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The Novel Ketoprofen Amides – Synthesis and Biological Evaluation as Antioxidants, Lipoxygenase Inhibitors and Cytostatic Agents

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The novel amides of ketoprofen and its reduced derivatives (5a–f, 4a–n, 6a–g) with aromatic and cycloalkyl amines or hydroxylamines were prepared and screened for their reducing and cytostatic activity as well as for their ability to inhibit soybean lipoxygenase and lipid peroxidation. 1,1-Diphenyl-picrylhydrazyl test for reducing ability revealed that ketoprofen amides were more potent antioxidants than the amides of the reduced ketoprofen derivatives. The most active compound was benzhydryl ketoprofen amide 5f. Lipoxygenase inhibition of the tested compounds varied from strong to very weak. The most potent compound was benzhydryl derivative 6f (IC_{50} = 20.5 μM). Aromatic and cycloalkyl amides 4 and 5 were more potent lipoxygenase inhibitors than derivatives with carboxylic group. Aromatic amides of series 4 and 5 showed excellent lipid peroxidation inhibition (92.2–99.9%). On the other hand, the most pronounced cytostatic activity was exerted by *O*-benzyl derivative 4i, although in general all tested reduced and non-reduced lipophilic derivatives showed similar activity.

Key words: amide, antioxidant activity, cytostatic activity, ketoprofen derivatives, soybean lipoxygenase

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; Bt, *N*-1-benzotriazolyl, COX, cyclooxygenase; DMEM, Dulbecco's modified Eagle's medium; DPPH, 1,1-diphenyl-picrylhydrazyl; FBS, fetal bovine serum; LOX, soybean lipoxygenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NDGA, nordihydroguaiaretic acid; NSAID, non-steroidal anti-inflammatory drug; PG, percentage of growth; TEA, triethylamine.

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Ketoprofen, 2-(3-benzoylphenyl)propionic acid, is a non-steroidal anti-inflammatory drug (NSAID) with pronounced analgesic and antipyretic properties. Numerous derivatives of ketoprofen and other NSAIDs have been synthesized in order to minimize side-effects, prolong plasma half-life and increase water solubility or lipophilicity (1–3). It has been demonstrated that amidation of NSAIDs improves selectivity toward COX-2 (4), while modification of carboxylic group to hydroxamic acid leads to inhibition of both cyclooxygenase and 5-lipoxygenase, two enzymes crucial in inflammatory processes (5,6). On the other hand, NSAIDs are potential anticancer drugs and effective chemopreventive agents (7–12). A combination of anti-inflammatory agents as biochemical modulators with several anticancer drugs has been considered useful as an auxiliary means for combined modality therapy in cancer (13).

In our previous articles (14–16), potency of amides, NSAID hydroxamic acids and *O*-alkylated NSAID hydroxamic acids of ketoprofen and related NSAIDs as cytostatic and antioxidative agents and as inhibitors of lipoxygenase and linoleic acid lipid peroxidation was screened. It was shown that ketoprofen amidation significantly enhanced antiproliferative activity of the parent compound and that lipophilicity of the compounds had a significant influence on their antiproliferative activity. Following these findings, a new series of lipophilic amides of ketoprofen was prepared. In order to evaluate the importance of hydroxyl group in the amide moiety, a number of hydroxylamides was designed. In this article, we report their synthesis and full chemical characterization, together with their biological screening.

Methods

Chemistry

Melting points were determined on a Stuart Melting Point Apparatus SMP3 (Barworld Scientific, Staffordshire, UK) and were uncorrected. IR spectra were recorded on a FTIR Perkin Elmer Paragon 500 spectrometer (Perkin Elmer, Waltham, MA, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer (Varian, Palo-Alto, CA, USA), operating at 300 and 75.5 MHz for the ^{1}H and ^{13}C nuclei, respectively. Samples were measured in dimethyl sulphoxide (DMSO)-d_6 solutions at 20 °C in 5-mm NMR tubes. Chemical shifts (δ) in ppm were referred to tetramethylsilane (TMS). Coupling constants (J) are given in Hz. Elemental analyses were performed on CHNS LECO analyzer and mass spectra on HPLC-MS/MS (HPLC, Agilent Technologies 1200 Series; MS, Agilent Technologies 6410 Triple Quad, Santa

Clara, CA, USA). For thin-layer chromatography, precoated Merck silica gel 60 F_{254} (Merck, Darmstadt, Germany) and solvent systems cyclohexane/ethyl acetate/methanol (3:1:0.5), dichloromethane/methanol (95:5) and ethyl acetate were used. Spots were visualized by short-wave UV light and iodine vapor. Column chromatography was performed with silica gel 0.063–0.200 mm (Merck), with ethyl acetate, cyclohexane/ethyl acetate (1:1, 2:1, 3:1, 4:1), ethyl acetate/methanol (8:1), cyclohexane/ethyl acetate/methanol (3:1:0.5), dichloromethane/methanol (93:7, 97:3) or chloroform/methanol (93:7, 95:5) as eluents. Benzotriazole, triphosgene, triethylamine, cyclopentylamine, cyclohexylamine, cyclohexanemethylamine, benzylamine, 2-phenylethylamine, benzhydrylamine, 2-hydroxyethylamine, 3-hydroxypropylamine, diethanolamine, ethylenediamine, and 10% Pd/C were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ketoprofen was obtained as a gift sample from PLIVA (Zagreb, Croatia). All solvents were of analytical grade purity and were dried prior to use.

1-Benzotriazole carboxylic acid chloride (**1**), benzotriazolide **3b**, 2-(3-benzoylphenyl)-*N*-cyclohexylpropanamide (**5b**), *N*-benzyloxy-2-(3-benzoylphenyl)propanamide (**5g**), *N*-hydroxy-2-(3-benzylphenyl) propanamide (**4m**) and 10% Pd/C(en) were prepared following the published procedures (16–19). 2-(3-Benzylphenyl)propanoic acid (**2a**) and 2-(3-(hydroxy(phenyl)methyl)phenyl)propanoic acid (**2b**) were prepared by reduction of ketoprofen using H_2/Pd/C/methanol and H_2/Pd/C(en)/tetrahydrofurane systems, respectively (the modified published procedures 21,22).

2-(3-Benzylphenyl)propanoic acid benzotriazolide (3a)

To a solution of 2.403 g (10 mmol) **2a** and 1.393 mL (10 mmol) triethylamine in dry toluene (30 mL) was added dropwise (0.25 h) a solution of 1.696 g (10 mmol) chloride **1** in dry toluene (30 mL). The reaction mixture was stirred at room temperature for 1 h and extracted with water (3 × 60 mL). After drying of the organic layer (anhydrous sodium sulfate), filtration, evaporation of the solvent, and trituration of the residue with ether, 2.663 g (78%) of **3a** was obtained; mp 59–60 °C; IR (KBr, ν/cm^{-1}) 3027, 2937, 2918, 2852, 1730, 1597, 1485, 1452, 1390, 1372, 1168, 955, 780, 756, 738, 708, 692. Elemental analysis: calcd for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31. Found: C, 77.48; H, 5.88; N, 12.64.

Ketoprofen derivatives 4a–i and 5a–f. General procedure

A solution of the appropriate benzotriazolide **3a**,**b** (1 mmol), the appropriate amine (1.1 mmol) and 0.696 mL (5 mmol) triethylamine in toluene (10 mL) was stirred at room temperature for 1–48 h or at 78 °C for 11–16 h. The reaction mixture was extracted with brine (5 × 10 mL), hydrochloric acid (w = 1%, 2 × 10 mL) and washed with water till pH 7. After drying (anhydrous sodium sulfate) and evaporation of the solvent, the crude product was obtained.

2-(3-Benzylphenyl)-*N*-cyclopentylpropanamide (4a)

From the reaction of 0.341 g (1 mmol) **3a**, 0.109 mL (1.1 mmol) cyclopentylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 5 h and purification of the product by trituration with ether was obtained 0.252 g (82%) of **9a**; mp 92–93 °C; IR (KBr, ν/cm^{-1}) 3274, 3061, 3027, 2962, 2936, 2864, 1635, 1599, 1548, 1448, 785, 731, 711, 699; ^{1}H NMR (300 MHz, DMSO) δ 7.85 (d, J = 7.15, 1H), 7.30–7.04 (m, 9H), 3.99–3.87 (m, 3H), 3.52 (q, J = 7.01, 1H), 1.83–1.16 (m, 8H), 1.27 (d, J = 7.03, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.02, 143.11, 141.69, 141.42, 129.09, 128.83, 128.68, 128.03, 127.21, 126.39, 125.26, 50.64, 45.22, 41.67, 32.68, 32.63, 23.96, 23.92, 19.01; MS/MS m/z 308.3 $(M + H)^+$, 330.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{21}H_{25}NO$: C, 82.04; H, 8.20; N, 4.56. Found: C, 82.00; H, 8.36; N, 4.68.

2-(3-Benzylphenyl)-*N*-cyclohexylpropanamide (4b)

From the reaction of 0.341 g (1 mmol) **3a**, 0.126 mL (1.1 mmol) cyclohexylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 7 h and purification of the product by trituration with ether was obtained 0.273 g (85%) of **4b**; mp 89–93 °C; IR (KBr, ν/cm^{-1}) 3297, 3062, 3026, 2980, 2936, 2854, 1639, 1585, 1546, 1448, 779, 750, 698; ^{1}H NMR (300 MHz, DMSO) δ 7.74 (d, J = 7.79, 1H), 7.29–7.04 (m, 9H), 3.90 (s, 2H), 3.52 (q, J = 7.04, 1H), 3.49–3.40 (m, 1H), 1.73–1.54 (m, 6H), 1.21–0.93 (m, 4H), 1.27 (d, J = 7.03, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 172.59, 143.15, 141.69, 141.41, 129.10, 128.82, 128.67, 128.00, 127.19, 126.37, 125.26, 47.84, 45.27, 41.66, 32.81, 32.78, 25.69, 25.04, 24.96, 19.00; MS/MS m/z 322.3 $(M + H)^+$, 344.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{22}H_{27}NO$: C, 82.20; H, 8.47; N, 4.36. Found: C, 82.09; H, 8.53; N, 4.42.

2-(3-Benzylphenyl)-*N*-(cyclohexanemethyl)propanamide (4c)

From the reaction of 0.341 g (1 mmol) **3a**, 0.143 mL (1.1 mmol) cyclohexanemethylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 8 h and purification of the product by column chromatography (eluent cyclohexane/ethyl acetate 3:1) was obtained 0.275 g (82%) of **4c**; mp 50–54 °C; IR (KBr, ν/cm^{-1}) 3295, 3061, 3027, 2968, 2918, 2851, 1647, 1602, 1585, 1558, 1442, 1221, 1155, 711, 698; ^{1}H NMR (300 MHz, DMSO) δ 7.84 (t, J = 5.31, 1H), 7.30–7.03 (m, 9H), 3.90 (s, 2H), 3.56 (q, J = 6.97, 1H), 2.85 (dd, J = 6.11, J = 6.50, 2H), 1.66–1.51 (m, 6H), 1.40–0.72 (m, 4H), 1.28 (d, J = 7.02, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.55, 143.10, 141.67, 141.36, 129.07, 128.83, 128.66, 128.11, 127.27, 126.39, 125.31, 45.46, 45.24, 41.65, 37.88, 30.81, 30.77, 26.49, 25.85, 19.02; MS/MS m/z 336.3 $(M + H)^+$, 358.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{29}NO$: C, 82.34; H, 8.71; N, 4.18. Found: C, 82.66; H, 8.65; N, 4.37.

N-Benzyl-2-(3-benzylphenyl)propanamide (4d)

From the reaction of 0.341 g (1 mmol) **3a**, 0.120 mL (1.1 mmol) benzylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 3.5 h, purification of the product by column chromatography (eluent cyclohexane/ethyl acetate 2:1) and trituration with ether was obtained 0.290 g (88%) of **4d**; mp 70–74 °C; IR (KBr, ν/cm^{-1}) 3292, 3062, 3028, 2971, 2930, 1648, 1602, 1546, 1495, 1453, 722, 697; ^{1}H NMR (300 MHz, DMSO) δ 8.44 (t, J = 5.65, 1H), 7.31–7.06

(m, 14H), 4.24 (d, J = 5.52, 2H), 3.91 (s, 2H), 3.64 (q, J = 6.96, 1H), 1.34 (d, J = 7.01, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.69, 142.88, 141.66, 141.48, 140.00, 129.11, 128.86, 128.67, 128.16, 127.23, 126.41, 125.41, 128.76, 127.51, 127.41, 127.14, 45.52, 42.48, 41.64, 19.04; MS/MS m/z 330.3 $(M + H)^+$, 352.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{23}NO$: C, 83.85; H, 7.04; N, 4.25. Found: C, 83.74; H, 6.99; N, 4.56.

2-(3-Benzylphenyl)-*N*-phenethylpropanamide (4e)

From the reaction of 0.341 g (1 mmol) **3a**, 0.139 mL (1.1 mmol) phenylethylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 2 h and purification of the product by trituration with ether/petrolether was obtained 0.295 g (86%) of **4e**; mp 60–64 °C; IR (KBr, ν/cm^{-1}) 3306, 3064, 3026, 2925, 1642, 1548, 1496, 1454, 753, 712, 698; ^{1}H NMR (300 MHz, DMSO) δ 7.97 (t, J = 5.43, 1H), 7.29–7.05 (m, 14H), 3.91 (s, 2H), 3.52 (q, J = 7.00, 1H), 3.29–3.16 (m, 2H), 2.64 (t, J = 7.23, 2H), 1.27 (d, J = 7.04, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.53, 142.90, 141.67, 141.47, 139.91, 129.13, 128.86, 128.71, 128.12, 127.30, 126.40, 125.40, 129.10, 128.67, 126.44, 45.50, 41.66, 40.83, 35.37, 19.02; MS/MS m/z 344.3 $(M + H)^+$, 366.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{24}H_{25}NO$: C, 83.93; H, 7.34; N, 4.08. Found: C, 84.08; H, 7.39; N, 4.18.

N-Benzhydryl-2-(3-benzylphenyl)propanamide (4f)

From the reaction of 0.341 g (1 mmol) **3a**, 0.190 mL (1.1 mmol) benzhydrylamine and 0.696 mL (5 mmol) triethylamine at 78 °C for 16 h, purification of the product by column chromatography (eluent cyclohexane/ethyl acetate 4:1) and trituration with ether was obtained 0.284 g (70%) of **4f**; mp 122–123 °C; IR (KBr, ν/cm^{-1}) 3297, 3087, 3064, 3025, 2979, 1667, 1641, 1601, 1586, 1529, 1494, 1452, 757, 741, 718, 703; ^{1}H NMR (300 MHz, DMSO) δ 8.86 (d, J = 8.45, 1H), 7.36–7.04 (m, 19H), 6.07 (d, J = 8.45, 1H), 3.90 (s, 2H), 3.80 (q, J = 6.94, 1H), 1.32 (d, J = 7.00, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 172.97, 142.95, 141.67, 141.40, 142.86, 142.74, 129.09, 128.85, 128.72, 128.23, 127.28, 126.40, 125.38, 128.68, 127.85, 127.55, 127.41, 127.40, 56.28, 45.06, 41.65, 18.86; MS/MS m/z 406.3 $(M + H)^+$, 428.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{29}H_{27}NO$: C, 85.89; H, 6.71; N, 3.45. Found: C, 85.69; H, 7.00; N, 3.76.

2-(3-Benzylphenyl)-*N*-methoxypropanamide (4g)

From the reaction of 0.341 g (1 mmol) **3a**, 0.092 g *O*-methylhydroxylamine hydrochloride and 0.696 mL (5 mmol) triethylamine at room temperature for 48 h and purification of the product by column chromatography (eluent dichloromethane/methanol 97:3) was obtained 0.158 g (59%) of **4g**; mp 61–64 °C; IR (KBr, ν/cm^{-1}) 3153, 3083, 2971, 938, 2854, 1661, 1640, 493, 1069, 1054, 720, 706; ^{1}H NMR (300 MHz, DMSO) δ 11.19 (s, 1H), 7.30–7.07 (m, 9H), 3.91 (s, 2H), 3.38–3.32 (m, 4H), 1.30 (d, J = 7.09, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 170.48, 141.99, 141.67, 141.62, 129.13, 128.87, 128.00, 127.57, 126.42, 125.30, 63.48, 42.52, 41.59, 18.65; MS/MS m/z 270.2 $(M + H)^+$, 292.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.50; H, 7.49; N, 5.53.

2-(3-Benzylphenyl)-*N*-ethoxypropanamide (4h)

From the reaction of 0.341 g (1 mmol) **3a**, 0.107 g *O*-ethylhydroxylamine hydrochloride and 0.696 mL (5 mmol) triethylamine at room temperature for 46 h and purification of the product by column chromatography (eluent cyclohexane/ethyl acetate 1:1) was obtained 0.130 g (46%) of **4h**; mp 114–116 °C; IR (KBr, ν/cm^{-1}) 3170, 3023, 2987, 1671, 1650, 1600, 1492, 1047, 721, 707; ^{1}H NMR (300 MHz, DMSO) δ 11.07 (s, 1H), 7.31–7.07 (m, 9H), 3.91 (s, 2H), 3.71 (q, J = 7.03, 1H), 3.37 (q, J = 7.07, 2H), 1.30 (d, J = 7.04, 3H), 1.07 (t, J = 7.03, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 170.55, 142.11, 141.62, 129.11, 128.86, 128.82, 127.99, 127.55, 126.41, 125.28, 70.85, 42.51, 41.60, 18.65, 13.83; MS/MS m/z 284.2 $(M + H)^+$, 306.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{18}H_{21}NO_2$: C, 76.29; H, 7.47; N, 4.94. Found: C, 76.01; H, 7.31; N, 4.99.

N-Benzyloxy-2-(3-benzylphenyl)propanamide (4i)

From the reaction of 0.341 g (1 mmol) **3a**, 0.176 g *O*-benzylhydroxylamine hydrochloride and 0.696 mL (5 mmol) triethylamine at 78 °C for 11 h and purification of the product by column chromatography (eluent cyclohexane/ethyl acetate/methanol 3:1:0.5) was obtained 0.231 g (67%) of **4i**; oil; IR (film, ν/cm^{-1}) 3181, 3062, 3028, 2975, 2934, 2876, 1658, 1601, 1495, 1454, 1027, 749, 725, 698; ^{1}H NMR (300 MHz, DMSO) δ 11.18 (s, 1H), 7.34–7.07 (m, 14H), 4.70 (s, 2H), 3.92 (s, 2H), 3.38 (q, J = 7.01, 1H), 1.30 (d, J = 7.03, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 170.71, 141.99, 141.62, 136.34, 129.36, 128.71, 129.12, 128.86, 128.82, 128.04, 127.56, 126.42, 125.36, 77.06, 42.44, 41.60, 18.59; MS/MS m/z 346.3 $(M + H)^+$, 368.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.08; H, 6.99; N, 4.11.

2-(3-Benzoylphenyl)-*N*-cyclopentylpropanamide (5a)

From the reaction of 0.355 g (1 mmol) **3b**, 0.109 mL (1.1 mmol) cyclopentylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 1.5 h and trituration with ether was obtained 0.257 g (80%) of **5a**; mp 94–95 °C; IR (KBr, ν/cm^{-1}) 3355, 3066, 2964, 2870, 1663, 1648, 1596, 1528, 1449, 1312, 1284, 723, 702, 642; ^{1}H NMR (300 MHz, DMSO) δ 7.99 (d, J = 7.01, 1H), 7.72–7.45 (m, 9H), 3.99–3.87 (m, 1H), 3.66 (q, J = 6.73, 1H), 1.83–1.17 (m, 8H), 1.32 (d, J = 7.00, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 196.23, 172.71, 143.34, 137.53, 137.28, 133.14, 131.99, 130.04, 129.03, 128.97, 128.88, 128.41, 50.73, 45.08, 32.68, 23.94, 23.90, 18.95; MS/MS m/z 322.3 $(M + H)^+$, 344.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{21}H_{23}NO_2$: C, 78.47; H, 7.21; N, 4.36. Found: C, 78.60; H, 7.56; N, 4.68.

2-(3-Benzoylphenyl)-*N*-(cyclohexanemethyl)propanamide (5c)

From the reaction of 0.355 g (1 mmol) **3b**, 0.143 mL (1.1 mmol) cyclohexanemethylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 2 h and trituration with ether was obtained 0.315 g (90%) of **5c**; mp 96–98 °C; IR (KBr, ν/cm^{-1}) 3298, 3094, 2919, 2853, 1676, 1660, 1642, 1597, 1563, 1447, 1289, 1271, 1234, 729, 707, 640; ^{1}H NMR (300 MHz, DMSO) δ 7.98 (t, J = 7.98, 1H),

7.74–7.47 (m, 9H), 3.72 (q, J = 6.99, 1H), 2.87 (dd, J = 6.18, J = 4.38, 2H), 1.67–1.51 (m, 6H), 1.41–0.73 (m, 4H), 1.35 (d, J = 7.00, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 196.22, 173.24, 143.41, 137.53, 137.30, 133.17, 132.00, 130.04, 129.03, 128.92, 128.44, 45.30, 45.26, 37.89, 30.78, 30.75, 26.46, 25.84, 18.92; MS/MS m/z 350.3 $(M + H)^+$, 372.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{27}NO_2$: C, 79.05; H, 7.79; N, 4.01. Found: C, 79.49; H, 8.01; N, 4.13.

N-Benzyl-2-(3-benzoylphenyl)propanamide (5d)

From the reaction of 0.355 g (1 mmol) **3b**, 0.120 mL (1.1 mmol) benzylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 1 h and trituration with ether was obtained 0.285 g (83%) of **5d**; mp 97–99 °C; IR (KBr, ν/cm^{-1}) 3314, 3287, 3066, 3033, 2926, 2870, 1644, 1551, 1449, 1349, 1015, 712, 701; ^{1}H NMR (300 MHz, DMSO) δ 8.56 (t, J = 5.75, 1H), 7.76–7.49 (m, 9H), 7.29–7.14 (m, 5H), 4.25 (d, J = 5.84, 2H), 3.80 (q, J = 7.03, 3H), 1,40 (d, J = 7.03, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 196.22, 173.37, 143.15, 137.51, 137.38, 139.86, 133.17, 132.08, 130.07, 129.04, 129.02, 128.58, 128.70, 127.50, 127.21, 45.36, 42.54, 18.91; MS/MS m/z 344.2 $(M + H)^+$, 366.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{21}NO_2$: C, 80.44; H, 6.16; N, 4.08. Found: C, 80.67; H, 6.30; N, 4.29. Synthesis of the compound **5d** was reported previously (23), but no spectroscopic data were given.

2-(3-Benzoylphenyl)-*N*-phenethylpropanamide (5e)

From the reaction of 0.355 g (1 mmol) **3b**, 0.139 mL (1.1 mmol) phenylethylamine and 0.696 mL (5 mmol) triethylamine at room temperature for 1 h and trituration with ether/petrolether was obtained 0.329 g (92%) of **5e**; mp 66–69 °C; IR (KBr, ν/cm^{-1}) 3255, 3062, 2936, 2876, 1661, 1634, 1599, 1559, 1543, 1448, 1288, 748, 702; ^{1}H NMR (300 MHz, DMSO) δ 8.11 (t, J = 5.45, 1H), 7.75–7.48 (m, 9H), 7.24–7.07 (m, 5H), 3.69 (q, J = 6.94, 1H), 3.32–3.18 (m, 2H), 2.66 (t, J = 7.13, 2H), 1.34 (d, J = 7.02, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 196.22, 173.22, 143.19, 137.54, 137.32, 139.85, 133.16, 132.09, 130.08, 129.04, 128.99, 128.97, 128.52, 129.10, 128.64, 126.44, 45.33, 40.71, 35.40, 18.91; MS/MS m/z 358.2 $(M + H)^+$, 380.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92. Found: C, 80.90; H, 6.70; N, 4.04.

N-Benzhydryl-2-(3-benzoylphenyl)propanamide (5f)

From the reaction of 0.355 g (1 mmol) **3b**, 0.190 mL (1.1 mmol) benzhydrylamine and 0.696 mL (5 mmol) triethylamine at 78 °C for 11 h and trituration with ether was obtained 0.306 g (73%) of **5f**; mp 115–119 °C; IR (KBr, ν/cm^{-1}) 3316, 3251, 3057, 3030, 2932, 1656, 1649, 1598, 1528, 1494, 1320, 1285, 723, 704, 696; ^{1}H NMR (300 MHz, DMSO) δ 8.98 (d, J = 8.46, 1H), 7.78–7.48 (m, 9H), 7.37–7.08 (m, 10H), 6.08 (d, J = 8.40, 1H), 3.96 (q, J = 6.99, 1H), 1.39 (d, J = 7.01, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 196.23, 172.64, 143.01, 137.51, 137.35, 142.80, 142.77, 133.16, 132.07, 130.05, 129.03, 128.98, 128.53, 128.88, 128.68, 127.86, 127.45, 127.33, 56.36, 44.98, 18.81; MS/MS m/z 420.2 $(M + H)^+$, 442.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{29}H_{25}NO_2$: C, 83.03; H, 6.01; N, 3.34. Found: C, 83.30; H, 6.09; N, 3.58.

Ketoprofen derivatives 4j–l. General procedure

A solution of 0.341 g (1 mmol) **3a**, the appropriate amine (1.1 mmol) and 0.139 mL (1 mmol) triethylamine in toluene/ethyl acetate or toluene/acetonitrile (2:1, 15 mL) was stirred at room temperature for 0.25 or 5 h. After evaporation of the solvent and column chromatography, the crude product was obtained.

2-(3-Benzylphenyl)-*N*-(2-hydroxyethyl)propanamide (4j)

From the reaction of 0.341 g (1 mmol) **3a**, 0.066 mL (1.1 mmol) 2-hydroxyethylamine and 0.139 mL (1 mmol) triethylamine in toluene/ethyl acetate for 0.25 h and purification of the product by column chromatography (eluent ethyl acetate) was obtained 0.238 g (84%) of **4j**; oil; IR (film, ν/cm^{-1}) 3414, 3312, 3084, 3027, 2972, 2931, 1648, 1601, 1560, 1540, 1494, 1453, 1363, 1072, 723, 700; ^{1}H NMR (300 MHz, DMSO) δ 7.92 (t, J = 5.24, 1H), 7.31–7.04 (m, 9H), 4.64 (t, J = 5.36, 1H), 3.91 (s, 2H), 3.57 (q, J = 7.03, 1H), 3.39–3.33 (m, 2H), 3.17–3.01 (m, 2H), 1.28 (d, J = 7.03, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.81, 143.11, 141.67, 141.47, 129.13, 128.86, 128.71, 128.14, 127.27, 126.41, 125.37, 60.30, 45.40, 41.98, 41.65, 19.21; MS/MS m/z 284.2 $(M + H)^+$, 306.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{18}H_{21}NO_2$: C, 76.29; H, 7.47; N, 4.94. Found: C, 76.62; H, 7.40; N, 4.82.

2-(3-Benzylphenyl)-*N*-(3-hydroxypropyl)propanamide (4k)

From the reaction of 0.341 g (1 mmol) **3a**, 0.084 mL (1.1 mmol) 3-hydroxypropylamine and 0.139 mL (1 mmol) triethylamine in toluene/ethyl acetate for 0.25 h and purification of the product by column chromatography (eluent ethyl acetate) was obtained 0.229 g (77%) of **4k**; oil; IR (film, ν/cm^{-1}) 3301, 3062, 3027, 2970, 2935, 2877, 1652, 1601, 1558, 1548, 1494, 1453, 721, 699; ^{1}H NMR (300 MHz, DMSO) δ 7.89 (t, J = 5.29, 1H), 7.31–7.04 (m, 9H), 4.38 (t, 1H), 3.91 (s, 2H), 3.53 (q, J = 7.00, 1H), 3.34 (q, J = 6.19, 2H), 3.06 (q, J = 6.51, 2H), 1.54–1.45 (m, 2H), 1.28 (d, J = 7.04, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.64, 143.02, 141.66, 141.47, 129.12, 128.86, 128.72, 128.08, 127.28, 126.40, 125.30, 58.80, 45.50, 41.65, 36.20, 32.83, 19.07; MS/MS m/z 298.3 $(M + H)^+$, 320.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71. Found: C, 76.80; H, 7.66; N, 4.70.

2-(3-Benzylphenyl)-*N,N*-bis(2-hydroxyethyl)propanamide (4l)

From the reaction of 0.341 g (1 mmol) **3a**, 0.116 mL (1.1 mmol) diethanolamine and 0.139 mL (1 mmol) triethylamine in toluene/acetonitrile for 5 h and purification of the product by column chromatography (eluent ethyl acetate $\rightarrow$ ethyl acetate/methanol 8:1) was obtained 0.249 g (76%) of **4l**; oil; IR (film, ν/cm^{-1}) 3370, 3060, 3026, 2931, 1622, 1494, 1473, 1454, 1442, 1061, 745, 699; ^{1}H NMR (300 MHz, DMSO) δ 7.31–7.04 (m, 9H), 4.82 (s, 1H), 4.46 (s, 1H), 4.10 (q, J = 6.74, 1H), 3.91 (s, 2H), 3.56–3.10 (m, 8H), 1.24 (d, J = 6.77, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.73, 143.10, 141.89, 141.63, 129.09, 128.86, 128.00, 127.32, 126.40, 125.40, 59.73, 59.07, 50.64, 48.93, 41.64, 41.60, 21.26; MS/MS m/z 328.3 $(M + H)^+$, 350.3, $(M + Na)^+$; Elemental analysis: calcd for

$C_{20}H_{25}NO_3$: C, 73.37; H, 7.70; N, 4.28. Found: C, 73.44; H, 7.88; N, 4.20.

2-(3-Benzylphenyl)propanamide (4n)

A suspension of 0.359 g (0.5 mmol) **5g** and 0.050 g Pd/C in methanol (10 mL) was hydrogenated at atmospheric pressure and room temperature for 17 h. After filtration of the catalyst and evaporation of the solvent, 0.239 g (100%) of **4n** was obtained; mp 76–78 °C; IR (KBr, ν/cm^{-1}) 3393, 3177, 3084, 3026, 2980, 2926, 1653, 1599, 1451, 1406, 1368, 1288, 1123, 786, 710, 694, 646, 628; ^{1}H NMR (300 MHz, DMSO) δ 7.41–7.04 (m, 9H), 6.78 (s, 2H), 3.91 (s, 2H), 3.53 (q, J = 7.01, 1H), 1.28 (d, J = 7.06, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 175.69, 143.02, 141.68, 141.46, 129.12, 128.87, 128.15, 127.30, 126.41, 125.41, 45.33, 41.65, 18.97; MS/MS m/z 240.2 $(M + H)^+$, 262.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.67; H, 7.08; N, 5.60.

Ketoprofen derivatives 6a–f. General procedure

A suspension of the amide **5a**–**f** (0.5 mmol) and 0.020 g 10% Pd/C(en) in methanol (10 mL) was hydrogenated at atmospheric pressure and room temperature for 5–9 h. After filtration of the catalyst and evaporation of the solvent under the reduced pressure, the crude product was obtained.

N-Cyclopentyl-2-(3-(hydroxy(phenyl)methyl)phenyl)propanamide (6a)

From the reaction of 0.161 g (0.5 mmol) **5a** for 5 h and purification by trituration with ether was obtained 0.126 (78%) of **6a**; mp 120–125 °C; IR (KBr, ν/cm^{-1}) 3274, 3062, 3031, 2964, 2933, 2866, 1645, 1603, 1549, 1453, 1231, 1195, 1021, 730, 701; ^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, J = 6.92, 1H), 7.36–7.13 (m, 9H), 5.86 (d, J = 3.83, 1H), 5.65 (d, J = 3.04, 1H), 3.98–3.87 (m, 1H), 3.54 (q, J = 6.94, 1H), 1.81–1.18 (m, 8H), 1.27 (d, J = 6.99, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.00, 146.16, 145.99, 142.76, 128.47, 128.29, 127.11, 126.69, 126.01, 125.56, 124.81, 74.84, 50.63, 45.29, 32.69, 32.65, 23.97, 23.93, 19.10; MS/MS m/z 324.3 $(M + H)^+$, 346.2, $(M + Na)^+$; Elemental analysis: calcd for $C_{21}H_{25}NO_2$: C, 77.98; H, 7.79; N, 4.33. Found: C, 78.08; H, 7.85; N, 4.69.

N-Cyclohexyl-2-(3-(hydroxy(phenyl)methyl)phenyl) propanamide (6b)

From the reaction of 0.166 g (0.5 mmol) **5b** for 6 h and purification by column chromatography (eluent dichloromethane/methanol 93:7) was obtained 0.127 g (75%) of **6b**; mp 94–98 °C; IR (KBr, ν/cm^{-1}) 3366, 3308, 3058, 2973, 2924, 2855, 1641, 1603, 1539, 1449, 1015, 712, 701; ^{1}H NMR (300 MHz, DMSO) δ 7.74 (dd, J = 5.24, J = 2.39, 1H), 7.35–7.11 (m, 9H), 5.84 (d, J = 3.84, 1H), 5.64 (d, J = 3.57, 1H), 3.53 (q, J = 6.98, 1H), 3.48–3.38 (m, 1H), 1.72–1.46 (m, 6H), 1.27–0.92 (m, 4H), 1.25 (d, J = 6.56, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 172.59, 146.16, 145.98, 142.82, 128.45, 128.28, 127.11, 126.71, 126.02, 125.55, 124.81, 74.84, 47.83, 45.34, 32.83, 32.80, 25.70, 25.05, 24.98, 19.10; MS/MS m/z 338.3 $(M + H)^+$, 360.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{22}H_{27}NO_2$: C, 78.30; H, 8.06; N, 4.15. Found: C, 78.55; H, 8.44; N, 4.36.

N-Cyclohexanemethyl-2-(3-(hydroxy(phenyl)methyl) phenyl)propanamide (6c)

From the reaction of 0.175 g (0.5 mmol) **5c** for 7 h and purification by column chromatography (eluent chloroform/methanol 95:5) was obtained 0.141 g (80%) of **6c**; oil; IR (film, ν/cm^{-1}) 3312, 3063, 2925, 2852, 1649, 1604, 1550, 1493, 1450, 1025, 731, 701; ^{1}H NMR (300 MHz, DMSO) δ 7.87 (t, J = 5.56, 1H), 7.37–7.14 (m, 9H), 5.86 (dd, J = 2.84, J = 1.05, 1H), 5.65 (d, J = 3.50, 1H), 3.58 (q, J = 7.01, 1H), 2.85 (t, J = 6.26, 2H), 1.68–1.51 (m, 6H), 1.39–0.73 (m, 4H), 1.28 (d, J = 6.85, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.58, 146.15, 145.93, 142.71, 128.47, 128.28, 127.13, 126.69, 126.03, 125.62, 124.90, 74.82, 45.53, 45.28, 37.87, 30.83, 30.81, 26.49, 25.85, 19.13; MS/MS m/z 352.3 $(M + H)^+$, 374.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{29}NO_2$: C, 78.59; H, 8.32; N, 3.99. Found: C, 78.67; H, 8.49; N, 3.70.

N-Benzyl-2-(3-(hydroxy(phenyl)methyl)phenyl)propanamide (6d)

From the reaction of 0.172 g (0.5 mmol) **5d** for 6 h and purification by column chromatography (eluent chloroform/methanol 93:7) was obtained 0.142 g (82%) of **6d**; mp 100–103 °C; IR (KBr, ν/cm^{-1}) 3331, 3285, 1648, 1603, 1539, 1494, 1454, 1238, 1017, 730, 701; ^{1}H NMR (300 MHz, DMSO) δ 8.46 (t, J = 5.57, 1H), 7.41–7.15 (m, 14H), 5.88 (d, J = 3.21, 1H), 5.66 (s, 1H), 4.24 (d, J = 5.74, 2H), 3.66 (q, J = 6.97, 1H), 1.34 (d, J = 6.97, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.71, 146.11, 146.02, 142.50, 139.96, 128.69, 127.53, 128.50, 128.38, 127.14, 126.73, 126.09, 125.67, 124.04, 74.83, 45.59, 42.51, 19.11; MS/MS m/z 368.2 $(M + Na)^+$; Elemental analysis: calcd for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.08; H, 6.99; N, 3.92.

2-(3-(Hydroxy(phenyl)methyl)phenyl)-*N*-phenethylpropanamide (6e)

From the reaction of 0.179 g (0.5 mmol) **5e** for 6 h and purification by column chromatography (eluent chloroform/methanol 93:7) was obtained 0.131 g (73%) of **6e**; oil; IR (film, ν/cm^{-1}) 3412, 3312, 3062, 3028, 2973, 2932, 1652, 1603, 1558, 1540, 1522, 1454, 1368, 1239, 1025, 750, 700; ^{1}H NMR (300 MHz, DMSO) δ 8.04 (t, J = 5.47, 1H), 7.42–7.14 (m, 14H), 5.91 (dd, J = 3.25, J = 0.63, 1H), 5.70 (d, J = 3.75, 1H), 3.58 (q, J = 6.93, 1H), 3.26 (q, J = 7.22, 2H), 2.68 (t, J = 7.28, 2H), 1.32 (d, J = 6.89, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.55, 146.16, 146.02, 142.55, 139.90, 129.10, 128.71, 126.45, 128.49, 128.34, 127.14, 126.73, 126.17, 125.63, 124.95, 74.83, 45.58, 40.74, 35.53, 19.12; MS/MS m/z 382.2 $(M + Na)^+$; Elemental analysis: calcd for $C_{24}H_{25}NO_2$: C, 80.19; H, 7.01; N, 3.90. Found: C, 79.96; H, 7.25; N, 4.02.

N-Benzhydryl-2-(3-(hydroxy(phenyl)methyl)phenyl) propanamide (6f)

From the reaction of 0.210 g (0.5 mmol) **5f** for 9 h and crystallization from acetone/petrolether was obtained 0.190 g (73%) of **6f**; mp 51–54 °C; IR (KBr, ν/cm^{-1}) 3410, 3304, 3062, 3029, 2975, 2930, 1649, 1602, 1531, 1494, 1451, 1028, 743, 699; ^{1}H NMR (300 MHz, DMSO) δ 8.88 (d, J = 8.39, 1H), 7.44–7.10 (m, 19H), 6.06 (d, J = 8.38, 1H), 5.87 (d, J = 3.86, 1H), 5.65 (d, J = 3.19, 1H), 3.82 (q, J = 6.78, 1H),

1.32 (d, J = 6.20, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 173.00, 146.13, 145.97, 142.82, 143.01, 142.36, 128.85, 128.70, 127.81, 127.58, 127.55, 127.41, 127.26, 128.49, 128.33, 127.13, 126.73, 126.05, 125.76, 125.04, 74.83, 56.30, 45.14, 18.95; MS/MS m/z 422.2 $(M + H)^+$, 444.3, $(M + Na)^+$; Elemental analysis: calcd for $C_{29}H_{27}NO_2$: C, 82.63; H, 6.46; N, 3.32. Found: C, 82.88; H, 6.69; N, 3.01.

N-Hydroxy-2-(3-(hydroxy(phenyl)methyl)phenyl) propanamide (6g)

A suspension of 0.180 g (0.5 mmol) **5g** and 0.020 g Pd/C in methanol (10 mL) was hydrogenated at atmospheric pressure and room temperature for 3 h. After filtration of the catalyst, evaporation of the solvent, and crystallization from cyclohexane/ethyl acetate 0.099 g (90%) of **6g** was obtained; mp 148–152 °C; IR (KBr, ν/cm^{-1}) 3378, 3214, 2979, 2915, 1645, 1520, 1492, 1447, 1022, 945, 724, 701; ^{1}H NMR (300 MHz, DMSO) δ 10.62 (s, 1H), 8.77 (s, 1H), 7.38–7.16 (m, 9H), 5.87 (d, J = 3.90, 1H), 5.66 (d, J = 3.60, 1H), 3.41 (q, J = 7.00, 1H), 1.31 (d, J = 7.00, 3H); ^{13}C NMR (75.5 MHz, DMSO) δ 170.65, 146.12, 146.02, 142.09, 128.52, 128.33, 127.16, 126.73, 126.13, 125.64, 125.11, 74.80, 42.62, 18.77; MS/MS m/z 294.1 $(M + Na)^+$; Elemental analysis: calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.64; H, 6.06; N, 5.39.

Biological evaluation

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), nordihydroguaiaretic acid (NDGA), sodium linoleate, soybean lipoxygenase, caffeic acid and Trolox were purchased from Sigma-Aldrich.

Interaction with DPPH (24)

To a solution of DPPH (0.05 mM) in absolute ethanol an equal volume of ethanolic solution of the tested compound (final concentrations 0.05 or 0.1 mM) was added. After 20 and 60 min, the absorbance was recorded at 517 nm and compared with the appropriate standard NDGA (Table 1). Ethanol was used as a control. Each experiment *in vitro* was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean.

Soybean lipoxygenase inhibition

Dimethyl sulphoxide solution of the tested compound was incubated with sodium linoleate (0.1 mM) and soybean lipoxygenase solution (0.2 mL, 1000 U/mL in saline) at room temperature. The inhibitory activity of the compounds was measured at the concentration range 10–1000 μM and IC_{50} values were determined. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared with the standard inhibitor caffeic acid (Table 1), according to the procedure previously reported (23).

Inhibition of linoleic acid lipid peroxidation (25)

Oxidation of linoleic acid to conjugated diene hydroperoxide in an aqueous dispersion was monitored at 234 nm. 2,2′-Azobis(2-amidinopropane) dihydrochloride was used as a free radical initiator. Linoleic acid dispersion (10 μL, 16 mM) was added to the UV cuvette containing phosphate buffer pH 7.4 (0.93 mL, 0.05 M) at room temperature. The oxidation reaction was initiated under air by the addition of AAPH solution (50 μL, 40 mM). Oxidation was carried out in the presence of compounds (10 μL, 0.1 mM). In the assay with no antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation was monitored at 37 °C by recording the increase of absorption at 234 nm caused by conjugated diene hydroperoxides. The results were compared to the standard inhibitor Trolox.

Calculation of lipophilicity

Lipophilicity was theoretically calculated as Clog P values in *n*-octanol-buffer by CLOGP Programme of Biobyte Corp.[a]

Cytostatic activity

The H 460 (lung carcinoma), MCF-7 (breast carcinoma), HCT 116 and SW620 (colon carcinoma) and MOLT-4 (acute lymphoblastic leukemia) cells (obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). MCF-7, SW620, HCT 116 and H 460 cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM, Gibco, Carlsbad, CA, USA), while MOLT-4 cells were cultured in suspension in RPMI medium, both supplemented with 10% fetal bovine serum (FBS, Gibco), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco) 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 (14,16). Briefly, the cells were cultured as monolayers and maintained in DMEM supplemented with 10% 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 cells 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 specific cell line. Test agents were then added in consecutive 10-fold dilutions (10^{-8}–10^{-4} M) and incubated for a further 72 h. Working dilutions were freshly prepared on the day of testing. The cell growth rate was evaluated by performing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma, Taufkirchen, Germany) after 72 h of incubation, which detects mitochondrial dehydrogenase activity in viable cells.

Each test was performed in quadruplicate in three 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 concentration–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%). If however, for a given cell line 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.

Clonogenic assay

Cell survival after treatment with the compound **4i** was determined by clonogenic assay. Briefly, HCT 116 cells were plated at

Table 1: Interaction with DPPH, *in vitro* inhibition of soybean lipoxygenase and inhibition of lipid peroxidation by ketoprofen derivatives **4**–**6**

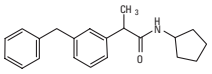
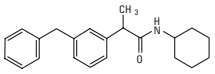
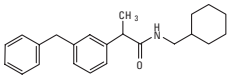
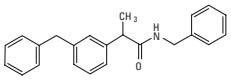
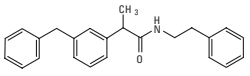
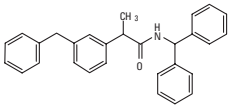
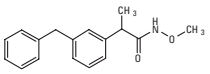
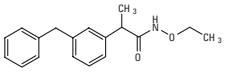
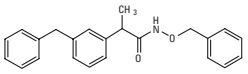
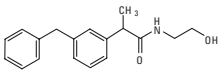
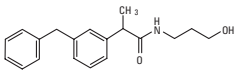
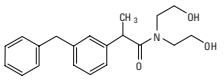
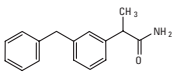
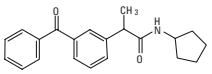
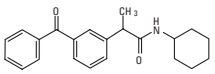
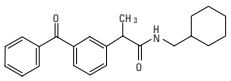
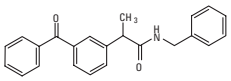
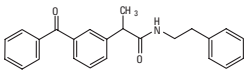
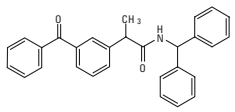
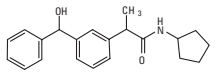
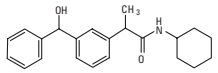
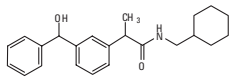
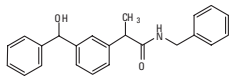
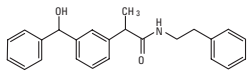
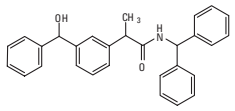
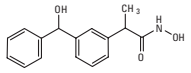
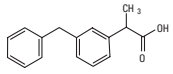
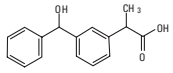
Compound		DPPH 20 min[a] (%)	DPPH 60 min[a] (%)	LOX IC_{50} (μM)	LP inhib.[a] (%)	Clog *P*
4a		28.5	27.6	82	63.2	4.54
4b		30.9	29.5	37.5	80.4	5.10
4c		31.4	33.9	80	87.9	5.72
4d		19.9	26.6	74.5	97.0	4.63
4e		32.9	21.7	43	99.9	5.01
4f		34.0	28.5	50	98.8	5.98
4g		22.8	26.1	n.d.[b]	n.a.[c]	3.04
4h		27.6	28.6	385	n.a.	3.57
4i		29.4	29.3	100	98.7	4.98
4j		33.0	22.5	270	66.7	2.61
4k		32.2	27.7	280	32.6	2.14
4l		32.7	23.5	400	22.8	2.83
4n		30.0	24.1	325	23.6	2.83
5a		38.1	33.8	61.5	55.8	3.51
5b		36.1	31.6	120	69.7	4.07
5c		29.4	35.4	53	92.2	4.69
5d		36.8	31.2	87.5	99.0	3.61
5e		35.2	30.8	65	92.2	3.98

Table 1: Continued

Compound	DPPH 20 min[a] (%)	DPPH 60 min[a] (%)	LOX IC_{50} (μM)	LP inhib.[a] (%)	Clog *P*
5f 	38.5	32.5	64.5	98.6	4.95
6a 	1.9	2.7	230	23.6	2.78
6b 	2.9	2.6	425	43.0	3.34
6c 	2.9	3.4	295	28.1	3.96
6d 	3.9	2.6	200	31.2	2.88
6e 	1.2	5.4	120	35.1	3.26
6f 	3.8	2.1	20.5	n.a.	4.22
6g 	26.6	43.0	200	44.3	1.56
2a 	6.9	4.8	415	31.1	3.79
2b 	n.a.	5.5	200	51.8	2.03
Ketoprofen	8.1	7.2	130	69.3	2.76
Trolox				63	
Caffeic acid			600		

DPPH, 1,1-diphenyl-picrylhydrazyl; LOX, soybean lipoxygenase; LP, lipid peroxidation.
[a]Concentration: 1×10^{-4} M.
[b]n.d. – IC_{50} value could not be determined: percent of inhibition was 12% and 27.5% at concentrations 100 and 500 μM, respectively.
[c]n.a. – No activity, under the reported experimental conditions.

3000 cell/100-mm dish and 18 h after seeding cells were treated with **4i** at the concentration of 10 μM ($\approx IC_{50}$) and 50 μM. The cultures were washed after the desired length of time (6 or 24 h), fed with complete medium, and 1–2 weeks later surviving colonies were fixed with ice-cold methanol, stained with 0.1% w/v crystal violet and counted. Survival was calculated as the ratio of the number of colonies from treated versus untreated samples.

Results and Discussion

Chemistry

Ketoprofen was the starting compound in the synthetic pathway leading to reduced analogs **2a**,**b** and their novel amides (Scheme 1). Compounds **2a**,**b** were synthesized by the catalytic hydrogenation of ketoprofen under different reaction conditions. Further on, **2a** and ketoprofen in the reaction with 1-benzotriazolide

carboxylic acid chloride (**1**) gave the corresponding NSAID benzotriazolides **3a**,**b** (17), which were transformed to amides **4a**–**l** and **5a**–**g**. In general, the amidation was performed with 1.1 equivalent of the appropriate amine in the presence of five equivalents of triethylamine in toluene. Triethylamine formed a water-soluble salt with benzotriazole, a by-product of the reaction, which was readily extracted with water. All amides **4** and **5** were prepared at room temperature except compounds **4f**, **4i** and **5f**: the reactions with benzhydrylamine and *O*-benzylhydroxylamine were too slow at room temperature, probably because of the steric hindrance of the first and lower nucleophilicity of the second amine. Reactions with hydrophilic bifunctional amines (2-hydroxyethylamine, 3-hydroxypropylamine, diethanolamine) were performed in solvent mixtures toluene/ethyl acetate or toluene/acetonitrile with 1.1 equivalent of the amine and one equivalent of triethylamine. It was established that under given reaction conditions hydroxy groups of the amines were not reactive.

Derivatives **6a**–**f** were synthesized by the catalytic hydrogenation of the compounds **5a**–**f**. Selective reduction of keto to hydroxy group was achieved using heterogeneous Pd-ethylenediamine [Pd/C(en)] complex catalyst (25). Completely reduced products **4a**–**f** were obtained as by-products in small amounts only.

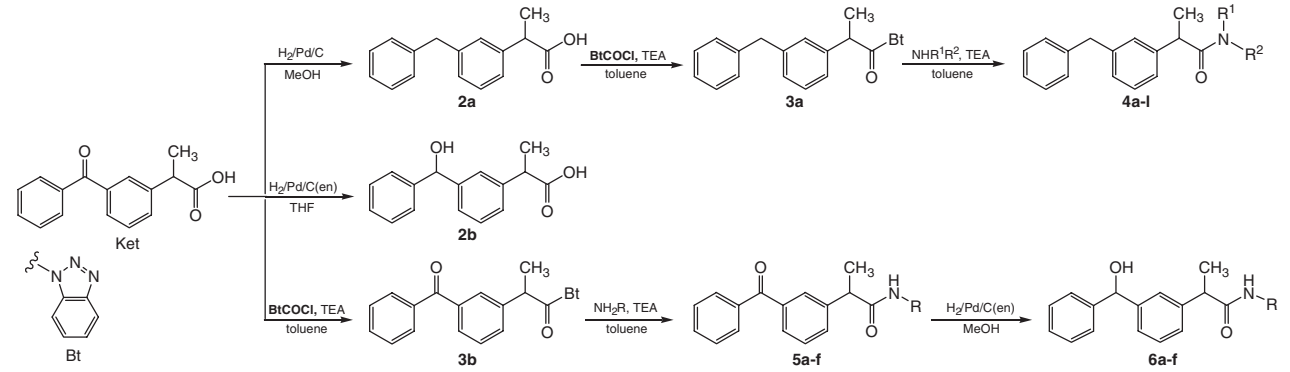
On the other hand, hydrogenolysis of the *N*-benzyloxy-2-(3-benzoylphenyl)propanamide (**5g**) with 10% Pd/C(en) was too slow (Scheme 2). When 10% Pd/C was used, the reaction rate was satisfactory and the product **6g** was obtained in 90% yield. The amount of the catalyst determined the selectivity of the reaction: a higher amount of the catalyst led first to hydroxamic acid **4m** (16), and finally to amide **4n**.

Structures of the compounds **4a**–**n**, **5a**–**g** and **6a**–**g** were deduced from the analysis of their IR, ^{1}H and ^{13}C NMR spectra and confirmed by elemental analysis. The chemical shifts were consistent with the proposed structures of the novel compounds.

Biological studies

Antioxidant activity

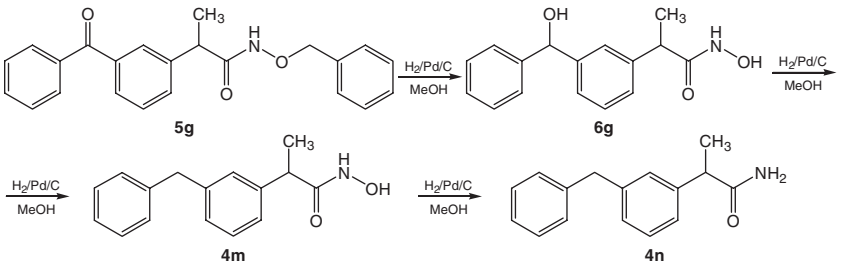
The interaction of the examined compounds with the stable free radical DPPH was studied. Interaction with DPPH indicates radical scavenging ability in an iron-free system. Interactions were monitored after 20 and 60 min at two concentrations of DPPH (0.05 and 0.1 mM). Ketoprofen, the prototype compound, as well as the



	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l
R^1												
R^2	H	H	H	H	H	H	H	H	H	H	H	

	5a, 6a	5b, 6b	5c, 6c	5d, 6d	5e, 6e	5f, 6f
R						

Scheme 1: Synthesis of ketoprofen derivatives **4a**–**l**, **5a**–**f** and **6a**–**f**.



Scheme 2: Hydrogenation of **5g**.

reduced derivatives **2a** and **2b** presented very low interaction values. In general, it seemed that all the tested compounds interacted with DPPH in a concentration-dependent manner. No significant changes were observed with time. Compounds **5f**, **5a**, **5d** and **5e** were the most active in the 0.1 mM assay. Lipophilicity does not seem to influence their interaction with DPPH. The non-reduced ketoprofen amides were more potent than the reduced ones (e.g., **5a** > **4a**, **5b** > **4b**, **5d** > **4d**). The lowest antioxidant activity showed the hydroxy bearing derivatives **6**. The results are presented in Table 1.

Soybean lipoxygenase inhibition

Compounds were further evaluated for inhibition of soybean lipoxygenase (LOX) by the UV absorbance-based enzyme assay (26). Lipoxygenases oxidize certain fatty acids at specific positions to hydroperoxides, precursors of leukotrienes, which contain a conjugated triene structure, i.e., soybean lipoxygenase converts linoleic to 13-hydroperoxylinoleic acid. Leukotrienes play a role as mediators of a variety of inflammatory and allergic processes. Lipoxygenases are involved in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer (27). Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases (28). Most of the LOX inhibitors are antioxidants or free radical scavengers, as lipoxygenation occurs via a carbon-centered radical (29). Perusal of IC_{50} values shows that compound **6f** is the most active by far, followed by compounds **4b**, **4e**, **4f**, **5c**, **5a**, **5e** ≈ **5f**, **4d**, **4c** and **4a** (IC_{50} = 20.5–82 μM). From Table 1, it is obvious that aromatic and cycloalkyl amides **4** and **5** are more potent lipoxygenase inhibitors (IC_{50} = 37.5–120 μM) than derivatives with carboxylic group (IC_{50} of ketoprofen, **2b**, and **2a** are 130, 200 and 415 μM, respectively). Lipophilicity is referred as an important physicochemical parameter for lipoxygenase inhibition (30,31). However, in this data set lipophilicity does not seem to affect absolutely the LOX inhibition, as the most potent compound **6f** does not possess the highest Clog *P* value within the set.

Inhibition of linoleic acid lipid peroxidation

Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies *in vitro*. The water soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals. In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. The results indicated that compounds **4e**, **5d**, **4f**, **4i**, **5f**, **4d**, **5e**, and **5c** are excellent inhibitors of lipid peroxidation (92.2–99.9%), significantly higher than ketoprofen (69.3%) and reduced derivatives **2b** and **2a** (51.8% and 31.1%, respectively) (Table 1). In general, methylene derivatives **4** (with the exception of **4k**, **4l**, **4n**) and keto derivatives **5** were more potent than the hydroxy bearing derivatives **6**. Comparison of the amide substituents revealed that in the series **4** the activity decreased as follows: aromatic substituent > cycloalkyl > aliphatic ≈ non-substituted amide > *O*-substituted hydroxamic acids. In the series **5**, aromatic amides were also more active than the cycloalkyl ones.

Using the C-QSAR program, an analysis was performed to discover whether any correlation between antioxidant activity and several physicochemical parameters (lipophilicity, steric and electronic variables) existed. For the inhibition of lipid peroxidation, the following equation was derived:

$$\log \mathrm{LP\%} = 0.153(0.0550)\mathrm{Clog}\,P + 0.217(0.130)\mathrm{Ico} + 1.086(0.225) \quad (1)$$

$N = 24$, $r = 0.829$, $r^2 = 0.688$, $q^2 = 0.594$, $s = 0.14$, $F_{2,21} = 26.43$, $\alpha = 0.01$

Lipophilicity is the most significant parameter followed by the indicator variable Ico (taking the value 1 for the compounds having the characteristic carbonyl group of ketoprofen and the value 0 for the congeners without this group). According to the leave-one-out procedure of the program compounds **4j** and **6g** were not included in the regression analysis. We may speculate that these compounds operate by a different biochemical mechanism from the rest. Further investigation is in progress to delineate the physicochemical properties implicated in the lipid peroxidation inhibition.

Furthermore, the results indicate that the LOX inhibition *in vitro* is correlated with the lipid peroxidation inhibition ($r^2 = 0.672$). Lipoxygenases play a role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer. Under our experimental conditions, both activities are correlated according to the following equation:

$$\log 1/\mathrm{IC_{50}LOX} = 1.182(0.348)\mathrm{LP\%} + 1.825(0.612) \quad (2)$$

$N = 26$, $r = 0.820$, $r^2 = 0.672$, $q^2 = 0.627$, $s = 0.194$, $F_{1,24} = 53.15$, $\alpha = 0.01$

Cytostatic activity

Compounds **4b**,**c**,**e**,**f**,**i**, **5c**,**e**,**f** and **6c**,**f**–**g** were screened for their potential antiproliferative (cytostatic) effects on a panel of five human cell lines, which were derived from different cancer types including HCT 116 and SW620 (colon carcinoma), MCF-7 (breast carcinoma), MOLT-4 (acute lymphoblastic leukemia), and H 460 (lung carcinoma). The tested compounds showed very similar antiproliferative effect on the presented panel cell lines (Table 2), except **6g**, which was the least active, coinciding again with its significantly lower lipophilicity, as discussed in our previous paper (14). Even so, differences in Clog *P* between all other compounds do not seem to have an impact on the slight differences in their antiproliferative activity. Besides, the cytostatic activities do not correlate with either antioxidative, lipoxygenase inhibitory activity or lipid peroxidation inhibition. However, some compounds, especially **5f**, precipitated from the cell culture medium, thus reducing their cell growth–inhibitory activity and causing larger deviations between experiments. Still, in spite of the precipitation, compound **4i** showed the highest and the most consistent activity.

Table 2: Inhibitory effects of ketoprofen derivatives **4**–**6** on the growth of malignant tumor cell lines

	Tumor cell growth [IC_{50}[a] (μM)]				
Compound	HCT 116	SW620	MCF-7	MOLT-4	H 460
4b[b]	12 ± 3	56 ± 43	14 ± 1	19 ± 3	53 ± 46
4c	11 ± 0.3	24 ± 2	14 ± 0.1	17 ± 0.2	19 ± 1
4e	15 ± 0.6	25 ± 5	20 ± 2	11 ± 8	21 ± 0.7
4f[b]	4 ± 3	87 ± 10	14 ± 4	40 ± 15	52 ± 40
4i[b]	7 ± 5	16 ± 0.2	15 ± 1	5 ± 3	7 ± 1
5c	13 ± 5	53 ± 42	13 ± 0.4	15 ± 0.8	20 ± 1
5e	15 ± 2	30 ± 3	17 ± 5	15 ± 3	22 ± 4
5f[b]	≥10[c]	>10[c]	≥10[c]	>10[c]	≥10[c]
6c	13 ± 0.8	37 ± 8	13 ± 0.1	15 ± 0.4	32 ± 8
6e[b]	18 ± 15	50 ± 40	23 ± 16	27 ± 15	57 ± 37
6f[b]	13 ± 2	18 ± 2	13 ± 2	11 ± 4	15 ± 0.1
6g	≥100	>100	>100	49 ± 31	≥100

[a]50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.
[b]Precipitates from the media at 1×10^{-4} μM concentration.
[c]The IC_{50} concentrations could not be calculated precisely because of the precipitation.

Table 3: Survival of HCT 116 cells after treatment with **4i**, measured by the clonogenic assay (see Methods for details)

	Survival of HCT 116 cells (%)	
Treatment with **4i**	10 μM	50 μM
6 h	61	1
24 h	4	0

Furthermore, we performed a clonogenic assay. This assay additionally determines the effectiveness of cytotoxic agents to understand the long-term fate of treated cells (32). We tested the most active compound **4i** for its ability to inhibit HCT 116 tumor cell proliferation. Therefore, we treated the HCT 116 cells with **4i** at 10 and 50 μM concentration for either 6 or 24 h. The results showed that the compound **4i** was absorbed by the cells and exerts a long-term cytostatic effect (Table 3, Figure S1). After six hours of treatment with a 10 μM solution of **4i**, 61% of cells survived and formed colonies during nine following days. Six-hours of treatment with higher concentration (50 μM) almost completely abolished the growth of cells. Moreover, when the cells were treated for 24 h with lower (10 μM) concentration, the survival of cells was even more drastically inhibited (only 4% of cells formed colonies). This experiment confirmed the long-term cytostatic effect of compound **4i** that was both dose- and time-dependant. This long-term effect could be undetected by other methods (such as MTT assay after 72 h in our previous experiments). Still, further investigations should be assessed to shed some light on the mechanisms of cell cycle inhibition, or cell death in the treated cells.

Conclusions

1,1-Diphenyl-2-picrylhydrazyl test for antioxidant screening revealed that ketoprofen amides were more potent than the amides of reduced ketoprofen derivatives. The most active compound was the benzhydryl ketoprofen amide **5f**. Lipoxygenase inhibition of the tested compounds varied from strong to very weak. The most potent compound was benzhydryl derivative **6f** (IC_{50} = 20.5 μM). Aromatic and cycloalkyl amides **4** and **5** were more potent lipoxygenase inhibitors (IC_{50} = 37.5–120 μM) than derivatives with carboxylic group. Aromatic amides of series **4** and **5** showed as excellent lipid peroxidation inhibitors (92.2–99.9%). In general, methylene derivatives **4** and keto derivatives **5** were more potent than the hydroxy bearing derivatives **6**. Comparison of the amide substituents revealed that in the series **4** the activity decreased as follows: aromatic substituent > cycloalkyl > aliphatic = non-substituted amide > *O*-substituted hydroxamic acids. In the series **5**, aromatic amides were also more active than the cycloalkyl ones. On the other hand, the most pronounced cytostatic activity was exerted by *O*-benzyl derivative **4i**, although in general all tested reduced and non-reduced lipophilic derivatives showed similar activity.

Acknowledgments

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Note

[a]C-QSAR Database, 201 West 4th Str, Suite 204, Claremont CA, CA 91711, USA: Biobyte Corp.

Supporting Information

Additional supporting information may be found in the online version of this article.

Figure S1. Long-term survival of HCT 116 cells following treatment with **4i** at 10, or 50 μM.

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