 149.86, 145.66, 131.92, 136.74, 130.78, 126.31, 120.42, 113.79, 129.20, 128.68, 128.54, 78.11. Anal. ($C_{15}H_{14}N_6O_3$) C, H, N.

*2.1.4. 1-Acyl-5-benzyloxy and 1-acyl-5-hydroxycarbamoylcarbazides (**8a**–**f**): general procedure*

Method A: A mixture of 0.326 g (1 mmol) compound **7** and NSAID hydrazide **3a**–**c** (1 mmol) was melted at 90 °C and stirred for 20 min, cooled, dissolved in ethyl acetate (20 ml) and extracted with 0.1% sodium hydroxide solution (20 ml × 3). The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Method B: A solution of 1-acyl-5-benzyloxycarbamoylcarbazides **8a**–**c** (0.5 mmol) in methanol (10 ml) was hydrogenated for 2 h in the presence of Pd/C (50 mg). The catalyst was filtered off and the solvent was evaporated under reduced pressure.

*2.1.4.1. 5-Benzyloxycarbamoyl-1-[2-(4-isobutylphenyl)propanoyl]carbazide (**8a**).* Method A; from the reaction of 0.220 g (1 mmol) compound **3a** and after purification by column chromatography (mobile phase dichloromethane/methanol 9.5:0.5) and recrystallization from ether/petroleum ether 0.304 g (71%) of **8a** was obtained; mp 87–90 °C; IR (KBr): ν_{max} 3266, 3032, 2955, 2930, 2869, 1677, 1513, 1466, 1455, 1366, 1309, 1271, 1212, 1188, 1075, 1030, 1002, 934, 909, 849, 751, 699, 620, 547 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.75, 9.43, 8.54, 8.15, 8.03 (5s, 5H), 7.44–7.30 (m, 5H), 7.26 (d, 2H), 7.08 (d, 2H), 4.77 (s, 2H), 3.62 (q, 1H), 2.41 (d, 2H), 1.85–1.76 (m, 1H), 1.35 (d, 3H), 0.86 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 173.52, 160.03, 158.37, 139.75, 139.34, 136.97, 129.17, 129.14, 128.61, 127.59, 128.41, 77.87, 44.71, 43.00, 30.09, 22.65, 18.85. Anal. ($C_{22}H_{29}N_5O_4$) C, H, N.

*2.1.4.2. 5-Benzyloxycarbamoyl-1-[2-(3-phenoxyphenyl)propanoyl]carbazide (**8b**).* Method A; from the reaction of 0.256 g (1 mmol) compound **3b** and after purification by column chromatography (mobile phase dichloromethane/methanol 9.5:0.5) and recrystallization from ether/petroleum ether 0.338 g (73%) of **8b** was obtained; mp 74–76 °C (decomp.); IR (KBr): ν_{max} 3251, 3062, 3034, 2971, 2933, 2873, 1677, 1583, 1523, 1488, 1455, 1369, 1312, 1243, 1211, 1163, 1073, 1023, 1002, 942, 914, 816, 752, 694, 614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.78, 9.43, 8.55, 8.18, 8.05 (5s, 5H), 7.44–6.82 (m, 14H), 4.77 (s, 2H), 3.65 (q, 1H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.03, 160.03, 158.32, 157.05, 156.87, 144.28, 136.97, 130.50, 129.14, 128.61, 118.99, 130.16, 128.41, 123.83, 123.06, 118.33, 117.13, 77.88, 43.25, 18.79. Anal. ($C_{24}H_{25}N_5O_5$) C, H, N.

*2.1.4.3. 5-Benzyloxycarbamoyl-1-[2-(3-benzylphenyl)propanoyl]carbazide (**8c**).* Method A; from the reaction of 0.254 g (1 mmol) compound **3c** and after purification by column chromatography (mobile phase dichloromethane/methanol 9.5:0.5) and recrystallization from ether/petroleum ether 0.337 g (73%) of **8c** was obtained; mp 75–77 °C; IR (KBr): ν_{max} 3248, 3060, 3028, 2880, 2836, 1675, 1602, 1519, 1494, 1453, 1369, 1310, 1270, 1210, 1075, 1030, 974, 941, 908, 751, 699, 620, 556 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.76, 9.43, 8.55, 8.16, 8.03, 7.44–7.05 (m, 14H), 4.78 (s, 2H), 3.92 (s, 2H), 3.62 (q, 1H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.35, 160.02, 158.36, 142.21, 141.64, 141.48, 136.96, 129.15, 128.87, 128.61, 128.70, 128.41, 128.31, 127.42, 126.40, 125.58, 77.88, 43.34, 41.65, 18.91. Anal. ($C_{25}H_{27}N_5O_4$) C, H, N.

*2.1.4.4. 5-Hydroxycarbamoyl-1-[2-(4-isobutylphenyl)propanoyl]carbazide (**8d**).* Method B; from the reaction of 0.214 g (1 mmol) compound **8a** and after trituration with ether 0.120 g (71%) of **8d** was obtained; mp 145–148 °C (decomp.); IR (KBr): ν_{max} 3261, 2955, 2928, 2870, 1670, 1514, 1466, 1383, 1367, 1281, 1206, 1077, 1002, 934, 850, 783 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.73, 8.66, 8.57, 8.23, 8.08, 7.92 (5s, bs, 6H), 7.24 (d, 2H), 7.07 (d, 2H), 3.61 (q, 1H), 2.40 (d, 2H), 1.82–1.78 (m, 1H), 1.33 (d, 3H), 0.85 (d, 6H); ^{13}C NMR (DMSO-d_6) δ 172.90, 160.70, 157.83, 139.24, 138.85, 128.6, 127.07, 44.22, 42.49, 29.55, 22.14, 18.35. Anal. ($C_{15}H_{23}N_5O_4$) C, H, N.

*2.1.4.5. 5-Hydroxycarbamoyl-1-[2-(3-phenoxyphenyl)propanoyl]carbazide (**8e**).* Method B; from the reaction of 0.232 g (1 mmol) compound **8b** and after trituration with ether 0.159 g (85%) of **8e** was obtained; IR (KBr): ν_{max} 3263, 2978, 2925, 2855, 1670, 1583, 1540, 1488, 1456, 1445, 1376, 1313, 1244, 1210, 1163, 1074, 943, 917, 755, 692 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.80, 8.67, 8.29, 8.14, 7.96 (5s, bs, 6H), 7.42–6.81 (m, 9H), 3.64 (q, 1H), 1.34 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 172.92, 161.24, 158.30, 157.03, 156.88, 144.28, 130.50, 119.01, 130.16, 123.84, 123.03, 118.30, 117.11, 43.22, 18.78. Anal. ($C_{17}H_{19}N_5O_5$) C, H, N.

*2.1.4.6. 1-[2-(3-Benzylphenyl)propanoyl]-5-hydroxycarbamoylcarbazide (**8f**).* Method B; from the reaction of 0.231 g (1 mmol) compound **8c** and after trituration with ether 0.147 g (79%) of **8f** was obtained; mp 102–105 °C; IR (KBr): ν_{max} 3258, 3060, 3027, 2981, 2934, 1670, 1601, 1538, 1494, 1453, 1375, 1321, 1195, 1075, 1030, 943, 784, 728, 699, 622, 557 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.78. 8.67, 8.28, 8.11, 7.95 (5s, bs, 6H), 7.31–7.04 (m, 9H), 3.91 (s, 2H), 3.61 (q, 1H), 1.33 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 173.23, 161.24, 158.34, 142.22, 141.64, 141.48, 129.16, 128.88, 128.71, 128.29, 127.42, 126.41, 125.56, 43.32, 41.65, 18.90. Anal. ($C_{18}H_{21}N_5O_4$) C, H, N.

2.2. Biological tests

2.2.1. Cytostatic activity assays

The cytostatic activity of the test compounds against murine leukemia L1210 and human lymphocyte CEM and cervix carcinoma HeLa cells was determined as follows: L1210, CEM and HeLa cells were suspended at 300,000–500,000 cells/ml of culture medium, and 100 μl of a cell suspension was added to 100 μl of an appropriate dilution of the test compounds in 200 μl-wells of 96-well microtiter plates. After incubation at 37 °C for two (L1210) or three (CEM, HeLa) days, the cell number was determined using a Coulter counter [29]. Whereas L1210 and CEM cells could be counted directly in the Coulter counter, HeLa cells were detached from the microtiter plate wells by trypsin treatment prior to counting. The IC_{50} was defined as the compound concentration required to inhibit cell proliferation by 50%.

The cytostatic activity of the test compounds against HCT 116 (colon carcinoma), H 460 (lung carcinoma), MCF-7 (breast carcinoma) and HaCaT (human immortalized keratinocytes) was determined as follows: the cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) 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 [30,31]. The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1×10^4 to 3×10^4 cells/ml, depending on the doubling times of a specific cell line. Test agents were then added in ten-fold dilutions (10^{-8}–10^{-4} M) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. After 72 h of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenize activity in viable cells. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the number of living, metabolically active cells. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$\text{If}(\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}}) \geq 0, \text{then PG} = 100 \times (\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}})/(\text{mean } A_{\text{ctrl}} - \text{mean } A_{\text{tzero}}).$$

$$\text{If}(\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}}) < 0, \text{then PG} = 100 \times (\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}})/(A_{\text{tzero}}),$$

where the mean A_{tzero} is the average of optical density measurements before exposure of cells to the test compound, the mean A_{test} is the average of optical density measurements after the desired period of time and the mean A_{ctrl} is the average of optical density measurements after the desired period of time with no exposure of cells to the test compound. 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 concentration–response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (i.e. 50%). If however, all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g. PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each test was performed in quadruplicate in at least two individual experiments.

2.2.2. Antiviral activity assays

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathic effect in human lung fibroblast [herpes simplex virus type 1 (HSV-1) [strain KOS], herpes simplex virus type 2 (HSV-2) [strain G], vaccinia virus (VV) and vesicular stomatitis virus (VSV)], African green monkey kidney (Vero, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), human cervix carcinoma (vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus (RSV)), feline Crandell-Rees kidney (CRFK) (feline herpes virus, feline corona virus (FIPV)) or Madin-Darby canine kidney (MDCK) (influenza A [H1N1; H3N2] and influenza B) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations (100, 40, 8, 1.6 and 0.32 μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The methodology of the anti-HIV assays was as follows: human T-lymphocyte CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were exposed to 100 $CCID_{50}$ of HIV-1(III_B) or HIV-2(ROD)/ml and seeded in 200 μl wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

2.2.3. 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. All tests were undertaken on three replicates, and the results were averaged (Table 2).

2.2.4. 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 absorption at 234 nm caused by conjugated diene hydroperoxides. Each experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean.

2.2.5. Soybean LOX inhibition study in vitro

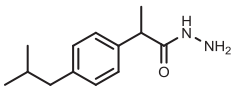
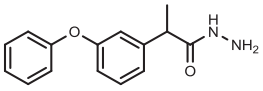
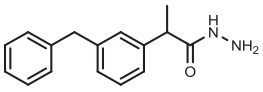
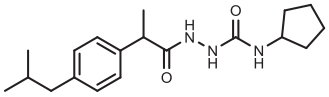
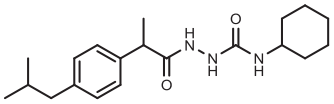
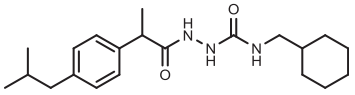
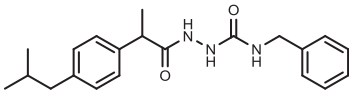
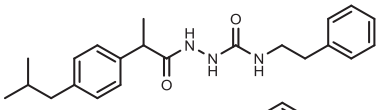
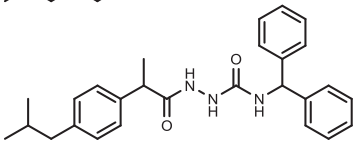
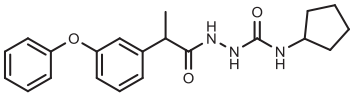
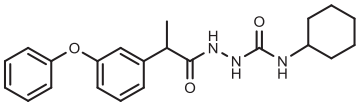
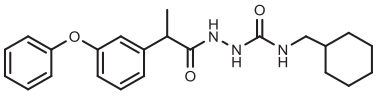
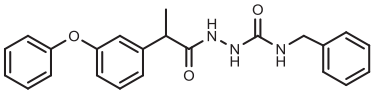
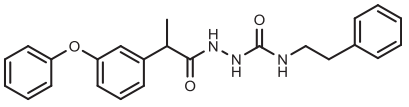
The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 ml) and 0.2 ml of the enzyme solution (1 part of enzyme/9 parts of saline $\times 10^{-4}$, w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor. Each experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean.

3. Results and discussion

3.1. Chemistry

Two series of nitrogen/oxygen-rich derivatives of the NSAIDs ibuprofen, fenoprofen and reduced ketoprofen **5** and **8** were

Table 1
Inhibitory effects of 1-acyl-4-cycloalkyl(or 4-aryl)semicarbazides **5a**–**y**, 1-acyl-5-benzyloxy(or 5-hydroxy)carbamoylcarbazides **8a**–**f** and intermediate compounds **3a**–**c**, **6** and **7** on the proliferation of malignant tumor cell lines.

Compd.	Structural formula	Tumor cell growth IC_{50} (μM)[a]						
		L1210	CEM	HeLa	HCT 116	MCF-7	H460	HaCaT
3a		170 ± 26	147 ± 22	129 ± 24	>100	>100	>100	–[b]
3b		212 ± 9	154 ± 6	120 ± 4	>100	>100	>100	–
3c		212 ± 26	150 ± 23	142 ± 17	>100	>100	>100	–
5a		103 ± 62	74 ± 22	80 ± 44	32 ± 4	18 ± 1	32 ± 8	–
5b		30 ± 7	53 ± 6	69 ± 45	28 ± 0.2	19 ± 3	35 ± 0.01	53 ± 14
5c		44 ± 29	26 ± 6	142 ± 109	21 ± 7	13 ± 0.7	29 ± 2	27 ± 12
5d		110 ± 10	74 ± 18	173 ± 8	34 ± 1	29 ± 12	59 ± 11	–
5e		180 ± 12	148 ± 45	≥ 250	26 ± 2	23 ± 5	51 ± 14	–
5f		20 ± 0	23 ± 4	22 ± 12	13 ± 0.06	6 ± 1	16 ± 1	13 ± 2
5g		55 ± 16	73 ± 27	39 ± 20	36 ± 20	20 ± 5	33 ± 17	18 ± 7
5h		35 ± 7	53 ± 8	48 ± 5	22 ± 7	17 ± 2	24 ± 2	19 ± 2
5i		22 ± 1	24 ± 1	24 ± 0	15 ± 0.1	13 ± 0.5	19 ± 1	20 ± 4
5j		63 ± 1	72 ± 14	70 ± 14	27 ± 0.2	17 ± 1	19 ± 13	46 ± 12
5k		53 ± 1	71 ± 9	64 ± 8	18 ± 1	19 ± 4	22 ± 1	15 ± 3

(*continued on next page*)

Table 1 (*continued*)

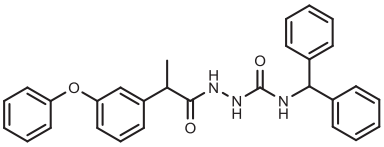
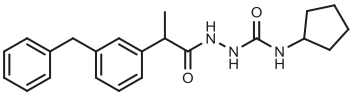
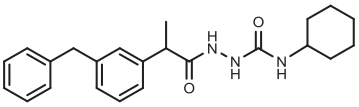
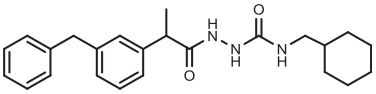
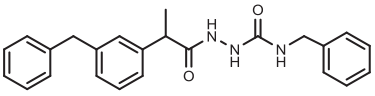
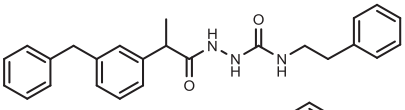
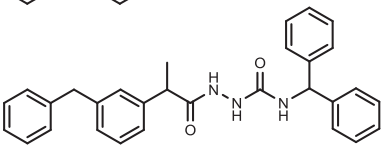
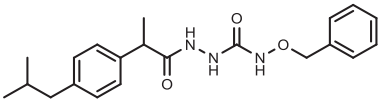
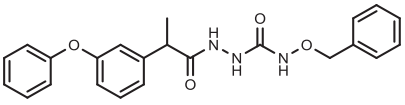
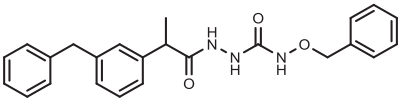
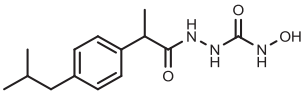
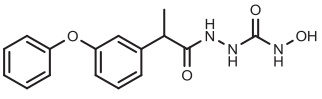
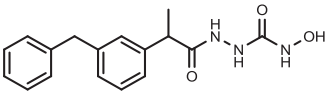
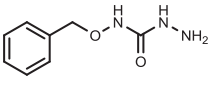
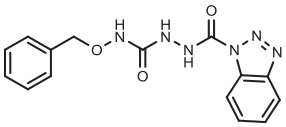
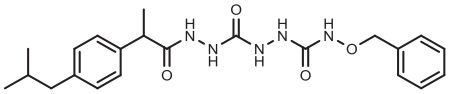
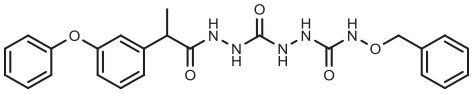
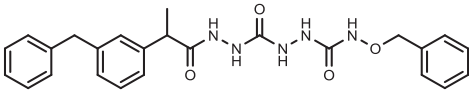
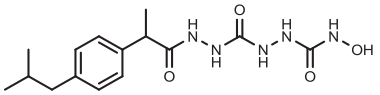
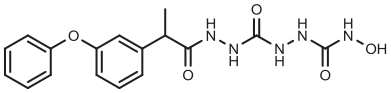
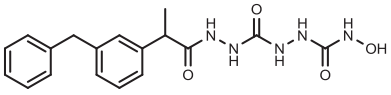
Compd.	Structural formula	Tumor cell growth IC_{50} (μM)[a]						
		L1210	CEM	HeLa	HCT 116	MCF-7	H460	HaCaT
5l		15 ± 1	11 ± 7	5.7 ± 0.2	5 ± 0.7	7 ± 3	3 ± 0.4	10 ± 0.03
5m		69 ± 12	66 ± 4	43 ± 12	33 ± 8	19 ± 2	38 ± 2	50 ± 2
5n		25 ± 1	25 ± 5	45 ± 12	22 ± 1	16 ± 3	23 ± 0.4	25 ± 4
5o		22 ± 1	20 ± 1	20 ± 0	16 ± 3	18 ± 10	18 ± 0.5	12 ± 3
5p		31 ± 3	49 ± 6	94 ± 42	24 ± 0.4	12 ± 8	28 ± 2	25 ± 3
5r		31 ± 3	29 ± 3	58 ± 7	20 ± 5	15 ± 0.8	22 ± 2	16 ± 2
5s		19 ± 0	15 ± 0	5.0 ± 0.5	8 ± 2	5 ± 1	4 ± 0.6	11 ± 0.01
5t		192 ± 81	≥250	178 ± 3	76 ± 10	50 ± 11	>100	–
5u		84 ± 10	91 ± 9	76 ± 4	76 ± 21	81 ± 7	>100	–
5v		79 ± 4	101 ± 11	78 ± 2	56 ± 10	41 ± 18	82 ± 19	–
5w		108 ± 11	205 ± 63	160 ± 13	>100	>100	>100	–
5x		125 ± 11	183 ± 95	≥ 250	>100	>100	>100	–
5y		113 ± 11	155 ± 71	176 ± 26	>100	>100	>100	–
6		>250	>250	>250	>100	>100	>100	–

Table 1 (*continued*)

Compd.	Structural formula	Tumor cell growth IC_{50} (μM)[a]						
		L1210	CEM	HeLa	HCT 116	MCF-7	H460	HaCaT
7		>250	>250	>250	>100	>100	>100	–
8a		105 ± 10	106 ± 34	98 ± 55	56 ± 31	19 ± 8	71 ± 30	–
8b		103 ± 5	90 ± 10	88 ± 4	84 ± 16	26 ± 10	>100	–
8c		102 ± 1	96 ± 3	76 ± 20	64 ± 21	19 ± 10	>100	–
8d		>250	>250	>250	>100	>100	>100	–
8e		>250	>250	≥250	>100	>100	>100	–
8f		>250	>250	≥250	>100	>100	>100	–
Cisplatin		–	–	–	7 ± 2	10 ± 1	1 ± 0.1	2 ± 0.1
5-Fluorouracil		0.49 ± 0.2	18 ± 5	0.54 ± 0.1	4 ± 0.7	15 ± 2	3 ± 0.3	0.3 ± 0.08

[a] 50% inhibitory concentration.
[b] Not tested.

prepared. They contain a region with five to nine electronegative atoms (three nitrogen plus two or three oxygen atoms or five nitrogen plus four oxygen atoms) framed by aryl, cycloalkyl or hydroxy residue on one or both terminal ends. Therefore, they are closely related to semicarbazides, carbazides, ureas, hydroxyureas and hydroxamic acids.

The first series involved semicarbazides bearing a NSAID acyl residue at position 1 and cycloalkyl or aryl (**5a**–**s**), benzyloxy (**5t**–**v**) or hydroxy (**5w**–**y**) substituents at position 4 of the semicarbazide backbone. Compounds **5a**–**v** were prepared from NSAID hydrazides **3a**–**c** and the corresponding 1-(*N*-alkyl/arylcarbamoyl)benzotriazoles (1-benzotriazole carboxylic acid amides) **4a**–**g** (Scheme 1). Hydrazide **3a** was previously described, while **3b** and **3c** are new compounds. Here we report a new method for efficient hydrazide preparation from NSAID benzotriazolides **2a**–**c** and hydrazine hydrate. 1-(*N*-Alkyl/arylcarbamoyl)benzotriazoles **4a**–**g** were synthesized from 1-benzotriazole carboxylic acid chloride (BtcCl, **1**) and corresponding amine, following our previously published procedure [27]. Benzyloxysemicarbazides **5t**–**v** after hydrogenolysis of the benzyl group gave 1-acyl-4-hydroxysemicarbazides **5w**–**y**.

The carbazide series of compounds was prepared by the reaction of NSAID hydrazides **3a**–**c** and 1-(1-benzotriazolecarbonyl)-4-benzyloxysemicarbazide (**7**) (compounds **8a**–**c**) or by hydrogenolysis of such obtained carbazides (**8d**–**f**). Compound **7** was prepared from BtcCl (**1**) and 4-benzyloxysemicarbazide (**6**), which was obtained from 1-(*N*-benzyloxycarbamoyl)benzotriazole (**4g**) and hydrazine (Scheme 1).

The synthetic pathways applied for preparation of the majority of compounds involved benzotriazole as synthetic auxiliary. The benzotriazole moiety in the compounds **2a**–**c**, **4a**–**g** and **7** activated the molecules for nucleophilic substitution with hydrazine hydrate or hydrazides, allowing the preparation of hydrazides **3a**–**c**, semicarbazides **5a**–**v** and **6** or carbazides **8a**–**c** under mild conditions. In our reactions, the hydrazides act as acyl donors but also as nucleophiles via the terminal nitrogen atom. Benzotriazole was introduced in the intermediate products by means of 1-benzotriazole carboxylic acid chloride (BtcCl, **1**), which was first described by our research group and now is commercially available [32]. TEA was used as a catalyst and/or HCl acceptor in several synthetic steps, but not in reactions *g* and *k* to avoid possible cyclization. The final step in preparations of **5a**–**s** and **8a**–**c** was performed in a solvent-free system. The reactions were run at a minimal temperature to afford melting (75–120 °C). These reaction conditions allowed completion of the reactions in less than 20 min and in good yields.

Structures of the synthesized compounds are supported by IR, ^{1}H and ^{13}C NMR spectra and confirmed by elemental analysis. The chemical shifts are consistent with the proposed structures of the novel compounds (detailed NMR data are given in Tables 3–7 in the Supporting Information).

Table 2
Interaction with DPPH, *in vitro* inhibition of lipid peroxidation (LP), soybean lipoxygenase (LOX), and theoretically calculated *C* log *P* values [45].

Compd.	*C* log *P*	DPPH 20 min[a] (%)	DPPH 60 min[a] (%)	LP inhibition (%)	LOX inhibition[a] (%)
3a	2.41	na	1	6	8
3b	2.55	na	1	10	3
3c	2.52	na	na	6	10
5a	4.04	4	7	26	na
5b	4.60	na	na	33	na
5c	5.22	3	6	16	25
5d	4.34	na	na	12	na
5e	4.67	na	na	33	na
5f	6.11	na	na	74	46
5g	4.19	16	19	21	na
5h	4.74	19	22	63	13
5i	5.36	18	21	64	12
5j	4.48	22	24	11	68[c]
5k	4.81	4	7	51	20
5l	5.83	2	4	99	81[d]
5m	4.15	4	12	na	na
5n	4.71	3	10	31	10
5o	5.33	5	12	61	9
5p	4.45	3	9	48	na
5r	4.78	8	15	71	17
5s	5.80	4	9	88	96[b]
5t	4.09	na	na	100	na
5u	4.24	na	na	8	6
5v	4.20	3	6	na	na
5w	1.57	29	54	20	7
5x	1.71	39	66	22	3
5y	1.68	36	59	8	8
6	0.27	6	14	36	na
7	1.67	85	85	31	na
8a	2.93	11	18	13	na
8b	3.07	8	15	26	na
8c	3.04	10	19	100	na
8d	0.41	24	46	15	na
8e	0.55	23	48	13	na
8f	0.52	28	53	28	1
NDGA		80	91		84[e]
Trolox				63	

Concentrations of the tested compounds: [a] 1×10^{-4} mol l^{-1}, [b] $IC_{50} = 51.5$ μM, [c] $IC_{50} = 75$ μM, [d] $IC_{50} = 60$ μM, [e] $IC_{50} = 28$ μM, na – no activity.

3.2. Biological evaluations

3.2.1. Cytostatic activity

The target compounds **5a**–**y** and **8a**–**f**, as well as their synthetic precursors, were evaluated for inhibitory activities against proliferation of the following malignant tumor cell lines: murine leukemia (L1210), human T-lymphocyte (CEM), human cervical carcinoma (HeLa), human colon carcinoma (HCT 116), human breast carcinoma (MCF-7) and human lung carcinoma (H460) (Table 1). The tested compounds do not discriminate very much between the different tumor cell lines regarding their cytostatic activity, except for the human mammary carcinoma MCF-7 cells that are generally more sensitive to the antiproliferative activity of the compounds than the other carcinoma cell lines (Table 1). The most active compounds 4-benzhydryl-1-[2-(3-phenoxyphenyl)propanoyl]semicarbazide (**5l**), 4-benzhydryl-1-[2-(3-benzylphenyl)propanoyl]semicarbazide (**5s**), and 4-benzhydryl-1-[2-(4-isobutylphenyl)propanoyl]semicarbazide (**5f**) inhibited tumor cell proliferation at IC_{50} values ranging from 3 to 23 μM. It is striking to observe that those three most active compounds consistently contain the same unique structural motif (i.e. a 4-benzhydryl-1-propanoyl-semicarbazide), whereas lack of any bulky aromatic or cycloalkyl group resulting in a free end-standing $-CONHNH_2$ or $-CONHOH$ group virtually annihilates the cytostatic activity. Thus, the presence of a bulky lipophilic group, preferably a benzhydryl group, is strictly required at this terminal end. It would be interesting to introduce additional substituents on the benzhydryl moiety (i.e. alkyl, alkoxy, halogene, amine, carboxylic acid and/or hydroxyl groups) to further explore the cytostatic potential and selectivity of these test compounds. The NSAID terminal of these molecules seems to be less important (i.e. isobutyl, phenoxyphenyl, benzyl) to determine the antiproliferative potency. It would be also interesting to explore an additional SAR by modifying the latter part of the molecule.

Additional testing of the selected compounds on a non-tumor cell line HaCaT (human keratinocytes) showed that the most active derivatives **5l** and **5s** may exert a slight selectivity toward tumor cells (specifically solid tumor cells), compared to non-tumor cells, having up to two times lower IC_{50} concentrations for tumor cell lines. All other compounds generally showed the same inhibitory effect toward tumor and non-tumor cell lines, except toward breast cancer cells MCF-7, which were shown to be slightly more sensitive. Still, these results should be interpreted with caution without detailed experiments and/or specific target identification, because although these cells are not tumorigenic, they are immortalized and have a relatively short doubling time. However, all tested NSAID derivatives show much lower toxicity toward HaCaT, compared to the reference compounds 5-fluorouracil and cisplatin.

3.2.2. Antiviral activity

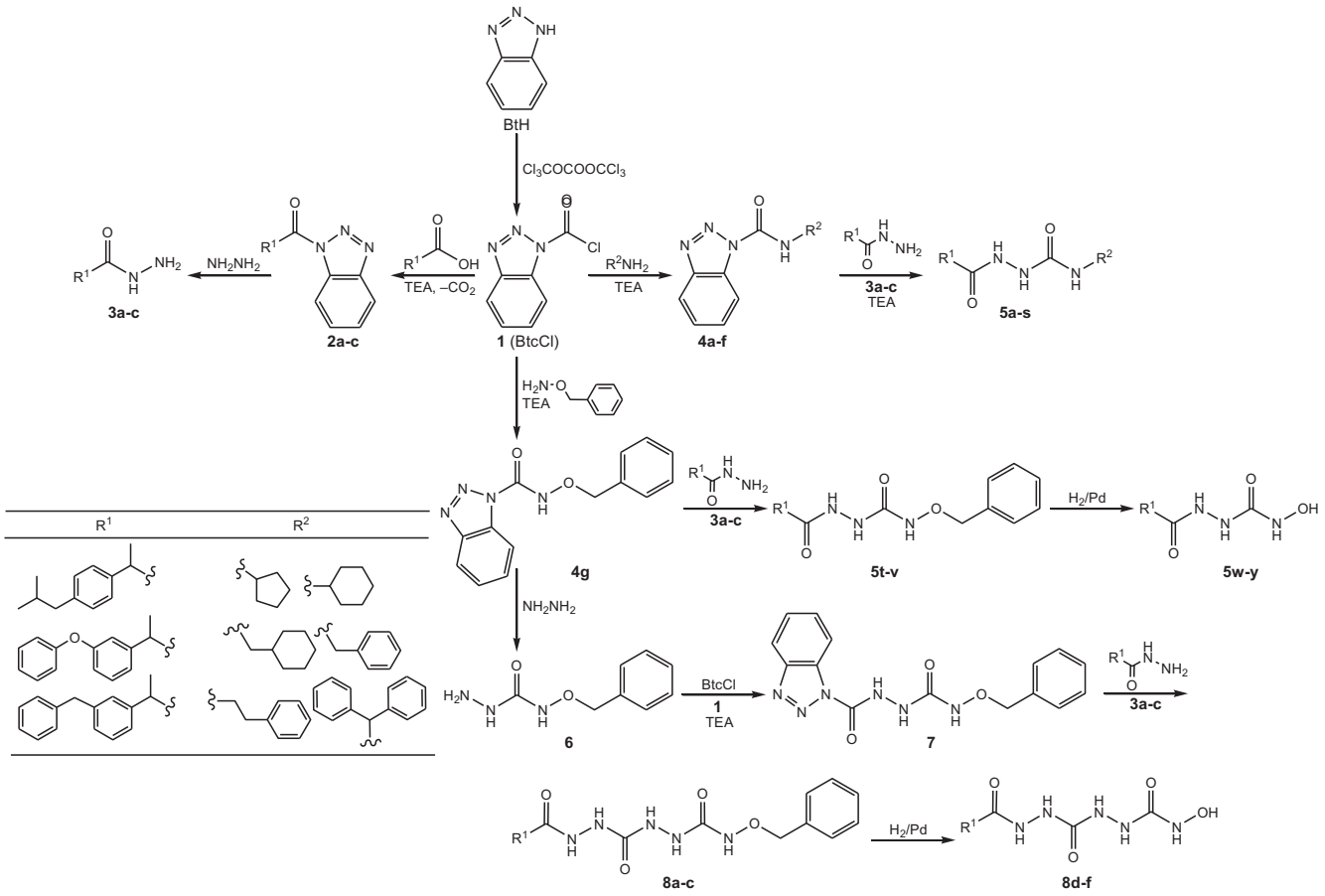
None of the tested compounds were inhibitory at subtoxic concentrations against a broad panel of DNA and RNA viruses in cell culture.

3.2.3. Antioxidative activity

It has been reported that many NSAIDs act either as inhibitors of free radical production or as radical scavengers [33], while those showing reduction of lipid peroxidation, also show less ulcerogenic activity [34,35]. In the present investigation, in vitro antioxidant ability of new NSAID derivatives **5a**–**y** and **8a**–**f** and precursors in their synthesis was studied and compared to the well-known antioxidant agents such as nordihydroguaiaretic acid (NDGA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). Two different antioxidant assays were used: (i) interaction with the stable free radical 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [36] and (ii) interaction with the water-soluble azo compound 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), used as a source of peroxyl radicals [37,38]. DPPH interaction of the tested compounds was examined at 100 μM concentration after 20 and 60 min. The results are shown in Table 2. 1-(1-Benzotriazolecarbonyl)-4-benzyloxysemicarbazide (**7**) showed the best DPPH interaction value (85%), similar to that of the reference compound NDGA (91%) at the same concentration. In the semicarbazide series **5** and carbazide series **8**, only compounds with low lipophilicity values bearing hydroxamic acid/hydroxyurea moiety, **5w**–**y** and **8d**–**f**, showed weak interaction with DPPH (23–39%) and their antioxidant activity increased after 60 min (46–66%).

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. The results showed that **5t**, **8c** and **5l** were almost equipotent and completely inhibited lipid peroxidation (LP). Two other compounds, **5s** and **5f**, also inhibited LP better than Trolox (88 and 74%, respectively). No significant difference was observed between the other compounds of the series **5**. Hydrazides **3a**–**c** were completely inactive. Our results, like some reported data [39], indicate that LP inhibition is not always accompanied by DPPH radical scavenging activity. Thus, compounds that inhibited LP potently, exerted low (if any) DPPH scavenging activity.

Carcinogenesis is a multistage process consisting of at least three separate, but closely linked processes: initiation, promotion and progression. Promotion is closely linked to oxidative and



Scheme 1. Synthesis of 1-acyl-4-cycloalkyl(or 4-aryl)semicarbazides **5a**–**y** and 1-acyl-5-benzyloxy(or 5-hydroxy)carbamoylcarbazides **8a**–**f**, derived from NSAIDs ibuprofen, fenoprofen and reduced ketoprofen.

inflammatory tissue damage. Conversely, substances with potent anti-inflammatory and/or antioxidant activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the stage of promotion and to act as antitumor promoters. Taking under consideration the above mentioned as well as the antioxidant and cytostatic activities of our compounds, it seems that three out of five of the most active LP inhibitors are the strongest anti-proliferative agents as well. These findings support the idea that the cytostatic activity of **5l**, **5s** and **5f** is highly correlated to their antioxidant potential (anti-lipid peroxidation activity).

In general, information obtained from the antioxidant activity may help to understand the anticancer properties of the compounds. It is known that metals, especially iron and copper, can play a key role in free radical generation and propagation. Therefore, metal chelators can act as protectors. This could be partly a possible mechanism of action of **5l**, **5s**, **5f**.

Lipoxygenases (LOXs) play a significant role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer from the biotransformation of arachidonic acid catalyzed by LOX [40]. LOX inhibitors have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, certain types of cancer, and cardiovascular diseases [41,42]. In order to diminish the pro-cancerous mechanisms a key treatment strategy is to reduce the free radical load. Antioxidant activity by the scavenging of reactive oxygen species is important in preventing potential damage to cellular macromolecules such as DNA, proteins and lipids. Moreover, it has been found that LOX inhibitors may have chemopreventive activity in lung carcinogenesis [43]. Reports published over the past three decades support a growth-regulatory role of arachidonic acid metabolites in the etiology of mammary carcinogenesis [44]. Studies have demonstrated that levels of several eicosanoids are increased in breast cancer in comparison to benign breast tumors [45].

In this context, we decided to evaluate the newly prepared NSAID derivatives for their ability to inhibit soybean LOX. 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 [46].

From the tested derivatives, **5s**, **5l**, and **5j** presented interesting IC_{50} values (51.5, 60 and 75 μM) in comparison to the IC_{50} value of the reference NDGA (28 μM, Table 2). Two of them, **5s** and **5l**, contain benzhydryl and the third one benzyl substituent at semicarbazide position 4, indicating that the presence of a bulky lipophilic group at this terminal end is strictly required for better LOX inhibitory activity. The fact that these compounds have high *C* log *P* values (5.80, 5.83, and 4.48, respectively) support the findings that lipophilicity is an important physicochemical property for LOX inhibitors [47]. However, compound **5f** with the highest *C* log *P* value (6.11) was not the most active and presented 46% inhibition at 100 μM concentration.

3.2.4. Physicochemical studies. Determination of lipophilicity as C log P values

Since lipophilicity is a significant physicochemical property determining distribution, bioavailability, metabolic activity, and elimination, we have calculated the lipophilicity values of the prepared compounds as *C* log *P* values by the *C* log *P* programme of Biobyte Corp. [48].

4. Conclusions

Two series of compounds **5a**–**y** and **8a**–**f** derived from three selected arylpropionic acids namely, ibuprofen, fenoprofen and reduced ketoprofen, were synthesized and evaluated for their cytostatic potential. The tested compounds varied very much in cytostatic activity. All intermediate products **3a**–**c**, **6** and **7** displayed no or very weak inhibitory activity against proliferation of all the tested tumor cell lines. Opposite to some reports [49,50], carbazides **8d**–**f** with the pharmacophore –CONHOH identified in hydroxamic acids and hydroxyureas, where also completely inactive, while their *O*-benzyl derivatives **8a**–**c** showed a slightly better activity with IC_{50} values ranging from 19 to 105 μM. Semicarbazides with a free (**5w**–**y**) or a benzyl protected hydroxy group (**5t**–**v**) showed no or low activity as well. However, 5-acylsemicarbazides **5a**–**s** bearing cycloalkyl or aryl substituents (cyclopentyl, cyclohexyl, cyclohexylmethyl, benzyl, phenylethyl, benzhydryl) at position 4 exerted a cytostatic activity against all examined malignant tumor cell lines, with a slight selectivity to MCF-7. In particular, the benzhydryl-substituted compounds showed the most pronounced cytostatic potential. Indeed, **5l** and **5s**, both bearing the highly lipophilic benzhydryl group, showed marked antiproliferative activity in vitro against a variety of cancer cell lines ($IC_{50} = 3–19$ μM). The same compounds profoundly inhibited soybean lipoxygenase and lipid peroxidase (81–99%). The ibuprofen benzhydryl analog **5f** was slightly less active with $IC_{50} = 6–23$ μM cytotoxicity, 46% inhibition of LOX and 74% LP inhibition, respectively.

The best antiscavenger activities between semicarbazide and carbazide derivatives showed **5w**–**y** and **8d**–**f** with hydroxamic acid/hydroxyurea functional groups and low calculated lipophilicity value, which is in accordance with the reported findings [51,52]. However, compound **7** showed the best DPPH interaction value (85%) from all the tested compounds. The highest LP inhibition showed compounds **5t**, **8c** and **5l**, followed by **5s** and **5f**. Our results indicate that LP inhibitory activity is not always accompanied by DPPH radical scavenging activity. Compounds **5s**, **5l** and **5j** showed as the best LOX inhibitors. The cytostatic activity of **5l**, **5s** and **5f** is highly correlated to their antioxidant potential. The compounds depict the same activity pattern, which may suggest similar mechanisms of action correlated to their antioxidant activities. The results of our biological tests point to compounds **5s** and **5l** as the leading compounds for further derivatization in our search for effective antiproliferative and LP/LOX inhibitory agents. A further detailed study of antioxidant activity of **5s** and **5l**, along with the elucidation of specific target(s) and pathway(s) that mediate these activities, especially in a breast cancer model, should be the next steps toward novel antioxidant/chemopreventive compounds discovery.

Acknowledgments

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Appendix. Supporting information

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.02.046.

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