Benzochromenopyrimidines: Synthesis, Antiproliferative Activity against Colorectal Cancer and Physicochemical Properties

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Abstract: Ten new differently substituted 3-benzyl-5-aryl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidin-4,6,11-triones 3 were synthesized by a simple and cost-efficient procedure in a one-pot, three-component reaction from readily available ethyl 2-amino-4-aryl-5,10-dioxo-5,10-dihydro-4H-benzo[g]chromene-3-carboxylates, benzylamine and triethyl orthoformate under solvent- and catalyst-free conditions. All the new compounds were screened for their antiproliferative activity against two colorectal-cancer-cell lines. The results showed that the compounds 3-benzyl-5-phenyl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3a) and 3-benzyl-5-(3-hydroxyphenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3g) exhibited the most potent balanced inhibitory activity against human LoVo and HCT-116 cancer cells.

Keywords: anticancer; anti-colorectal cancer; benzochromenopyrimidines

1. Introduction

Cancer is inherently a genetic disease. The accumulation of hereditary and/or acquired defects in genes that regulate cell proliferation and survival are responsible for the development of cancer [1]. Cancer is one of the leading causes of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths [2]. The most common cancers are breast, lung, colorectal, and prostate. Colorectal cancer (CRC) is the most frequently diagnosed cancer in Europe and the US, and the second leading cause of cancer-related death [3].

Despite the availability of developed drugs, including targeted tumor therapies, the World Health Organization has announced that it is quite possible that the global burden of cancer will continue to increase in the coming years without real effective responses [4]. Therefore, the development of new anti-cancer drugs is a major goal and challenge for modern medicinal chemistry.

Benzog[γ]chromenes bearing the naphthoquinone structural moiety occur in a variety of natural products that show a broad spectrum of biological activities [5–8]. On the other hand, several synthetic benzog[γ]chromene derivatives have received growing interest in the pharmaceutical industry and in the study of organic synthesis due to their various biological and pharmacological properties, including antimicrobial [9,10], antileishmanial [11,12], and anticancer activities [13–17]. Pyrimidines and fused pyrimidines are also known to be preferred structures with diverse biological activities, and many of them are used as antimicrobial [18–21], anti-inflammatory [22,23], and anticancer therapeutic agents [24–29].
The association of these two scaffolds in a single molecule could create a synergistic effect in terms of activity and better drug-likeness properties.

This type of fused system has already been reported in the literature as an anti-Alzheimer’s [30,31], antioxidant [32], or antibacterial agent [33–35].

In light of the above considerations, herein, we describe the synthesis of novel 3-benzyl-5-aryl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidin-4,6,11-triones 3 (Scheme 1) bearing both biologically active benzo[g]chromene and pyrimidine functional motifs, by a simple and cost-effective procedure, in a one-pot, three-component reaction. All the synthesized products were evaluated as inhibitors of colon-cancer-cell proliferation.

![Scheme 1. Synthesis of benzochromenopyrimidines 3a–j.](image)

### 2. Results

#### 2.1. Synthesis

The synthesis of racemic benzochromenopyrimidine derivatives 3 was achieved by using a synthetic route shown in Scheme 1. In the first step, the preparation of the precursor ethyl 2-amino-4-aryl-5,10-dioxo-5,10-dihydro-4H-benzo[g]chromene-3-carboxylates was performed by mixing commercial 2-hydroxy-1,4-naphthoquinone 1 with ethyl 2-cyano-3-arylacylates in the presence of a catalytic amount of triethylamine, at reflux [36–39]. Next, the one-pot condensation between compounds 2a–j, benzylamine, and triethyl orthoformate, which were used as both reagent and solvent, at reflux, led to the corresponding 3-benzyl-5-aryl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-triones 3a–j (Table 1). The desired new compounds were obtained from modest-to-good yields (37–60%), and their analytical and spectroscopic data were in good agreement with their structures. In particular, the $^1$H NMR spectra showed the appearance of two doublets attributed to the methylene group protons –CH$_2$–Ph between 4.95 and 5.15 ppm, with coupling constants for a typical AB system due to the magnetic non-equivalence of the methylene-group protons (see Supplementary Data).

Interestingly, we can note that the nature of the aryl group can have a significant impact on the yield of the reaction.

Indeed, a chlorine in position 2 of the aryl group decreased the yield from 59 to 43%, compared to the absence of substituent. This effect was enhanced when the aryl group bore a chlorine in position 2 and another in position 4, where the yield decreased, this time to 37%.

On the other hand, no significant effect was observed when the different substituents were in position 3 or 4, including chlorine.
Table 1. Synthesis of benzo[6,7]chromeno[2,3-d]pyrimidine derivatives under one-pot solvent- and catalyst-free conditions.

| Entry | Product | Ar          | Time (h) | Yield (%) |
|-------|---------|-------------|----------|-----------|
| 1     | 3a      | C₆H₅       | 3        | 59        |
| 2     | 3b      | 4-CH₃C₆H₄  | 4        | 53        |
| 3     | 3c      | 4-OCH₃C₆H₄ | 3        | 60        |
| 4     | 3d      | 4-ClC₆H₄   | 4        | 55        |
| 5     | 3e      | 2-ClC₆H₄   | 5        | 43        |
| 6     | 3f      | 2,4-Cl₂C₆H₃| 5        | 37        |
| 7     | 3g      | 3-OH₂C₆H₄  | 3        | 57        |
| 8     | 3h      | 4-NO₂C₆H₄  | 5        | 50        |
| 9     | 3i      | 3-NO₂C₆H₄  | 4        | 52        |
| 10    | 3j      | 4-FC₆H₄    | 3        | 51        |

2.2. Biological Evaluation

2.2.1. Cytotoxicity Test

The ten newly synthesized benzochromenopyrimidines 3a–j were evaluated for their in vitro antiproliferative activity against two representative cell lines of human colon cancer, LoVo and HCT-116, using the standard 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The cytotoxicity of each compound was assessed at different concentrations of 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.12 µM, 1.5 µM, 0.8 µM, and 0.4 µM. Oxaliplatin and 5-FU, the most common chemotherapy drugs used to treat colorectal cancer, were used as standards. The curve of the cell survival of LoVo and HCT-116 after treatment was obtained by the relation plotting of surviving fraction and drug concentration. All the results showed that the concentration of the cytotoxic compounds 3a–j was inversely proportional to the percentage of cell viability. The value of concentration required to inhibit 50% of the cell viability (IC₅₀) was determined and compared to those of the standard drugs, as shown in Table 2.

Table 2. Results of in vitro cytotoxic activity of the synthesized compounds on human-colon-cancer-cell lines (LoVo and HCT-116) *.

| Compounds      | LoVo IC₅₀ (µM) | HCT-116 IC₅₀ (µM) |
|----------------|---------------|-------------------|
| 3a             | 14.99 ± 0.59  | # 15.92 ± 0.30    |
| 3b             | 19.55 ± 3.17  | 53.93 ± 7.52      |
| 3e             | 24.35 ± 3.30  | 40.32 ± 3.28      |
| 3f             | 30.11 ± 3.16  | 42.65 ± 3.41      |
| 3g             | 18.49 ± 0.57  | # 13.70 ± 0.74    |
| 3h             | 39.32 ± 5.13  | 56.03 ± 0.94      |
| 3i             | 47.12 ± 3.83  | 16.45 ± 2.32      |
| 3j             | 33.14 ± 2.18  | # 7.15 ± 0.67 *   |
| Oxaliplatin    | 39.03 ± 2.19  | 23.85 ± 3.08      |
| 5-FU           | 19.51 ± 2.35  | 13.48 ± 1.78      |

*The results are expressed as the mean number of IC₅₀ ± SD (n = 3), (*) and (#) indicates a significant difference between standards (oxaliplatin and 5-FU respectively) and the synthesized compounds (at p ≤ 0.05, ANOVA; post hoc Dunnett test).

Several benzochromenopyrimidines showed good activities compared to the oxaliplatin and 5-FU against both types of cancer-cell line.

Concerning the LoVo cell lines, four compounds (3a: IC₅₀ = 14.99 µM, 3b: IC₅₀ = 19.55 µM, 3e: IC₅₀ = 18.49 µM and 3g: IC₅₀ = 11.79 µM) showed activity that was better than or comparable to those of the two standards. In particular, compound 3g, which showed an IC₅₀ equal to 11.79 µM, was 1.7 times more active than the oxaliplatin and 5-FU. Thus, from
the point of view of the structure–activity relationship (SAR), several conclusions can be drawn: (i) It is clear that the cytotoxic effect was related to the nature of the substituent in the aryl group. (ii) With the exception of compound 3b, the substitution in position 4 of the aryl group did not enhance the activity. (iii) The two most promising compounds were the chlorine in position 2 and the hydroxy in position 3. This suggests that for better activity, a polar group in position 2 or 3 is necessary.

For the HCT-116 lines, six compounds showed better activity than the 5-FU (3a: IC50 = 15.92 µM, 3e: IC50 = 13.70 µM, 3g: IC50 = 13.61 µM, 3h: IC50 = 16.45 µM, 3i: IC50 = 7.15 µM and 3j: IC50 = 23.85 µM), of which three (3e: IC50 = 13.70 µM, 3g: IC50 = 13.61 µM and 3i: IC50 = 7.15 µM) also showed activity that was better than or comparable to that of oxaliplatin (IC50 = 13.48 µM).

In this case, the SAR was also related to the nature of the aryl group. The comparison of the results of these compounds with oxaliplatin showed that the substitution in position 4 did not favor the activity. This unfavorable effect was related to the nature of the substituent (CH3 > OCH3 > Cl > F > NO2).

According to these results, the most balanced compounds were 3a and 3g, with an IC50 equal to 14.99 µM and 11.79 µM, respectively, against the Lovo cell lines, and 15.92 µM and 13.61 µM, respectively, against the HCT-116 cell lines. Compounds 3a and 3g were, thus, more active than oxaliplatin and 5-FU against the LoVo cell lines and showed activities that were almost comparable to those of the oxaliplatin and two-fold more active than those of the 5-FU against the HCT-116 cell line.

2.2.2. ADME Studies

Next, the physicochemical properties of the synthesized compounds were investigated by Data Warrior software, a chemical- and biological-data-visualization-and-analysis tool developed by Actelion/Idorsia Pharmaceuticals Ltd. (Table 3). This software utilizes different parameters of Lipinski’s rule of five (molecular weight, LogP, LogS, H-donors, H-acceptors, topological polar surface (TPSA) for the analysis of drug-like properties. All the compounds showed suitable MW values (MW < 500) for the pharmacokinetics of a drug in the human body with the exception of compound 3f, which had a slightly higher value, 515.351 g/mol.

Table 3. Physicochemical properties of the synthesized compounds calculated by Data Warrior.

| Name | Molweight (g/mol) | CLogP | CLogS | H-Donors | H-Acceptors | Lipinsky Violations | Drug-Likeness | TPSA (Å²) |
|------|-------------------|-------|-------|----------|-------------|---------------------|--------------|------------|
| 3a   | 446.461           | 4.237 | −7.271| 0        | 6           | 0                   | 4.8734       | 76.04      |
| 3b   | 460.488           | 4.5809| −7.615| 0        | 6           | 0                   | 4.83         | 76.04      |
| 3c   | 476.487           | 4.167 | −7.289| 0        | 7           | 0                   | 4.9122       | 85.27      |
| 3d   | 480.906           | 4.843 | −8.007| 0        | 6           | 0                   | 4.9183       | 76.04      |
| 3e   | 480.906           | 4.843 | −8.007| 0        | 6           | 0                   | 4.9183       | 76.04      |
| 3f   | 515.351           | 5.449 | −8.743| 0        | 6           | 0                   | 4.9183       | 76.04      |
| 3g   | 462.46            | 3.8913| −6.975| 1        | 7           | 0                   | 4.8783       | 96.27      |
| 3h   | 491.458           | 3.3154| −7.731| 0        | 9           | 0                   | −0.18075     | 121.86     |
| 3i   | 491.458           | 3.3154| −7.731| 0        | 9           | 0                   | −0.18075     | 121.86     |
| 3j   | 464.451           | 4.3378| −7.585| 0        | 6           | 0                   | 3.5334       | 76.04      |

MW < 500; LogP < 5; LogS > −4; H-donors < 5; H-acceptors < 10; Drug-likeness > 0; TPSA < 140 Å².

Lipophilicity is one of the properties of compounds that determine whether a molecule will cross the biological membrane, of which Log P (less than 5) is an important physicochemical example. Interestingly most of the compounds showed good lipophilicity, with Log P values between 3.3154 and 4.843. Only compound 3f, bearing two chlorines on
the aromatic ring, showed a Log P higher than 5, with a value equal to 5.449. This value remained lower than 6.5, which is the upper limit for druggable compounds, indicating that 3f was slightly lipophilic. Since lipophilicity plays a crucial role in determining the solubility of drug candidates in biological systems, we also calculated the Log S values of these compounds. All the compounds showed low aqueous solubility, suggesting reduced bioavailability. Structural modifications could be considered by introducing more polar groups to improve hydrophilicity and, therefore, duggability. Introducing an additional hydroxyl in position 2 of the aryl group of compound 3g could be an option to enhance the solubility without a loss of activity. Indeed, the SAR showed that the presence of a polar group in 2 and 3 seems to be a necessary condition for the activity. Nevertheless, we can introduce polar groups, such as halogens or hydroxyls, on the aromatic rings of benzyl or benzochromene and study their impact on drugability and biological activity.

The number of donor and acceptor hydrogen bonds was also in agreement with Lipinski’s rule of five. Indeed, for all the compounds, the number of donor hydrogen bonds was lower than 5 and the number of acceptors was lower than 10. It can be noted, however, that compounds 3h and 3i had slightly more hydrogen acceptors than their analogues. Data Warrior also calculates drug-likeness as a qualitative concept to predict whether synthesized compounds are drug-like. This parameter is calculated by using several data, such as LogP, LogS, and molar mass, as well as other parameters, such as the presence of structures with specific pharmacological properties (such as enones, which can be mutagenic and carcinogenic). It can be noted that all the compounds had an interesting drug-likeness prediction, except for compounds 3h and 3i, for which it was <0.

The TPSA corresponded to the Van der Waals surface of the molecules’ polar atoms (usually oxygen and nitrogen) and their attached hydrogens. The polar surface area was no greater than 140 Å², as suggested by Veber’s Rule. Interestingly, all the compounds had a TPSA < 100 Å², except for compounds 3h and 3i, for which it was 121.86 Å².

3. Materials and Methods

Melting points (°C) were determined with a Kofler hot bench and were uncorrected. Analytical thin-layer chromatography (TLC) on silica-gel precoated aluminum sheets (Type 60 F254, 0.25-mm thickness; from Merck, Darmstadt, Germany) was employed to follow the progress of the reactions and to check the purity and homogeneity of the synthesized products. Nuclear-magnetic-resonance spectra (NMR) were recorded on a Bruker DRX-400 Avance spectrometer (at 400 MHz for 1H and 100 MHz for 13C), using dimethylsulfoxide (DMSO-d6) as the solvent and tetramethylsilane (TMS) as internal standard. The chemical shifts are expressed in parts per million (ppm) and the multiplicities of 1H NMR signals were designated as follows: s: singlet; d: doublet; t: triplet; q: quartet; and m: multiplet. Coupling constants were expressed in hertz (Hz). High-resolution mass spectra (HRMS) were carried out by using a Bruker micrOTOF-Q II spectrometer (Bruker Daltonics) in positive electrospray ionization time-of-flight at UCA Clermont Ferrand, France.

3.1. Synthesis of Compounds 2a–j

3.1.1. General Procedure for the Synthesis of Ethyl 2-Amino-4-(3-hydroxyphenyl)-5,10-dioxo-5,10-dihydro-4H-benzo[g]chromene-3-carboxylates (2a–j)

An equimolar mixture of ethyl 2-cyano-3-arylacrylates and 2-hydroxy-1,4-naphthoquinone 1 was dissolved in 30 mL of ethanol in the presence of triethylamine as a catalyst. The reaction mixture was heated at reflux for 2 h. The resulting precipitate was collected by filtration and recrystallized from ethanol.

All compounds 2a–j were previously described in the following studies: 2a–c and 2i–j [40], 2d [37], 2e [41], 2f [38] and 2g [42].

3.1.2. General Procedure for the Synthesis of 3-Benzyl-5-aryl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidin-4,6,11-triones (3a–j)

A mixture of ethyl 2-amino-4-aryl-5,10-dioxo-5,10-dihydro-4H-benzo[g]chromene-3-carboxylates 2a–j (0.5 g, 1.33 mmol), benzylation (0.14 g, 1.33 mmol), and triethyl
orthoformate (3 mL) was heated under catalyst-free and solvent-free conditions. The completion of the reaction required a time of 3 to 5 h, as highlighted by TLC analysis, leading to the formation of a precipitate, which was collected by filtration, washed with ethanol, and dried. The obtained products 3a–j were characterized by spectroscopic analysis (NMR and HRMS), which showed good agreement with the desired structure.

3-Benzyl-5-phenyl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3a)

Light-yellow solid; yield: 59%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d6) δ 8.73 (s, 1H, CH(pyrimidine)), 8.10–8.07 (m, 1H), 7.93–7.91 (m, 1H), 7.89–7.85 (m, 2H), 7.38 (d, J = 7.4 Hz, 2H), 7.32–7.24 (m, 7H), 7.19–7.15 (m, 1H), 5.14 (d, J = 14.7 Hz, 1H, CH2), 5.13 (s, 1H, CH(phenyl)), 4.98 (d, J = 14.7 Hz, 1H, CH2); 13C NMR (100 MHz, DMSO-d6) δ 183.0, 177.5, 160.4, 159.6, 152.1, 149.9, 139.5, 136.8, 136.4, 135.1, 134.7, 131.5, 131.0, 129.3 (2C), 129.1 (2C), 129.0 (2C), 128.7 (2C), 128.3, 128.2 (2C), 127.5, 126.6, 126.3, 123.3, 103.7, 49.8, 34.3. HRMS (ESI, M + H+) Calcd for C28H19O2N4: 447.1345. Found: 447.1339.

3-Benzyl-5-(p-tolyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3b)

Olive-green solid; yield: 53%; mp 252–4 °C; 1H NMR (400 MHz, DMSO-d6) δ 8.71 (s, 1H, CH(pyrimidine)), 8.09–8.07 (m, 1H), 7.90–7.86 (m, 3H), 7.30–7.24 (m, 7H), 7.05 (d, J = 7.1 Hz, 2H), 5.12 (d, J = 14.7 Hz, 1H, CH2), 5.08 (s, 1H, CH(pyran)), 4.98 (d, J = 14.7 Hz, 1H, CH2), 2.20 (s, 3H, CH3); 13C NMR (100 MHz, DMSO-d6) δ 183.0, 177.5, 160.4, 159.6, 152.1, 149.9, 139.5, 136.8, 136.4, 135.1, 134.7, 131.5, 131.0, 129.3 (2C), 129.1 (2C), 129.0 (2C), 128.3, 128.2 (2C), 126.6, 126.3, 123.5, 100.8, 49.8, 34.3, 21.0. HRMS (ESI, M + H+) Calcd for C29H21O2N4: 461.1501. Found: 461.1494.

3-Benzyl-5-(4-methoxyphenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3c)

Ochre-yellow solid; yield: 60%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d6) δ 8.71 (s, 1H, CH(pyrimidine)), 8.09–8.07 (m, 1H), 7.92–7.86 (m, 3H), 7.31 (m, 7H), 6.80 (d, J = 7.6 Hz, 2H), 5.12 (d, J = 14.2 Hz, 1H, CH2), 5.07 (s, 1H, CH(pyran)), 4.98 (d, J = 14.2 Hz, 1H, CH2), 3.68 (s, 3H, OCH3); 13C NMR (100 MHz, DMSO-d6) δ 183.1, 177.5, 160.4, 159.5, 158.7, 152.0, 149.8, 136.4, 135.1, 134.7, 134.6, 131.5, 131.1, 130.2 (2C), 129.1 (2C), 128.3, 128.2 (2C), 126.6, 126.3, 123.4, 114.1 (2C), 103.8, 55.4, 49.8, 33.9. HRMS (ESI, M + H+) Calcd for C28H21O2N4: 477.1450. Found: 477.1445.

3-Benzyl-5-(4-chlorophenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3d)

Pale yellow solid; yield: 55%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d6) δ 8.73 (s, 1H, CH(pyrimidine)), 8.08 (m, 1H), 7.90–7.86 (m, 3H), 7.42 (d, J = 6.7 Hz, 2H), 7.31 (m, 7H), 5.14–5.11 (m, 2H, CH2, CH(pyran)), 4.98 (d, J = 14.6 Hz, 1H, CH2); 13C NMR (100 MHz, DMSO-d6) δ 183.0, 177.4, 160.4, 159.6, 152.3, 150.2, 141.4, 136.4, 135.0, 134.7, 132.1 (2C), 131.4, 131.1 (2C), 129.0 (2C), 128.6 (2C), 128.3, 128.2 (2C), 128.6, 126.3, 122.6, 103.2, 49.8, 34.5. HRMS (ESI, M + H+) Calcd for C28H18ClN2O4: 481.0955. Found: 481.0948.

3-Benzyl-5-(2-chlorophenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3e)

Red solid brick; yield: 43%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d6) δ 8.73 (s, 1H, CH(pyrimidine)), 8.08–8.06 (m, 1H), 7.88–7.84 (m, 3H), 7.43–7.41 (m, 2H), 7.34–7.18 (m, 7H), 5.48 (s, 1H, CH(pyran)), 5.10 (d, J = 14.6 Hz, 1H, CH2), 4.95 (d, J = 14.6 Hz, 1H, CH2); 13C NMR (100 MHz, DMSO-d6) δ 182.9, 177.5, 160.1, 159.8, 152.4, 150.2, 139.8, 136.4, 131.5, 134.7, 133.9, 132.7, 131.4, 130.8, 129.8 (2C), 129.1 (2C), 128.3, 128.0 (2C), 127.6, 126.5, 126.4, 122.5, 102.9, 49.7, 33.5. HRMS (ESI, M + H+) Calcd for C28H18ClN2O4: 481.0955. Found: 481.0948.
3-Benzyl-5-(2,4-dichlorophenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3f)

Ochre-yellow solid; yield: 37%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.74 (s, 1H, CH$_3$pyrimidine), 8.08–8.06 (m, 1H), 7.88–7.85 (m, 3H), 7.49–7.44 (m, 2H), 7.33–7.26 (m, 6H), 5.46 (s, 1H, CH$_3$pyran), 5.11 (d, J = 14.7 Hz, 1H, CH$_2$), 4.96 (d, J = 14.7 Hz, 1H, CH$_2$); 13C NMR (100 MHz, DMSO-d$_6$) δ 183.0, 177.4, 160.1, 159.7, 152.4, 150.2, 139.1, 136.2, 135.3, 134.9, 134.8, 133.7, 132.7, 131.3, 130.7, 129.1 (2C), 129.0, 128.3, 128.0 (2C), 127.7, 126.6, 126.4, 122.0, 102.6, 49.7, 33.1. HRMS (ESI, M + H$^+$) Calcd for C$_{28}$H$_{17}$Cl$_2$N$_2$O$_4$: 515.0565. Found: 515.0559.

3-Benzyl-5-(3-hydroxyphenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3g)

Red solid brick; yield: 57%; mp 200–2 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 9.37 (s, 1H, OH), 8.74 (s, 1H, CH$_3$pyrimidine), 8.07 (m, 1H), 7.93–7.85 (m, 3H), 7.34–7.31 (m, 5H), 7.05–7.02 (m, 1H), 6.80–6.76 (m, 2H), 6.56 (d, J = 7.9 Hz, 1H), 5.15 (d, J = 14.6 Hz, 1H, CH$_2$), 5.04 (s, 1H, CH$_3$pyran), 5.00 (d, J = 14.6 Hz, 1H, CH$_2$); 13C NMR (100 MHz, DMSO-d$_6$) δ 183.0, 177.5, 160.4, 159.7, 157.6, 152.1, 149.9, 143.6, 136.4, 135.1, 134.7, 131.4, 130.7, 129.1 (2C), 128.3, 128.2 (2C), 126.6, 126.4, 123.5, 119.7, 116.1, 114.6, 103.7, 49.8, 34.5. HRMS (ESI, M + H$^+$) Calcd for C$_{28}$H$_{19}$N$_2$O$_3$: 463.1294. Found: 463.1286.

3-Benzyl-5-(3-nitrophenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3h)

Ochre-yellow solid; yield: 50%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.73 (s, 1H, CH$_3$pyrimidine), 8.10–8.08 (m, 3H), 7.87–7.81 (m, 3H), 7.70 (d, J = 8.5 Hz, 2H), 7.32–7.27 (m, 5H), 5.21 (s, 1H, CH$_3$pyran), 5.09 (d, J = 14.7 Hz, 1H, CH$_2$), 5.47 (d, J = 14.7 Hz, 1H, CH$_2$); 13C NMR (100 MHz, DMSO-d$_6$) δ 183.0, 177.3, 160.4, 159.7, 152.6, 150.4, 149.7, 146.9, 136.2, 135.1, 134.8, 131.3, 131.0, 130.8 (2C), 129.1 (2C), 128.4, 128.2 (2C), 126.6, 126.3, 123.7 (2C), 122.0, 102.6, 49.9, 35.3. HRMS (ESI, M + H$^+$) Calcd for C$_{28}$H$_{18}$N$_2$O$_3$: 492.1196. Found: 492.1192.

3-Benzyl-5-(3-nitrophenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3i)

Orange solid; yield: 52%; mp 258–260 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.76 (s, 1H, CH$_3$pyrimidine), 8.24 (s, 1H), 8.06–8.04 (m, 2H), 7.89–7.85 (m, 4H), 7.56 (m, 1H), 7.29 (m, 5H), 5.25 (s, 1H, CH$_3$pyran), 5.12 (d, J = 14.7 Hz, 1H, CH$_2$), 4.98 (d, J = 14.7 Hz, 1H, CH$_2$); 13C NMR (100 MHz, DMSO-d$_6$) δ 183.0, 177.3, 160.4, 159.7, 152.7, 150.5, 148.0, 144.4, 136.3, 136.1, 135.0, 134.7, 131.4, 131.2, 130.1, 129.0 (2C), 128.3, 128.2 (2C), 126.6, 126.3, 124.0, 122.6, 121.8, 102.8, 49.9, 35.2. HRMS (ESI, M + H$^+$) Calcd for C$_{28}$H$_{18}$N$_2$O$_3$: 492.1196. Found: 492.1190.

3-Benzyl-5-(4-fluorophenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3j)

Ochre-yellow solid, yield: 51%; mp > 260 °C; 1H NMR (400 MHz, DMSO-d$_6$) δ 8.73 (s, 1H, CH$_3$pyrimidine), 8.09–8.07 (m, 1H), 7.93–7.83 (m, 3H), 7.45–7.42 (m, 2H), 7.33–7.26 (m, 5H), 7.09–7.05 (m, 2H), 5.13 (d, J = 14.7 Hz, 1H, CH$_2$), 5.12 (s, 1H, CH$_3$pyran), 4.99 (d, J = 14.7 Hz, 1H, CH$_2$); 13C NMR (100 MHz, DMSO-d$_6$) δ 183.1, 177.4, 160.4, 159.6, 152.3, 150.1, 138.6, 136.4, 135.0, 134.7, 131.4 (2C), 131.2, 131.1, 129.0 (2C), 128.3, 128.2 (2C), 126.6, 126.3, 122.9, 115.5, 115.3, 103.5, 49.8, 34.2. HRMS (ESI, M + H$^+$) Calcd for C$_{28}$H$_{18}$FN$_2$O$_4$: 465.1251. Found: 465.1244.

3.2. Biological Evaluation
3.2.1. Materials and Methods

Two colon-cancer-cell lines (LoVo and HCT-116) were used in this study. Cells were cultured in RPMI-1640 media supplemented with 10% FBS and penicillin streptomycin. They were grown in a humidified incubator with 5% of CO$_2$ at 37 °C.
3.2.2. Cytotoxic Activity by MTT Assay

The synthesized compounds were solubilized in the DMSO as stock solutions (100 mM) and serial dilutions were prepared with cell-culture media just prior to use. A 96-well plate was taken and seeded with 5000 cells/well, after which it was incubated overnight in an incubator at 37 °C with 5% of CO₂. Next, the treatments were performed in triplicates and the plate was placed back in the incubator for 48h. After 48 h, the medium was removed and 100 µL of MTT was added into each well and incubated for 24 h. Subsequently, the MTT containing medium was removed from the wells. A total of 100 µL of SDS 10% was added into each well to dissolve the formazan crystals from the cells. Next, the plate was analyzed on micro plate reader (Varioskan Thermo Fisher) after 4 h. The absorbance was measured for each well with a wavelength of 570 nm. The IC₅₀ values were then calculated.

3.3. Statistical Data Analyses

All experiments were performed in triplicate. Data were expressed as mean ± SD. Statistical analyses were performed by Student’s test. The normality and Leven’s test for homogeneity of variances were applied prior to one-way analysis of variance (ANOVA) and multiple mean comparisons were performed with Duncan’s test at p values ≤ 0.05 to investigate the significance differences in factors between synthesized compounds and standards (oxaliplatin and 5-FU) at a confidence level of 95%.

4. Conclusions

In the present study, a new series of benzochromenopyrimidine derivatives 3 was synthesized in a single step, by reacting ethyl 2-amino-4-aryl-5,10-dioxo-5,10-dihydro-4H-benzo[g]chromene-3-carboxylates 2 with benzylamine and triethyl orthoformate, both of which were readily available, without a solvent or catalyst.

The evaluation of the newly synthesized compounds for antitumor activity against the human-colon-cancer-cell lines LoVo and HCT-116 exhibited good results. Among the tested compounds, 3-benzyl-5-(3-hydroxyphenyl)-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3g) showed strong activity against the LoVo cell line with an IC₅₀ value equal to 11.79 µM, and 3-benzyl-5-phenyl-3,5-dihydro-4H-benzo[6,7]chromeno[2,3-d]pyrimidine-4,6,11-trione (3a) also exhibited high antitumor activity against towards the LoVo cell line, with an IC₅₀ value of 14.99 µM, comparing very well with standards, oxaliplatin and 5-FU. In addition, ligands 3a and 3g showed good activities against the HCT-116 cell line, with IC₅₀ equal to 15.92 µM and 13.61 µM, respectively.

Interestingly, both compounds showed suitable physicochemical properties according to the drug-likeness score for druggability predicted by the Data Warrior software.

In summary, this preliminary study revealed that compounds 3a and 3g may be promising agents for further research into the treatment of colon cancer. It should be noted that products 3a and 3g were obtained in racemic form and that their antiproliferative activities could be attributed to one of the enantiomers.

Therefore, work is currently underway in our laboratories to develop analogues with better pharmacological profiles by identifying the contribution of each enantiomer to the biological activity. The results will be reported in due course.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27227878/s1, Supplementary Data S1: The NMR and HRMS spectras.

Author Contributions: E.C. carried out the synthesis of the molecules. F.E. and D.M. performed the biological study. P.J.B. provided the Physicochemical properties. S.A. supervised the biological assays and edited the manuscript. F.C. and J.M.-C. supervised the project and edited the manuscript and L.I. supervised the project and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.
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