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Synthesis and evaluation of chromone-2-carboxamide derivatives as cytotoxic agents and 5-lipoxygenase inhibitors

Fatima Bousejra-ElGarah¹ · Barbora Lajoie¹ · Jean-Pierre Souchard² · Geneviève Baziard¹ · Jalloul Bouajila² · Salomé El Hage¹

Abstract In the present study, we prepared a series of 21 chromone carboxamide derivatives bearing diverse amide side chains. Their potency to inhibit the proliferation of breast (MCF-7), ovarian (OVCAR and IGROV), and colon (HCT-116) cancer cell lines, was evaluated in vitro using the MTT assay. Among these compounds, 13 showed promising cytotoxic activity against at least one cancer cell line with IC₅₀ in the range 0.9–10 μM. Our compounds were also screened for their anti-inflammatory activity as putative inhibitors of 5-lipoxygenase.

Structure-activity relationships studies on our chromone carboxamide derivatives revealed that the presence of a 6-fluoro substituent on the chromone nucleus (R₁) or propyl and 3-ethylphenyl groups on the amide side chain (R₂) has a positive impact on the cytotoxic activity. In terms of the anti-inflammatory activity, hydrophilic chromone carboxamide derivatives showed greater 5-lipoxygenase inhibition.

The physico-chemical properties of chromone carboxamides are in accordance with the general requirements of

drug development process and ligand efficiency values allow further structure optimization, with compound **4b** as a lead.

Keywords Chromone carboxamides · Cytotoxic activity · Anti-inflammatory effect · Lipophilicity · SAR

Introduction

The chromone scaffold [(4H)-1-benzopyran-4-one] is well known as a pharmacophore of a large number of natural and synthetic bioactive molecules. This heterocycle constitutes the basic nucleus of flavones, an important and widespread class of compounds from plants with a large number of biological activities (Gaspar et al., 2014, Keri et al., 2014). Natural and synthetic chromones have shown a large spectrum of biological activities such as anti-inflammatory (Khan et al., 2010, Thanigaimalai et al., 2010), antimalarial (Auffret et al., 2007), and anticancer activity (Budzisz et al., 2002, Barve et al., 2006), associated with low toxicity (do Rocio Andrade Pires et al., 2016)

With 8.2 million deaths and 14.1 million new cases in 2012 (WHO, 2013), and given the fact that this number will reach 22.2 million by 2030, cancer has become one of the major health problems worldwide (Bray et al., 2012). The situation is expected to worsen because of the increase of multidrug resistances in various types of cancer (e.g., breast, ovarian, gastrointestinal, lung cancers) making the development of new active compounds a real challenge for the coming years (Pan et al., 2016).

In the search for new efficient anticancer drugs, chromones in particular carboxamides, can play an important

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role as demonstrated by the growing interest toward this class of compounds. Indeed, several chromone carboxamides have been reported as potent and selective cytotoxic agents (Bhatnagar et al., 2010, Nam et al., 2010, Valdameri et al., 2012, Redda et al., 2014) and others were evaluated as monoamine oxidase inhibitors (Gaspar et al., 2011) or adenosine receptor ligands (Gaspar et al., 2012).

Besides, the association between cancer and inflammation has long been established (Balkwill and Mantovani, 2012). For instance, the involvement of the 5-lipoxygenase enzyme (5-LOX) and its products have been implicated in the development and progression of colon cancer (Soumaoro et al., 2006). 5-LOX has been reported to induce inflammation *via* the catalysis of the production of leukotrienes from arachidonic acid (Shimizu et al., 1984) and it is therefore an important target for mechanism-based drugs for the treatment of inflammatory disorders, often associated with other diseases including cancer. Therefore, anti-inflammatory agents that inhibit leukotriene production may have a beneficial effect in both the prevention and treatment of cancer (Aggarwal et al., 2006).

In the present study, we synthesized 21 substituted chromone carboxamides, among which 12 are newly described, and evaluated their potency to inhibit the proliferation of breast, ovarian, and colon cancer cell lines. Our compounds were also screened for their anti-inflammatory activity as putative inhibitors of 5-LOX. Additionally, an *in silico* study of the “drug-likeness” properties for our derivatives was carried out by investigating their Lipinski parameters (Lipinski et al., 1997) and calculating their ligand efficiency values (Abad-Zapatero, 2007) to discuss structure–activity relationships (SAR).

Materials and methods

Chemistry

Melting points were determined on a DSC-50 Shimadzu apparatus (Kyoto, Japan). Infra-red spectra were recorded on a Perkin–Elmer Spectrum One fourier transform infrared spectrometer (Perkin–Elmer, USA). Proton nuclear magnetic resonance (^1H NMR) (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained in CDCl_3 or DMSO-d_6 on a DPX 300 spectrometer (Bruker Biosciences, USA), and peak positions are given as s (singlet), d (doublet), t (triplet), q (quadruplet) or m (multiplet). Chemical shift (δ) values are given in parts per million. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel plates 60 F-254. All yields are calculated for analytically pure materials. The microanalyses were performed in the Microanalytical Laboratory of ENSIACET in Toulouse, France, and the results obtained were within $\pm 0.4\%$ of the

theoretical values. Mass spectra, electrospray ionization-method, were recorded on DSQ Thermo Fisher Scientific mass spectrometer; signals were given as m/z .

Commercially available chromone-2-carboxylic acids **1a–1e**, amine derivatives and all other reagents were purchased from Acros Organics (Halluin, France) and used without additional purification. Chemical structures were confirmed by infrared (IR), ^1H NMR, ^{13}C NMR, and ESI–MS.

The physico-chemical properties of compounds previously described **3a**, **3c**, **3d**, **3e** (Ellis and Shaw, 1972), **4a**, **5a** (Gaspar et al., 2011), **8a** (Gaspar et al., 2011), **9a** (Gaspar et al., 2012), and **10a** (Gaspar et al., 2012) are in agreement with the literature data.

General procedure for the synthesis of acyl chloride 2

4-oxo-4H-1-benzopyran-2-carbonyl chloride derivatives were prepared according to a previously described method (Payard, 1973). Phosphorus pentachloride (0.028 mol; 1.1 equiv.) was added to a solution of substituted chromone-2-carboxylic acid (0.025 mol) in dry cyclohexane (50 mL). The mixture was stirred under reflux for 1 h with the formation of the corresponding acyl chloride. After cooling, the formed solid was filtered and used without purification.

General procedure for the synthesis of compounds 3–14

An equimolar amount of the suitable acyl chloride was slowly added to a stirred solution of the corresponding amine (0.015 mol) in dry dichloromethane (DCM) (ammonium hydroxide, aniline, propylamine, 3-ethylaniline, 4-methylaniline, 4-chloroaniline, 4-fluoroaniline, 4-hydroxyaniline, 4-amino- α -diethylamino-*O*-cresol dihydrochloride, *N,N*-diethylpentyl-2,5-diamine, aminomorpholine, 1-(pyrrolidinocarbonylmethyl)piperazine) in presence of triethylamine (1 equiv.). The solution was stirred at room temperature and monitored by TLC. The mixture was washed with water, 10 % Na_2CO_3 and brine. Purification by flash silica gel chromatography (0–8 % MeOH in DCM) provided the desired amides.

6-Fluoro-4-oxo-4H-1-benzopyran-2-carboxamide (**3b**)
Yield: 91 %; m.p.: 131 °C; IR (KBr) ν cm^{-1} : 3362 (NH_2), 3073, 3050, 2927 (CH), 1738, 1696 (CONH_2), 1635 (CO), 1624 (C=C); ^1H NMR (CDCl_3): δ 6.95 (s, 1H, H_3), 7.80–7.94 (m, 3H, H_5 , H_7 , H_8), 8.33, and 8.70 (2s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ : 177.30 (C_4), 161.02 (C_6), 158.45 (C_2), 156.51 (CO-NH_2), 152.04 (C_{8a}), 125.29 (C_{4a}), 123.47 (C_7), 122.16 (C_8), 110.17 (C_3), 109.88 (C_5); MS (m/z) 208.0 [$\text{M} + \text{H}$] $^+$. Anal. calcd. for $\text{C}_{10}\text{H}_6\text{FNO}_3$: C, 57.98; H, 2.92; N, 6.76 %. Found: C, 57.83; H, 2.97; N, 6.84 %.

6-Fluoro-*N*-propyl-4-oxo-4H-chromene-2-carboxamide (**4b**) Yield: 32 %; m.p.: 196 °C; IR (KBr) ν cm⁻¹: 3412 (NH), 3303, 2964, 2936, 2879 (CH, CH₂, CH₃), 1686 (CONH), 1642 (CO), 1618, 1540 (C=C); ¹H NMR (DMSO-d₆): δ 0.91 (t, 3H, CH₃, J = 7.5 Hz), 1.57 (m, 2H, CH₂-CH₂-CH₃), 3.26 (m, 2H, NH-CH₂), 6.83 (s, 1H, H₃), 7.71–7.86 (m, 3H, H₅, H₇, H₈), 9.14 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.25 (C₄), 160.89 (C₆), 158.45 (C₂), 156.46 (CO-NH), 152.00 (C_{8a}), 125.31 (C_{4a}), 123.72 (C₇), 122.14 (C₈), 110.15 (C₃), 109.91 (C₅), 41.47, 22.57 (2 CH₂), 11.83 (CH₃); MS (m/z) 250.0 [M+H]⁺. Anal. calcd. for C₁₃H₁₂FNO₃: C, 62.65; H, 4.85; N, 5.62 %. Found: C, 62.76; H, 4.97; N, 5.46 %.

6-Bromo-*N*-propyl-4-oxo-4H-chromene-2-carboxamide (**4d**) Yield: 33 %; m.p.: 210 °C; IR (KBr) ν cm⁻¹: 3291 (NH), 3075, 2965, 2932, 2873 (CH, CH₂, CH₃), 1687 (CONH), 1646 (CO), 1600, 1536 (C=C); ¹H NMR (DMSO-d₆): δ 0.90 (t, 3H, CH₃, J = 7.4 Hz), 1.57 (m, 2H, CH₂-CH₂-CH₃), 3.26 (m, 2H, NH-CH₂), 6.85 (s, 1H, H₃), 7.71 (d, 1H, H₈, J = 8.9 Hz), 8.06 (dd, 1H, H₇, J = 8.8, 2.5 Hz), 8.11 (d, 1H, H₅, J = 2.5 Hz), 9.14 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 176.61 (C₄), 159.07 (C₂), 156.40 (CO-NH), 154.51 (C_{8a}), 138.03 (C₇), 127.48 (C₅), 125.57 (C_{4a}), 121.90 (C₈), 118.85 (C₆), 110.85 (C₃), 41.49, 22.58 (2 CH₂), 11.85 (CH₃); MS (m/z) 310.0 and 312 [M+H]⁺. Anal. calcd. for C₁₃H₁₂BrNO₃: C, 50.34; H, 3.90; N, 4.52 %. Found: C, 50.45; H, 3.79; N, 4.63 %.

N-(3'-ethylphenyl)-4-oxo-4H-chromene-2-carboxamide (**6a**) Yield: 71 %; m.p.: 168 °C; IR (KBr) ν cm⁻¹: 3268 (NH), 2960, 2927, 2868 (CH, CH₂, CH₃), 1682 (CONH), 1640 (CO), 1615, 1540 (C=C); ¹H NMR (CDCl₃) δ : 1.29 (t, 3H, CH₃, J = 7.6 Hz), 2.71 (q, 2H, CH₂, J = 7.6 Hz), 7.09 (d, 1H, H₈, J = 7.5 Hz), 7.32 (s, 1H, H₃), 7.35 (t, 1H, H₅, J = 7.8 Hz), 7.49–7.64 (m, 4H, H₆, H₂, H₄, H₆'), 7.79 (td, 1H, H₇, J = 7.2, 1.7 Hz), 8.27 (dd, 1H, H₅, J = 7.9, 1.6 Hz), 8.54 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.79 (C₄), 158.08 (C₂), 156.19 (CO-NH), 155.62 (C_{8a}), 144.90 (C₃'), 137.99 (C₁'), 135.49 (C₇), 129.19 (C₅'), 126.56 (C₆), 125.39 (C₅), 124.94 (C₄'), 124.15 (C_{4a}), 120.89 (C₂'), 119.49 (C₈), 118.97 (C₆'), 111.48 (C₃), 28.68 (CH₂), 15.94 (CH₃); MS (m/z) 294.1 [M+H]⁺. Anal. calcd. for C₁₈H₁₅NO₃: C, 73.71; H, 5.15; N, 4.78 %. Found: C, 73.59; H, 5.28; N, 4.92 %.

6-Fluoro-*N*-(3'-ethylphenyl)-4-oxo-4H-chromene-2-carboxamide (**6b**) Yield: 54 %; m.p.: 198 °C; IR (KBr) ν cm⁻¹: 3280 (NH), 3081, 2962, 2929 (CH, CH₂, CH₃), 1685 (CONH), 1637 (CO), 1612, 1593, 1543 (C=C); ¹H NMR (DMSO-d₆): δ 1.21 (t, 3H, CH₃, J = 7.6 Hz), 2.64 (q, 2H, CH₂, J = 7.6 Hz), 6.98 (s, 1H, H₃), 7.06 (d, 1H, H₄, J = 7.6 Hz), 7.33 (t, 1H, H₅', J = 7.6 Hz), 7.64 (m, 2H, H₂', H₆'), 7.77 (dd, 1H, H₈, J = 8.3, 3.1 Hz), 7.85 (td, 1H, H₇, J = 8.1,

3.1 Hz), 7.95 (dd, 1H, H₅, J = 9.2, 4.4 Hz), 10.68 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.24 (C₄), 160.97 (C₆), 158.53 (C₂), 156.39 (CO-NH), 152.03 (C_{8a}), 144.94 (C₃'), 137.89 (C₁'), 129.22 (C₅'), 125.38 (C₄'), 125.31 (C_{4a}), 123.55 (C₇), 122.30 (C₈), 120.88 (C₂'), 118.96 (C₆'), 110.68 (C₃), 109.93 (C₅) 28.67 (CH₂), 15.94 (CH₃); MS (m/z) 312.1 [M+H]⁺. Anal. calcd. for C₁₈H₁₄FNO₃: C, 69.45; H, 4.53; N, 4.50 %. Found: C, 69.57; H, 4.65; N, 4.39 %.

6-Bromo-*N*-(3'-ethylphenyl)-4-oxo-4H-chromene-2-carboxamide (**6d**) Yield: 52 %; m.p.: 227 °C; IR (KBr) ν cm⁻¹: 3267 (NH), 3075, 2961, 2960, 2927 (CH, CH₂, CH₃), 1683 (CONH), 1637 (CO), 1608, 1597, 1541 (C=C); ¹H NMR (DMSO-d₆): δ 1.34 (t, 3H, CH₃, J = 7.6 Hz), 2.76 (q, 2H, CH₂, J = 7.6 Hz), 7.13 (s, 1H, H₃), 7.19 (d, 1H, H₄', J = 7.6 Hz), 7.46 (t, 1H, H₅', J = 7.7 Hz), 7.75 (m, 2H, H₂', H₆'), 7.96 (d, 1H, H₈, J = 8.9 Hz), 8.22 (dd, 1H, H₇, J = 8.9, 2.5 Hz), 8.28 (d, 1H, H₅, J = 2.5 Hz), 10.4 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 176.68 (C₄), 157.80 (C₂), 156.38 (CO-NH), 154.58 (C_{8a}), 144.95 (C₃'), 138.16 (C₇), 137.88 (C₁'), 129.23 (C₅'), 127.49 (C₅), 125.63 (C_{4a}), 125.04 (C₄'), 122.17 (C₈), 120.88 (C₂'), 119.04 (C₆), 118.97 (C₆'), 111.51 (C₃), 28.67 (CH₂), 15.94 (CH₃); MS (m/z) 372.0 and 374.0 [M+H]⁺. Anal. calcd. for C₁₈H₁₄BrNO₃: C, 58.08; H, 3.79; N, 3.76 %. Found: C, 58.29; H, 3.64; N, 3.89 %.

N-(3'-ethylphenyl)-6-methyl-4-oxo-4H-chromene-2-carboxamide (**6e**) Yield: 33 %; m.p.: 202 °C; IR (KBr) ν cm⁻¹: 3326 (NH), 3074, 2928, 2850 (CH, CH₂, CH₃), 1681 (CONH), 1637 (CO), 1610, 1594, 1541 (C=C); ¹H NMR (DMSO-d₆): δ 1.21 (t, 3H, CH₂-CH₃, J = 7.6 Hz), 2.48 (s, 3H, CH₃), 2.64 (q, 2H, CH₂, J = 7.6 Hz), 6.94 (s, 1H, H₃), 7.07 (d, 1H, H₄', J = 8.0 Hz), 7.33 (t, 1H, H₅', J = 8.0 Hz), 7.65 (m, 2H, H₂', H₆'), 7.75 (m, 2H, H₇, H₈), 7.87 (s, 1H, H₅), 10.66 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.72 (C₄), 158.13 (C₂), 156.04 (CO-NH), 153.88 (C_{8a}), 144.89 (C₃'), 137.99 (C₇), 136.56 (C₁'), 136.29 (C₆), 129.18 (C₅'), 124.92 (C₄'), 124.62 (C₅), 123.86 (C_{4a}), 120.86 (C₂'), 119.27 (C₈), 118.94 (C₆'), 111.31 (C₃), 28.68 (CH₂), 20.94 and 15.94 (2 CH₃); MS (m/z) 308.1 [M+H]⁺. Anal. calcd. for C₁₉H₁₇NO₃: C, 74.25; H, 5.58; N, 4.56 %. Found: C, 74.37; H, 5.42; N, 4.69 %.

N-(4'-fluorophenyl)-4-oxo-4H-chromene-2-carboxamide (**7a**) Yield: 48 %; m.p.: 222 °C; IR (KBr) ν cm⁻¹: 3338 (NH), 3138, 3072 (CH), 1697 (CONH), 1630 (CO), 1573, 1508 (C=C); ¹H NMR (DMSO-d₆): δ 6.98 (s, 1H, H₃), 7.27 (m, 2H, H₃', H₅'), 7.57 (m, 1H, H₆), 7.82–7.85 (m, 4H, H₇, H₈, H₂', H₆'), 8.08 (dd, 1H, H₅, J = 7.9, 1.6 Hz), 10.82 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.73 (C₄), 160.66 (C₄'), 158.25 (C₂), 156.01 (CO-NH), 155.60 (C_{8a}), 135.48 (C₇), 134.35 (C₁'), 126.55 (C₆), 125.39 (C₅), 124.14 (C_{4a}), 123.60 (C₆'), 123.52 (C₂'), 119.47 (C₈), 116.03 (C₅'), 115.80 (C₃'), 111.56 (C₃); MS (m/z) 284.0 [M+H]⁺. Anal.

calcd. for C₁₆H₁₀FNO₃: C, 67.84; H, 3.56; N, 4.94 %. Found: C, 67.53; H, 3.62; N, 4.88 %.

N-[3-((diethylamino)methyl)-4-hydroxyphenyl]-4-oxo-4H-chromene-2-carboxamide (**11a**) Yield: 26 %; m.p.: 186 °C; IR (KBr) ν cm⁻¹: 3470 (OH), 3339 (NH), 3075, 2975, 2827 (CH, CH₂, CH₃), 1685 (CONH), 1638 (CO), 1616, 1560, 1512 (C=C); ¹H NMR (DMSO-d₆): δ 1.05 (t, 6H, CH₂-CH₃, *J* = 6.8 Hz), 2.56 (m, 4H, N-CH₂-CH₃), 3.33 (s br, 1H, OH), 3.73 (s, 2H, Ar-CH₂-N), 6.73 (d, 1H, H₅', *J* = 8.4 Hz), 6.94 (s, 1H, H₃), 7.54 (m, 2H, H₆ and H₆'), 7.70 (s, 1H, H₂'), 7.85 (d, 1H, H₈, *J* = 8.7 Hz), 7.93 (t, 1H, H₇, *J* = 7.5 Hz), 8.08 (d, 1H, H₅, *J* = 6.9 Hz), 10.56 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.92 (C₄), 157.70 (C₂), 156.36 (CO-NH), 155.61 (C_{8a}), 154.92 (C₄'), 135.54 (C₇), 129.32 (C₁'), 129.30 (C₆'), 126.52 (C₆), 125.39 (C₅), 125.37 (C₃'), 124.07 (C_{4a}), 123.29 (C₂'), 119.48 (C₈), 115.91 (C₅'), 111.27 (C₃), 53.19 (Ar-CH₂-N), 46.65 (N-CH₂), 10.51 (CH₃); MS (*m/z*) 367.2 [M+H]⁺. Anal. calcd. for C₂₁H₂₂N₂O₄: C, 68.84; H, 6.05; N, 7.65 %. Found: C, 68.92; H, 6.24; N, 7.52 %.

N-(5-(diethylamino)pent-2-yl)-4-oxo-4H-chromene-2-carboxamide (**12a**) Yield: 57 %; m.p.: 110 °C; IR (KBr) ν cm⁻¹: 3475, 3428, 3370 (NH), 2978, 2939, 2633 (CH, CH₂, CH₃), 1679 (CONH), 1647 (CO), 1608, 1572, 1528 (C=C); ¹H NMR (DMSO-d₆): δ 1.19 (t, 6H, CH₂-CH₃, *J* = 7.2 Hz), 1.23 (d, 3H, CH-CH₃, *J* = 6.6 Hz), 1.62 (m, 4H, CH-CH₂-CH₂-N), 3.05 (m, 6H, 3 CH₂-N), 4.05 (m, 1H, CH), 6.85 (s, 1H, H₃), 7.55 (td, 1H, H₆, *J* = 7.9, 0.9 Hz), 7.82 (d, 1H, H₈, *J* = 7.8 Hz), 7.91 (td, 1H, H₇, *J* = 8.5, 1.6 Hz), 8.05 (dd, 1H, H₅, *J* = 7.9, 1.3 Hz), 10.13 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.86 (C₄), 159.02 (C₂), 156.27 (CO-NH), 155.60 (C_{8a}), 135.38 (C₇), 126.49 (C₆), 125.30 (C₅), 124.05 (C_{4a}), 119.51 (C₈), 110.97 (C₃), 50.76 and 46.50 (2 CH₂), 45.42 (CH), 32.96 (CH₂), 20.89 (CH₃) 20.48 (CH₂), 8.82 (CH₃); MS (*m/z*) 331.2 [M+H]⁺. Anal. calcd. for C₁₉H₂₆N₂O₃: C, 69.06; H, 7.93; N, 8.48 %. Found: C, 69.23; H, 7.72; N, 8.33 %.

N-morpholino-4-oxo-4H-chromene-2-carboxamide (**13a**) Yield: 57 %; m.p.: 271 °C; IR (KBr) ν cm⁻¹: 3219 (NH), 3061, 2962, 2938, 2874 (CH), 1684 (CONH₂), 1644 (CO), 1620, 1607, 1511 (C=C); ¹H NMR (DMSO-d₆): δ 2.94 (t, 4H, O-CH₂-CH₂-N, *J* = 4.6 Hz), 3.69 (t, 4H, O-CH₂-CH₂-N, *J* = 4.6 Hz), 6.82 (s, 1H, H₃), 7.55 (td, 1H, H₆, *J* = 8.0, 1.0 Hz), 7.76 (d, 1H, H₈, *J* = 7.9 Hz), 7.90 (m, 1H, H₇), 8.05 (dd, 1H, H₅, *J* = 7.9, 1.6 Hz), 10.20 (s br, 1H, NH); ¹³C NMR (DMSO-d₆): δ 177.69 (C₄), 157.30 (C₂), 156.15 (CO-NH), 155.57 (C_{8a}), 135.51 (C₇), 126.57 (C₆), 125.39 (C₅), 124.09 (C_{4a}), 119.34 (C₈), 111.25 (C₃), 66.39 (2 CH₂), 54.63 (2 CH₂); MS (*m/z*) 275.0 [M+H]⁺. Anal. calcd. for C₁₄H₁₄N₂O₄: C, 61.31; H, 5.14; N, 10.21 %. Found: C, 61.45; H, 5.06; N, 10.36 %.

2-[4(*N*-pyrrolidinylcarbonylmethyl)-piperazinylcarbonyl]-4H-chromen-4-one (**14a**) Yield: 55 %; m.p.: 117 °C; IR (KBr) ν cm⁻¹: 3052, 2973, 2955, 2867, 2820 (CH, CH₂, CH₃), 1662 (CONH), 1644 (CO), 1617, 1573, 1510 (C=C); ¹H NMR (CDCl₃): δ 1.89 and 1.98 (2m, 4H, H₃'', H₄''), 2.7 (m, 4H, H₂'', H₅''), 3.22 (s, 2H, N-CH₂-CO-), 3.48 (m, 4H, H₃', H₅'), 3.62, and 3.85 (2m, 4H, H₂', H₆'), 6.53 (s, 1H, H₃), 7.46 (t, 1H, H₆, *J* = 7.1 Hz), 7.52 (d, 1H, H₈, *J* = 8.6 Hz), 7.76 (td, 1H, H₇, *J* = 7.1, 1.7 Hz), 8.25 (dd, 1H, H₅, *J* = 8.0, 1.5 Hz); ¹³C NMR (DMSO-d₆): δ 177.08 (C₄), 167.66 (CO-CH₂), 160.52 (CO-NH), 158.46 (C₂), 155.91 (C_{8a}), 135.51 (C₇), 126.57 (C₆), 125.39 (C₅), 124.16 (C_{4a}), 119.08 (C₈), 111.01 (C₃), 60.22 (CO-CH₂-N), 53.06 and 52.23 (2C pyrrolidine), 46.99, 45.99, 45.81 and 42.20 (4C piperazine), 26.16 and 24.12 (2C pyrrolidine); MS (*m/z*) 370.2 [M+H]⁺. Anal. calcd. for C₂₀H₂₃N₃O₄: C, 65.03; H, 6.28; N, 11.37 %. Found: C, 65.21; H, 6.15; N, 11.25 %.

Pharmacology

Anti-inflammatory activity

The anti-inflammatory activity of compounds was determined by the inhibition of soybean lipooxygenase. Chromone derivatives were dissolved in dimethyl sulfoxide (DMSO) and solutions were diluted in sodium phosphate buffer (pH 7.4). Diluted solutions of compounds (20 μ L) were mixed individually with 30 μ L of sodium phosphate buffer containing 5-LOX and 60 μ L of linoleic acid (3.5 mM) in a 96-well microliter plate. Addition of 90 μ L buffer gave a total volume of 200 μ L and a final concentration of chromone carboxamide of 100 μ M. The DMSO concentration did not exceed 1 %. The mixture was incubated at 25 °C for 10 min, and the absorbance was recorded at 234 nm using a Thermo Scientific Multiskan GO Microplate Spectrophotometer. The absorption changes with the conversion of linoleic acid to 13-hydroperoxyoctadeca-9,11-dienoate. Nordihydroguaiaretic acid (NDGA) was used as positive control. Percentage inhibition of enzyme was determined by comparison of rates of reaction of samples relative to blank sample using the formula $(E-S)/E \times 100$, where *E* is the activity of enzyme without test sample and *S* is the activity of enzyme with test sample. The percentage of inhibition of each compound was determined in triplicate.

Cytotoxicity evaluation

The cytotoxicity of compounds was estimated on human cancer cell lines (MCF-7, HCT-116, OVCAR, IGROV) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Cells were distributed in 96-well plates at 3 \times 10⁴ cells/well in 100 μ L culture medium, and then 100 μ L of medium containing the chromone

carboxamide at various concentrations were added. MTT is a yellow water-soluble tetrazolium salt, which metabolically active cells convert to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. All compounds were dissolved in DMSO followed by dilution in buffer so that the DMSO concentration did not exceed 1%. The level of blue formazan is then used as indirect index of cell density. The optical density of each sample was measured with a Thermo Scientific Multiskan GO Microplate Spectrophotometer at 500 nm. Four replicates were performed for each sample. Tamoxifen was used as positive control.

Results and discussion

Chemistry

Chromone carboxamides derivatives were obtained by functionalization of the chromone nucleus at position C2 of the γ -pyrone ring.

Structural modifications have been made in order to evaluate the SAR on cytotoxicity and 5-LOX inhibition. Particularly, (i) the introduction of a halide or methyl group on the 6-position of the chromone nucleus (R1, scheme 1), (ii) the side chain nature, either aliphatic or aromatic (R2), (iii) the substitution on the aromatic moiety, and (iv) the functionalization of the hydroxyl group have been considered. The molecular diversity in this library of compounds was further increased by preparing analogs **12a**, **13a**, and **14a** bearing nitrogen-containing side chains.

Syntheses were carried out starting from 6-substituted 2-chromone carboxylic acids, prepared according to a previously described method (Payard et al., 1974). The carboxamide derivatives were prepared in a two-step process, involving first the conversion, in quantitative yield, of the carboxylic acid into the acylchloride **2** using PCl_5 (Payard, 1973), and then the condensation with the appropriate amine to give amides **3–14** (scheme 1). The main advantage of this synthetic procedure is that no coupling reagents are needed. Chromone carboxamides were synthesized in a range of 30–90% yields, similar to those reported with the use of coupling agents such as phosphonium salts (BOP or PyBOP) (Gaspar et al., 2011). Their chemical structures were confirmed by IR, ^1H NMR, ^{13}C NMR and ESI-MS.

Reactions conditions: i) PCl_5 /dry cyclohexane; ii) $\text{R}_2\text{-NH}_2$ /TEA/dry DCM.

Pharmacology

Cytotoxicity

Twenty one chromone carboxamide derivatives were assayed for their in vitro anti-proliferative effect against

four human cancer cell lines: MCF-7 (hormone dependent breast cancer), OVCAR and IGROV (ovary cancer) and HCT-116 (colon cancer) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as described by Mosmann (1983) with modifications (Bekir et al., 2013). The results, expressed as IC_{50} values (μM), i.e., the concentration required to inhibit cell proliferation by 50% after exposure of cells to test compounds, are summarized in Table 1 and tamoxifen was used as reference.

Among these compounds, 13 showed good cytotoxic activity against at least one cancer cell line ($\text{IC}_{50} \leq 10 \mu\text{M}$). In the case of the breast cancer cell line MCF-7, five compounds were active with the best antiproliferative activity observed for **4b** ($\text{IC}_{50} = 0.9 \mu\text{M}$). Eight compounds were active against OVCAR cells (lowest IC_{50} for **4b** and **11a** = $5.1 \mu\text{M}$), eight derivatives were cytotoxic against IGROV cells (lowest IC_{50} for **4b** and **10a** = $3.2 \mu\text{M}$), and four compounds were active against colon HTC-116 cells (lowest IC_{50} for **4d** = $4.5 \mu\text{M}$).

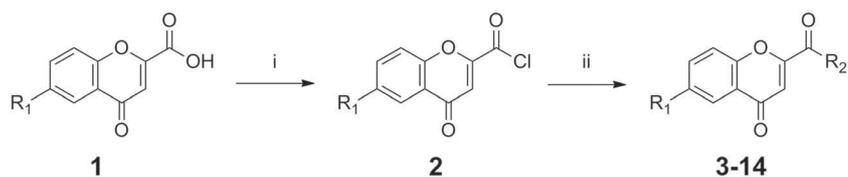
The tests showed that the primary amide analogs **3a–3e** are not cytotoxic, except the 6-fluorinated compound **3b**, which had an interesting activity against the two cancer cell lines OVCAR and IGROV ($\text{IC}_{50} = 10.1$ and $11.8 \mu\text{M}$, respectively).

Amide alkylation by a propyl side chain (compounds **4a–4d**) had a significantly positive impact on the cytotoxic activity. For instance the 6-fluorinated propyl carboxamide **4b** (IC_{50} range: $0.9–22.2 \mu\text{M}$) was more cytotoxic than its primary analog **3b** (IC_{50} range: $10.1–44.2 \mu\text{M}$). Compound **4b** was also the most potent compound against hormone dependent breast cancer cells MCF-7 with an IC_{50} value in the same range as tamoxifen ($\text{IC}_{50} = 0.9$ and $0.39 \mu\text{M}$, respectively). Regarding the non-substituted chromone series ($\text{R}_1 = \text{H}$), alkylation by a 3-ethylphenyl ring also had a positive impact as compound **6a** exhibited IC_{50} values against the four cell lines in the same range as the propyl carboxamide derivative **4a** (IC_{50} range: $5.3–18.0 \mu\text{M}$). However, the positive effect of the 6-fluoro substituent on activity showed with compounds **3b** and **4b** was not observed with derivative **6b**, less active than its 6-brominated and 6-methylated analogs (**6d** and **6e**).

The beneficial effect of fluorine seen with the 6-fluoro chromone series was not observed when the fluorine atom was placed on the phenylcarboxamide side chain (compound **7a**) as the toxicity towards the tested cell lines was not enhanced compared to the chloro (**8a**) and methyl (**9a**) analogs.

The presence of a hydroxyl group in the *para* position of the phenylcarboxamide produced a beneficial effect as compound **10a** showed IC_{50} values from 3.2 to $12.3 \mu\text{M}$. On adding a methyldiethylamino chain in the *meta* position (compound **11a**), only the activity against OVCAR cells was conserved ($\text{IC}_{50} = 5.1 \mu\text{M}$).

Scheme 1 Synthesis of compounds **3–14**



Reactions conditions: i) PCl_5 /dry cyclohexane; ii) $\text{R}_2\text{-NH}_2$ /TEA/dry DC

	R₁	R₂
3a	H	-NH ₂
3b	F	-NH ₂
3c	Cl	-NH ₂
3d	Br	-NH ₂
3e	CH ₃	-NH ₂
4a	H	-NH-C ₃ H ₇
4b	F	-NH-C ₃ H ₇
4d	Br	-NH-C ₃ H ₇
5a	H	-NH-Ph
6a	H	-NH-(3'-C ₂ H ₅)-Ph
6b	F	-NH-(3'-C ₂ H ₅)-Ph
6d	Br	-NH-(3'-C ₂ H ₅)-Ph
6e	CH ₃	-NH-(3'-C ₂ H ₅)-Ph
7a	H	-NH-(4'-F)-Ph
8a	H	-NH-(4'-Cl)-Ph
9a	H	-NH-(4'-CH ₃)-Ph
10a	H	-NH-(4'-OH)-Ph
11a	H	-NH-(4'-OH, 3'-CH ₂ -N(C ₂ H ₅) ₂)-Ph
12a	H	-NH-CH(CH ₃)-(CH ₂) ₃ -N(C ₂ H ₅) ₂
13a	H	-NH-morpholino
14a	H	-N-2-(4-pyrrolidinylcarbonylmethyl)-piperaziny

To widen the panel of structures, we also studied chromone carboxamides bearing various non-aromatic nitrogen-containing side chains: *N,N*-(diethyl)pentanamine, morpholine and *N*-substituted piperazine (**12a–14a**). The impact of these groups on the activity was not the same depending on the cell line but one can note that the IC₅₀ values were kept below 10 μM against at least one cell line.

Overall, while several compounds in the present library possess IC₅₀ values below 10 μM, none of them displays good selectivity, i.e., activity against one cell line 10-fold higher than the three other cell lines.

Anti-inflammatory activity

In order to study the ability of compounds **3–14** to directly inhibit the catalytic activity of 5-LOX, a cell-free assay using purified 5-LOX enzyme in the presence of 100 μM of the chromone derivative was applied, according to the method described by Baylac and Racine (Baylac and

Racine, 2003) with modifications. Among 20 chromone carboxamide derivatives tested as putative inhibitors of the 5-LOX enzyme, 10 were totally inactive or showed low activity (<20 %) and 10 showed moderate to good activity (Table 1).

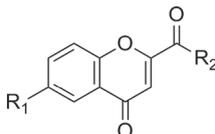
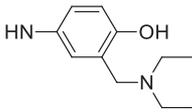
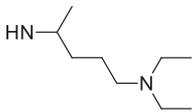
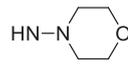
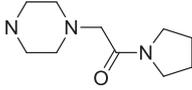
The 6-fluorinated compound **3b** showed interesting inhibition (79.9 %). Here again, the effect of a 6-fluoro substituent was positive for 5-LOX inhibition compared to its 6-Cl, 6-Br, and 6-methyl analogs (**3c–3e**). The presence of the propyl side chain (**4b**) led to a decreased activity (68.0 % instead of 79.9 % for **3b**).

Alkylation with a phenyl moiety, either substituted or not with ethyl, fluoro, chloro, or methyl groups (compounds **6a–e**, **7a–9a**) had a major negative impact on the anti-inflammatory activity. However, using a *para* phenol as alkylating group (compound **10a**) had a positive effect on the anti-inflammatory activity as 5-LOX inhibition increased up to 73.5 % compared to 55.2 % for the primary amide homolog **3a**. The beneficial effect of a *para* phenol

Table 1 In vitro cytotoxicity against four human cancer cell lines and anti-inflammatory activity of compounds **1**, **3–14**

			Antiproliferative activity IC ₅₀ (μM)				Anti-inflammatory activity % inhibition (100 μM) 5-Lipoxygenase
	R ₁	R ₂	MCF-7	OVCAR	IGROV	HCT-116	
1a	H	OH	>100	>100	20.2 ± 4.1	14.4 ± 1.3	ND
3a	H	NH ₂	>100	>100	>100	>100	55.2 ± 4.0
3b	F	NH ₂	44.2 ± 5.0	10.1 ± 1.3	11.8 ± 1.3	33.2 ± 2.2	79.9 ± 6.6
3c	Cl	NH ₂	89.2 ± 13.2	>100	>100	>100	41.8 ± 6.1
3d	Br	NH ₂	>100	>100	>100	>100	15.5 ± 1.2
3e	CH ₃	NH ₂	80.2 ± 13.2	>100	>100	>100	12.9 ± 0.6
4a	H		11.2 ± 1.1	10.1 ± 1.1	5.4 ± 0.3	20.2 ± 1.1	67.2 ± 3.4
4b	F		0.9 ± 0.0	5.1 ± 0.3	3.2 ± 0.1	22.2 ± 2.2	68.0 ± 1.8
4d	Br		12.6 ± 1.0	25.2 ± 2.3	7.2 ± 0.4	4.5 ± 0.2	30.5 ± 5.1
5a	H		>100	>100	>100	>100	ND
6a	H		5.3 ± 0.6	8.0 ± 0.4	18.0 ± 2.0	14.2 ± 0.9	NA
6b	F		56.1 ± 6.4	5.8 ± 0.3	13.2 ± 1.2	32.3 ± 4.4	NA
6d	Br		6.3 ± 0.4	20.1 ± 3.3	8.0 ± 1.2	>100	NA
6e	CH ₃		18.0 ± 2.2	9.2 ± 1.3	5.9 ± 0.0	>100	NA
7a	H		63.2 ± 5.3	>100	18.0 ± 2.1	25.2 ± 3.2	NA
8a	H		>100	>100	22.1 ± 1.2	9.0 ± 0.4	NA
9a	H		>100	>100	25.4 ± 2.2	11.3 ± 1.2	NA
10a	H		12.3 ± 1.0	6.3 ± 0.2	3.2 ± 0.0	9.0 ± 1.3	73.5 ± 3.4

Table 1 continued

	R ₁	R ₂	Antiproliferative activity IC ₅₀ (μM)				Anti-inflammatory activity % inhibition (100 μM) 5-Lipoxygenase
			MCF-7	OVCAR	IGROV	HCT-116	
							
11a	H		34.8 ± 3.2	5.1 ± 0.3	12.0 ± 0.4	40.0 ± 3.0	52.0 ± 4.9
12a	H		40.2 ± 2.0	10.4 ± 1.2	6.8 ± 0.4	36.2 ± 3.1	48.6 ± 2.3
13a	H		8.3 ± 0.1	25.3 ± 2.4	13.1 ± 1.2	5.8 ± 1.1	68.8 ± 4.6
14a	H		6.0 ± 0.5	28.0 ± 2.2	10.1 ± 0.2	31.3 ± 3.3	62.3 ± 2.9
Tamoxifen			0.39 ± 0.01	0.52 ± 0.04	0.82 ± 0.10	0.37 ± 0.02	—
NDGA (13 μM)			—	—	—	—	50.0 ± 2.7

NDGA nordihydroguaiaretic acid, NA not active, ND not determined

was reduced when a *N*-diethylmethyl side chain was added in the *meta* position of the phenyl group (52.0 % for compound **11a**).

Amidation using other nitrogen containing side chains (*N*-diethylpentyl **12a**, morpholinyl **13a**, and substituted piperazinyl **14a**) showed good activity with 5-LOX inhibition from 48.6 % up to 68.8 %.

Theoretical evaluation of “drug-likeness” properties

To predict their overall “drug-likeness”, an in silico computational study of the synthesized chromone carboxamides **3–14** was performed by determining Lipinski parameters (Lipinski et al., 1997) using the molinspiration online software (www.molinspiration.com) (Table 1 of supplementary material). To better understand the therapeutic potential of chromone derivatives, we also calculated their ligand efficiency (LE) for all the cell lines (Table 2 of supplementary material). LE is a measure of the in vitro biological activity corrected for the physico-chemical properties of the molecule and quantify how effectively the

molecule uses its structural features in binding the target. LE is therefore a useful metric to normalize the affinities of hits to identify the best starting points for optimization, i.e., those with the highest LE values (Hopkins et al., 2014).

From the data obtained, one can observe that all the chromone derivatives possess LogP values compatible with those required to cross membranes and no violations of Lipinski’s rules were found (Table 2), thus making our chromone derivatives promising therapeutic agents with a good potential for eventual development as orally active drug candidates. Lipophilicity does seem to play a significant role for 5-LOX inhibition as, except for **11a**, derivatives with logP ≥ 3.01 were totally inactive (compounds **6a–9a**) and the six most potent chromone carboxamides had a logP value < 2.47.

Overall, the lowest IC₅₀ value was displayed by compound **4b** against MCF-7 cell line. In order to see if this compound is the one which should be selected as a lead for further investigations, we calculated the LE values for all compounds against this cell line. Results showed that LE values are in the range 0.36–0.46 kcal/mol/non-H atom.

Table 2 LogP and LE values of the chromone derivatives

N°	LogP ^a	MW	IC ₅₀ (μM)	LE _{MCF-7} ^b
3b	0.04	207.16	44.2	0.40
3c	1.53	223.61	89.2	0.37
3e	1.30	203.19	80.2	0.38
4a	2.13	231.25	11.2	0.40
4b	1.30	249.24	0.9	0.46
4d	2.91	310.14	12.6	0.38
6a	3.84	293.32	5.3	0.33
6b	3.01	311.31	56.1	0.26
6d	4.62	372.21	6.3	0.31
6e	4.26	307.34	18.0	0.28
7a	3.11	283.25	63.2	0.28
10a	2.47	281.26	12.3	0.32
11a	3.42	366.41	34.8	0.23
12a	2.90	330.42	40.2	0.25
13a	0.09	274.27	8.3	0.35
14a	1.14	369.41	6.0	0.27

^a LogP values were determined with molinspiration software (www.molinspiration.com)

^b Ligand efficiency (LE) was calculated from the equation $LE = 1.4 (-\log(IC_{50})/N)$ where “IC₅₀” is the molar concentration required to inhibit MCF-7 cells proliferation by 50 % and “N” is the number of non-hydrogen atoms

The highest LE value (0.46 kcal/mol/non-H atom) was observed for compound **4b**. In addition, this compound also exhibits good LE values for the three other cell lines (0.36–0.42 kcal/mol/non-H atom). As compound **4b** is relatively small, this result means that this molecule can be further improved for enhanced potency.

Conclusion

In this study, 21 chromone carboxamide derivatives were synthesized using simple and affordable chemistry and evaluated for anti-proliferative activity against four human cancer cells. Among these compounds, 13 showed IC₅₀ values below 10 μM against at least one cancer cell line. Compound **4b** was the most promising one with cytotoxic effect against breast cancer cell line MCF-7 equivalent to tamoxifen's. Among the four cancer cell lines, the ovarian IGROV line was the most sensitive to the tested compounds. Chromone carboxamide derivatives with a 6-fluoro substituent on the chromone nucleus (R₁) or propyl or 3-ethylphenyl groups on the amide side chain (R₂) showed better cytotoxic effect. In terms of anti-inflammatory activity, hydrophilic chromone carboxamide derivatives showed greater 5-LOX inhibition.

Overall, the physico-chemical properties (lipophilicity, molecular weight, number of H acceptors/donors) of chromone carboxamides are in accordance with the general requirements of drug discovery and development process and LE values allow further structure optimization, with compound **4b** as a lead. Other compounds such as **6a** or **10a** are also promising compounds. Therefore, this study provides a solid base for further research on chromone carboxamides to get more potent and selective cytotoxic agents.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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