Green Synthesis of Spiro Compounds with Potential Anticancer Activity through Knoevenagel/Michael/Cyclization Multicomponent Domino Reactions Organocatalyzed by Ionic Liquid and Microwave-Assisted

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Abstract: In this work a microwave-assisted Knoevenagel/Michael/cyclization multicomponent domino methodology, using ethanol as solvent and the ionic liquid 1-methylimidazolium chloride as catalyst was developed for the synthesis of spiro compounds. The reaction conditions considered ideal were determined from a methodological study varying solvent, catalyst, amount of catalyst, temperature, and heating mode. Finally, the generality of the methodology was evaluated by exploring the scope of the reaction, varying the starting materials (isatin, malononitrile, and barbituric acid). Overall, the twelve spiro compounds were synthesized in good yields (43–98%) and the X-ray structure of compound 1b was obtained. In addition, the in vitro antiproliferative activities of the spirocycles against four types of human cancer cell lines including HCT116 (human colon carcinoma), PC3 (prostate carcinoma), HL60 (promyelocytic leukemia), and SNB19 (astrocytoma) were screened by MTT-based assay. It is noteworthy that spiro compound 1c inhibited the four cell lines tested with the lowest IC_{50} values: 52.81 µM for HCT116, 74.40 µM for PC3, 101 µM for SNB19, and 49.72 µM for HL60.

Keywords: spiro compound; domino reaction; multicomponent reaction; ionic liquid; anticancer activity

1. Introduction

Spiro compounds are organic substances formed by at least two rings linked through only one atom, called a spiro-atom. These molecules are present in many natural products isolated from various sources [1-5]. Furthermore, they have a wide range of biological activities [6] and, therefore, have attracted the attention of many researchers as a primary framework for the discovery of new drugs.

However, the synthesis of spiro compounds is a big challenge for synthetic organic chemists, given their 3D structural properties, conformational rigidity, and intrinsic complexity. In this sense, domino reactions have gained prominence in the literature to obtain this class of compounds [7], because they are direct processes that generally have a simplified operating procedure, reduced reaction time, lower waste generation, and lower cost [8].
Furthermore, as in the present work, in many cases, domino synthesis requires more than two starting materials, which participate simultaneously in the reaction. Thus, these reactions are like a branch of multicomponent reactions. Therefore, the advantages of multicomponent processes also apply to domino sequences, namely: the generation of structurally diverse products, varied possibilities of synthesis, highly convergent routes, and better reaction efficiency [8].

Regarding the use of catalysts in the synthesis of spiro compounds, most examples found in the literature involve metal catalysis, organocatalysis, and synergistic methodologies [9–11]. It is worth mentioning that the use of ionic liquids as organocatalysts has become increasingly pungent, although still incipient, given the characteristic physico-chemical properties of these substances, as well as the possibility of altering their solubility, hydrophilic/hydrophobic character, acidity/basicity, and coordination ability from the modification of their cations and anions, enabling the construction of ionic liquids with specific applications in several fields, including catalysis [12,13].

Thus, in the present work, a Knoevenagel/Michael/cyclization multicomponent domino methodology organocatalyzed by ionic liquid and microwave-assisted was used for the synthesis of twelve spiro compounds with good yields (Figure 1).

![Figure 1. Synthesized spiro compounds.](image-url)

2. Results and Discussion

This study started with the optimization of reaction conditions for the synthesis of spiro compounds. Therefore, the synthesis of the 1a, using as starting materials isatin 2a, malononitrile 3a, and barbituric acid 4a, in equimolar concentrations, was taken as a model reaction (Scheme 1).

![Scheme 1. Synthesis of spiro compound 1a.](image-url)
Initially, the evaluation of the best solvent for the reaction was carried out under conventional heating at reflux temperature, using the ionic liquid (IL) 1-methylimidazolium chloride 5 (0.3 mmol) as catalyst. Table 1 shows the solvents used and yields observed after 24 h of reaction. It is noted that higher yields were obtained in polar solvents (Entries 1, 2, and 7), probably due to the better solubility of the starting materials in them, as well as the possible stabilization of the charged reactive species generated in the reaction medium and of the polar transition states of Knoevenagel condensation and Michael addition [14]. As result, the activation energy of the process is reduced, favoring it and, consequently, providing higher conversions. Therefore, the best result was achieved using ethanol as solvent (Entry 7). Then, the study of the catalyst was carried out to evaluate the effectiveness of the IL 1-methylimidazolium chloride, used for the first time as a catalyst in this type of reaction, against other catalysts (Entries 7–11). To our delight, the best result was obtained with the IL. It is worth mentioning that the choice of the other catalysts (Entries 8–11) was based on the literature [15–18]. Once the effectiveness of the IL was confirmed, its stoichiometry was also evaluated (Entries 7 and 12–14). Thus, it was observed that when using a stoichiometry lower than 30 mol%, the reaction yield decreased, therefore, the ideal amount of IL is the one described in entry 7.

Table 1. Results of solvent and catalyst evaluation for the synthesis of 1a.

| Entry | Solvent | Catalyst | Amount of Catalyst (mmol) | Yield (%) |
|-------|---------|----------|---------------------------|-----------|
| 1     | CH₃CN   | IL       | 0.3                       | 81        |
| 2     | H₂O     | IL       | 0.3                       | 71        |
| 3     | Acetone | IL       | 0.3                       | 28        |
| 4     | CH₂Cl₂  | IL       | 0.3                       | 51        |
| 5     | Et₂O    | IL       | 03                        | 39        |
| 6     | Toluene | IL       | 0.3                       | 23        |
| 7     | EtOH    | IL       | 0.3                       | 85        |
| 8     | EtOH    | p-TsOH   | 0.3                       | 80        |
| 9     | EtOH    | Glucose  | 0.3                       | 39        |
| 10    | EtOH    | Citric acid | 0.3                      | 67        |
| 11    | EtOH    | TFA      | 0.3                       | 80        |
| 12    | EtOH    | IL       | 0.2                       | 55        |
| 13    | EtOH    | IL       | 0.1                       | 65        |
| 14    | EtOH    | IL       | 0.05                      | 58        |

p-TsOH: p-toluenesulfonic acid; TFA: trifluoroacetic acid; IL: ionic liquid.

Finally, the effect of the reaction temperature was evaluated, as shown in Table 2. Thus, the previously optimized reaction was carried out at 0 °C, through an ice bath, and at room temperature (Entries 1 and 2). Under these conditions longer reaction times were required, and lower yields were obtained when compared to the reaction under reflux (Entry 3). Finally, to reduce the reaction time, it was decided to carry out the model reaction under microwave irradiation at 80 °C (Entry 4), conditions that were considered optimal for the reaction because they provided the formation of the product after 2 h with 91% yield.

Table 2. Results of the evaluation of temperature and heating mode for the synthesis of 1a.

| Entry | Solvent | Heating | Temperature | Time (h) | Yield (%) |
|-------|---------|---------|-------------|----------|-----------|
| 1     | EtOH    | -       | 0 °C        | 34       | 36        |
| 2     | EtOH    | -       | r.t.        | 72       | 71        |
| 3     | EtOH    | Conventional | Reflux    | 24       | 85        |
| 4     | EtOH    | Microwave | 80 °C      | 2        | 91        |

With the reaction conditions fully optimized, the scope of the reaction was explored. Thus, initially, compound 2 was varied, as shown in Scheme 2, obtaining spirolactams 1a–j in yields from 43 to 98%. The standard reaction was also carried out with the isatin nitrogen atom protected by the di-tert-butyl dicarbonate (Boc) group, compound 2k. However, spiro
compound 1a was formed, probably due to the acidity of the medium promoted by the protic IL 5, resulting in nitrogen deprotection.

\[
\text{2a-k + NC-CN + 4a} \xrightarrow{\text{EtOH, microwave, 80°C, 2h}} \text{1a-j}
\]

Scheme 2. Reaction scope increase with compound isatins (2a–k) variation.

Then, compounds 3b–f (Scheme 3a) were used instead of malonitrile 3a. By replacing malononitrile 3a with ethyl cyanoacetate 3b, the desired spiro compound 1k could be obtained in trace amount. So, it was decided to repeat the reaction under conventional heating to verify if the formation of 1k would occur under such conditions. Surprisingly, the spirocycle was obtained in 36% yield. In addition, in an unprecedented way in the literature, an attempt has been made to replace 3a with dimethyl malonate 3c and diethyl malonate 3d diesters, as well as ethyl acetoacetate 3e and acetylacetone 3f, since such substances are normally used only in place of barbituric acid 4a [18–24]. However, the
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Scheme 3. (a) Reaction scope increase with compound 3 variation; (b) Knoevenagel adduct formation.

Analyzing the pKa values of 3a–f and 4a [25], it is noted that the methylene compounds 3c–f (pKa = 16.4, 16.4, 14.2, and 13.3, respectively), are less acidic than 4a (pKa = 8.4), which justifies the non-formation of spirocycles, but of the Knoevenagel product 6 (Scheme 3b). On the other hand, it is also observed that 3a and 3b (pKa = 11.0 and 13.1, respectively), are equally less acidic than 4a; however, in these cases, the formation of spiro compounds 1a–j and 1k occurred, probably due to greater nucleophilicity of 3a and 3b. This is because the resonance structures of conjugate bases of 3a and 3b are less stabilized than those of 4a, since the delocalization of the negative charge passes through nitrogen atoms, which are less electronegative than the oxygen atoms, through which the negative charge passes in the 4a resonance structures.

Finally, compound 4 was also varied, as shown in Scheme 4. The replacement of barbituric acid 4a by dimedone 4b enabled the formation of spirocycle 1l with a yield of 83%. However, a mixture of substances that was difficult to purify was obtained when Meldrum’s acid 4c was used in place of barbituric acid 4a. This was probably due to the thermal instability of Meldrum’s acid, which at temperatures close to 80 °C and long reaction times undergoes a retro-hetero-Diels-Alder reaction, giving rise to the highly reactive ketene 7, acetone 8, and carbon dioxide 9, as shown in Scheme 5 [26,27]. Another possibility is the degradation of Meldrum’s acid into its precursor, malonic acid, as reported by Dourado (2018) [28].

The proposed mechanism is illustrated for the synthesis of 1a, as described in Scheme 6. Initially, isatylidene–malononitrile intermediate 10 from the Knoevenagel condensation between 2a and 3a is formed. Then, a Michael addition between 4a and 10, followed by cyclization and tautomerization, gives 1a. The catalytic role of 5 is possibly due to the stabilization of the suggested polar transition state 11, as well as the activation of species 4a and 10 participating in the Michael addition step. It is worth mentioning that intermediate 10 could be isolated and characterized, which contributed to support this mechanistic proposal, which is also valid for the other synthesized compounds. In the infrared spectrum of 10, it was possible to highlight a frequency in infrared spectrum at 2230 cm⁻¹, referring to the nitriles present in the structure, as well as at 1619 cm⁻¹, referring to the C=C bond formed due to the Knoevenagel condensation. As expected, in the 1H-NMR spectrum, only the signals referring to the aromatic hydrogens and the NH of the isatin were identified. Finally, in the 13C-NMR spectrum, the presence of signals at 112.0 ppm and 81.0 ppm
referring to the nitrile carbons and the central carbon of malononitrile (added to isatin), respectively, were assigned.

\[
\text{2a} + \text{NC} = \text{CN} + \text{3a} \xrightarrow{\text{IL 5 (30 mol\%)} \text{ EtOH} \text{ microwave, 80°C, 2h}} \text{11-m}
\]

Scheme 4. Reaction scope increase with compound 4 variation.

\[
\text{4c} \xrightarrow{\Delta} \text{H}_2\text{C} = \text{C} = \text{O} + \text{8} + \text{CO}_2
\]

Scheme 5. Retro-hetero Diels-Alder of Meldrum’s Acid.

Scheme 6. Suggested mechanism for the synthesis of spiro compounds 1a–j, 1k, and 1l.
X-ray structure determination of compound **1b** was obtained from methanol under slow evaporation. The crystal structure has been described in the triclinic P1 space group. Three molecules of water of crystallization were observed in the asymmetric unit of the crystal. Crystalline packing is maintained by hydrogen bonding and π-π stacking. Figure 2 shows the Oak Ridge thermal ellipsoid plot (ORTEP) diagram of the compound. Further crystallographic details for the structure reported in this paper may be obtained from the Cambridge Crystallographic Data Center, on quoting the depository numbers CCDC-2213864.

The newly synthesized spiro compounds **1a–l** were screened for their in vitro antiproliferative activities against four types of human cancer cell lines including HCT116 (human colon carcinoma), PC3 (prostate carcinoma), HL60 (promyelocytic leukemia), and SNB19 (astrocytoma) by MTT-based assay. The results are represented as the half maximal inhibitory concentration (IC$_{50}$-µM) values in Table 3.

**Table 3.** Anti-proliferative activities of selected compounds against HCT116, PC3, HL60, and SNB19 cell lines.

| Compounds | HCT116 | PC3   | SNB19 | HL60 |
|-----------|--------|-------|-------|------|
| **1a**    | >200   | >200  | >200  | >200 |
| **1b**    | 89.45  | 110.7 | 124.9 | 77.38|
| **1c**    | 52.81  | 74.40 | 101.9 | 49.72|
| **1d**    | >200   | >200  | >200  | >200 |
| **1e**    | 67.29  | 117.6 | >200  | >200 |
| **1f**    | 165.8  | >200  | >200  | >200 |
| **1g**    | >200   | >200  | >200  | >200 |
| **1h**    | >200   | >200  | >200  | >200 |
| **1i**    | >200   | >200  | >200  | >200 |
| **1j**    | >200   | >200  | >200  | >200 |
| **1k**    | >200   | >200  | >200  | >200 |
| **1l**    | >200   | >200  | >200  | >200 |

Graph representation of inhibitory concentration mean (IC$_{50}$) of the compounds **1b–c** and **1e–g** against HCT116, PC3, HL60, and SNB19 tumoral cell lines are shown in the Figure 3.
Table 3. Anti-proliferative activities of selected compounds against HCT116, PC3, HL60, and SNB19 cell lines.

| Cell Lines | Compounds | HCT116 | PC3 | SNB19 | HL60 |
|------------|-----------|--------|-----|-------|------|
|            | 1a        | >200   | >200| >200  | >200 |
|            | 1b        | 89.45  | 110.7| 124.9 | 77.38|
|            | 1c        | 52.81  | 74.40| 101.9 | 49.72|
|            | 1d        | >200   | >200| >200  | >200 |
|            | 1e        | 67.29  | 117.6| >200  | 72.26|
|            | 1f        | 165.8  | >200| >200  | 117.2|
|            | 1g        | >200   | >200| >200  | 99.07|
|            | 1h        | >200   | >200| >200  | >200 |
|            | 1i        | >200   | >200| >200  | >200 |
|            | 1j        | >200   | >200| >200  | >200 |
|            | 1k        | >200   | >200| >200  | >200 |
|            | 1l        | >200   | >200| >200  | >200 |

Graph representation of inhibitory concentration mean (IC50) of the compounds 1b–c and 1e–g against HCT116, PC3, HL60, and SNB19 tumoral cell lines are shown in the Figure 3.

Figure 3. Inhibitory concentration means (IC50) of the synthesized compounds 1b–c and 1e–g against the (a) HCT116; (b) PC3; (c) HL60; and (d) SNB19 tumoral cell lines.

Unfortunately, the antiproliferative activity of the tested compounds was only moderate. The only spiro compounds that inhibited the four cell lines tested were 1b and 1c, the latter with the lowest IC50 values: 52.81 µM for HCT116; 74.40 µM for PC3; 101 µM for SNB19, and 49.72 µM for HL60.

3. Experimental Session
3.1. Materials and Methods

The necessary reagents and solvents were used without prior purification. The microwave reactor CEM Discover (CEM Corporation, Matthews, NC, USA) was used with a power of 150 W and temperature monitoring through an infrared monitoring system. The reactions were followed by thin-layer chromatography using aluminum coated with silica gel UV254 (250 µm, 20 × 20 cm). The determination of the decomposition/melting point of the synthesized compounds was carried out in the Fisatom 431D digital equipment (Fisatom, São Paulo, Brazil). Infrared spectra were obtained on the Agilent Cary 630 FTIR Spectrometer (Agilent, Santa Clara, CA, USA) using as parameters 16 scans and resolution of length of 4 cm⁻¹, in attenuated total reflectance (ATR) mode, with horizontal zinc selenide (ZnSe) crystal. All analyses were performed in a wavelength range of 4000 to 400 cm⁻¹. The ¹H-NMR spectra of the compounds 6 and 10 were obtained in DMSO-d₆ (Sigma-Aldrich, St. Louis, MO, USA) in a Varian 400 MHz spectrometer (Varian, Palo Alto, Santa Clara, CA, USA) with a 5 mm broadband 1H/X/D probe. The NMR spectra of the compounds 1a, 1h, and 1k were obtained in DMSO-d₆ (Sigma-Aldrich, St. Louis, MO, USA) in a Bruker Advance III 500 MHz (Bruker, Rheinstetten, Germany) equipped with a 5 mm smart BBO probe. The NMR spectra of the compounds 1b–g, 1i, and 1l were obtained in DMSO-d₆ (Sigma-Aldrich, St. Louis, MO, USA) in a Bruker Advance III 400 MHz (Bruker, Rheinstetten, Germany) equipped with a 5 mm BBI probe. Finally, the NMR spectra of the compound 1j were obtained in D₂O (Sigma-Aldrich, St. Louis, MO, USA).
USA) in a Bruker Advance III 600 MHz (Bruker, Rheinstetten, Germany) equipped with a 5 mm TBO probe. Chemical shifts δ were expressed in ppm relative to the TMS. Mass spectra were obtained using a high-resolution spectrometer (model 9.4 T Solarix, Bruker Daltonics, Bremen, Germany), operated in positive and negative ionization mode with ionizing electrospray, ESI(+)−FT-ICR (MS) and ESI(−)−FT-ICR (MS), respectively. The acquisition of FT-ICR MS spectra was performed with resolving power of m/Δm50% ≈ 500,000, where Δm50% is the entire peak with m/z 400 being half the maximum height and mass accuracy < 1 ppm. Infrared, NMR and mass spectra of all synthesized compounds are available in the Supplementary Material of this article.

3.2. General Procedure

In a 10 mL flask were added 1 mmol of 2a−j, 1 mmol of 3a−b, 1 mmol of 4a−b and 0.3 mmol of the ionic liquid 1-methylimidazolium chloride 5 in 6 mL of ethanol. The reaction was kept under stirring and heated by microwave irradiation at 80 °C for 2 h, with a power of 150 W. The solid obtained was filtered and washed with ice-cold acetonitrile.

7′-amino-2,2′,4′-trioxo-1′,2′,3′,4′-tetrahydrospiro[indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (1a); mp: 262−264 °C; IR νmax (cm−1, ATR): 3353 e 3304 (v NH2), 3133 (v NH), 2203 (v nitrile), 1666 (v C=O), 1336 (v C-N); 1H-NMR (500 MHz, DMSO-d6): δH 12.29 (1H, br s, H17), 11.10 (1H, s, H19), 10.46 (1H, s, H7), 7.35 (2H, s, H14), 7.16 (1H, dt, J = 7.7, 1.3 Hz, H2), 7.13 (1H, br d, J = 7.4 Hz, H6), 6.91 (1H, dt, J = 7.5, 0.9 Hz, H1), 6.78 (1H, br d, J = 7.7 Hz, H3); 13C-NMR (126 MHz, DMSO-d6): δC 158.3 (d, J = 236.7 Hz, C1), 153.5 (s, C11), 149.3 (s, C16), 158.2 (s, C12), 153.4 (s, C11), 149.2 (s, C18), 142.1 (s, C4), 133.5 (s, C5), 128.4 (s, C2), 123.7 (s, C6), 121.7 (s, C1), 116.9 (s, C15), 109.2 (s, C3), 86.8 (s, C10), 57.9 (s, C13), 46.7 (s, C9); ESI (+)−FT-ICR MS: [C15H14N4O6]⁺ exp = 322.05812 m/z, calc = 322.05818 m/z (err = 0.19 ppm), [C30H14N10O6]⁺ exp = 645.12356 m/z, calc = 645.12363 m/z (err = 0.01 ppm), [C45H26N5O12]− exp = 968.18860 m/z, calc = 968.18908 m/z (err = 0.05 ppm).

7′-amino-5-bromo-2,2′,4′-trioxo-1′,2′,3′,4′-tetrahydrospiro[indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (1b); mp: 226 °C; IR νmax (cm−1, ATR): 3142 (v NH), 2197 (v nitrile), 1685 (v C=O), 1338 (v C-N), 1116 (v C=O), 1151 (v C-O); 1H-NMR (400 MHz, DMSO-d6): δH 12.30 (1H, br s, H17), 11.14 (1H, s, H19), 10.61 (1H, s, H7), 7.44 (1H, d, J = 2.1 Hz, H6), 7.43 (2H, s, H14), 7.34 (1H, dd, J = 8.2, 2.1 Hz, H2), 6.76 (1H, d, J = 8.2 Hz, H3); 13C-NMR (101 MHz, DMSO-d6): δC 177.4 (s, C8), 161.5 (s, C18), 158.4 (s, C12), 153.6 (s, C11), 149.3 (s, C16), 141.5 (s, C4), 136.0 (s, C5), 131.1 (s, C2), 126.8 (s, C6), 116.9 (s, C15), 113.5 (s, C1), 111.2 (s, C3), 86.3 (s, C10), 57.1 (s, C13), 46.9 (s, C9); ESI (+)−FT-ICR MS: [C15H14BrN5O6]⁺ exp = 399.96852 m/z, calc = 399.96799 m/z (err = −2.31 ppm), [C30H18Br2N5O10]− exp = 800.94508 m/z, calc = 800.943559 m/z (err = −1.90 ppm).

7′-amino-6-bromo-2,2′,4′-trioxo-1′,2′,3′,4′-tetrahydrospiro[indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (1c); mp: 261 °C; IR νmax (cm−1, ATR): 3148 (v NH), 2201 (v nitrile), 1685 (v C=O), 1338 (v C-N), 1124 (v C=O); 1H-NMR (400 MHz, DMSO-d6): δH 12.34 (1H, br s, H17), 11.12 (1H, s, H19), 10.64 (1H, s, H7), 7.44 (2H, s, H14), 7.15 (1H, d, J = 7.9 Hz, H6), 7.10 (1H, dd, J = 7.9, 1.8 Hz, H2), 6.93 (1H, d, J = 1.8 Hz, H3); 13C-NMR (101 MHz, DMSO-d6): δC 177.6 (s, C8), 161.5 (s, C18), 158.4 (s, C12), 153.6 (s, C11), 149.2 (s, C16), 143.8 (s, C4), 132.9 (s, C5), 125.8 (s, C6), 124.4 (s, C1), 121.0 (s, C2), 116.8 (s, C15), 112.0 (s, C3), 86.3 (s, C10), 57.0 (s, C13), 46.5 (s, C9); ESI (+)−FT-ICR MS: [C15H12BrN5O4]⁺ exp = 399.96855 m/z, calc = 399.96799 m/z (err = −2.40 ppm), [C30H16Br2N5O10]− exp = 800.94542 m/z, calc = 800.943559 m/z (err = −2.32 ppm).

7′-amino-5-fluoro-2,2′,4′-trioxo-1′,2′,3′,4′-tetrahydrospiro[indoline-3,5′-pyrano[2,3-d]pyrimidine]-6′-carbonitrile (1d); mp: 210 °C (with decomposition); IR νmax (cm−1, ATR): 3135 (v NH), 2199 (v nitrile), 1685 (v C=O), 1325 (v C-N), 1182 (v C=O); 1H-NMR (400 MHz, DMSO-d6): δH 12.31 (1H, s, H17), 11.14 (1H, s, H19), 10.49 (1H, s, H7), 7.42 (2H, s, H14), 7.16 (1H, dd, J = 8.2, 2.7 Hz, H6), 6.98 (1H, dd, J = 9.6, 8.5, 2.7 Hz, H2), 6.77 (1H, dd, J = 8.5, 4.3 Hz, H3); 13C-NMR (101 MHz, DMSO-d6): δC 177.7 (s, C8), 161.5 (s, C18), 158.4 (s, C12), 158.3 (d, J = 236.7 Hz, C1), 153.5 (s, C11), 149.3 (s, C16), 138.3 (d, J = 1.6 Hz, C4), 135.3 (d, J = 7.7 Hz, C5), 116.8 (s, C15), 114.6 (d, J = 23.4 Hz, C2), 111.7 (d, J = 24.9 Hz, C3).
C6). 109.9 (d, J = 8.0 Hz, C3), 86.4 (s, C10), 57.3 (s, C13), 47.2 (d, J = 1.6 Hz, C9); ESI (-) FT-ICR MS: [C19H20F2N3O4]− exp = 340.04864 m/z, calc = 340.04876 m/z (err = 0.03 ppm); [C30H15F2N10O8]− exp = 681.10497 m/z, calc = 681.10497 m/z (err = −0.26 ppm).

7'-amino-5-ido-2',3',4'-trixo-1',2',3',4'-tetrahydrospiro[indoline-3,5'-pyrano[2,3-d]pyrimidine]-6'-carbonitrile (1e): mp: 236 °C; IR υmax (cm−1, ATR): 3122 (NH), 2201 (v nitrile), 1688 (v C=O), 1340 (v C-N), 1118 (v C-O). 

1H-NMR (400 MHz, DMSO-d6): δH 12.30 (1H, s, H7), 11.14 (1H, s, H19), 2.99 (3H, s, Me); δC 149.3 (s, C16), 139.7 (s, C12), 133.6 (s, C5), 128.7 (s, C6), 124.3 (s, C2), 117.0 (s, C13), 87.0 (s, C7), 61.9 (s, C12), 58.1 (s, C13), 46.7 (s, C9); ESI (-) FT-ICR MS: [C19H24N8O4]− exp = 447.95435 m/z, calc = 447.95482 m/z (err = 0.05 ppm); 

[C32H20N10O4Na]− exp = 896.91744 m/z, calc = 896.91692 m/z (err = −0.08 ppm).

7'-amino-5-methyl-2',3',4'-trixo-1',2',3',4'-tetrahydrospiro[indoline-3,5'-pyrano[2,3-d]pyrimidine]-6'-carbonitrile (1f): mp: 200–221 °C; IR υmax (cm−1, ATR): 3261 (v NH), 2199 (v nitrile), 1688 (v C=O), 1390 (v C-N), 1111 (v C=O); 

1H-NMR (400 MHz, DMSO-d6): δH 12.27 (1H, s, H7), 11.10 (1H, s, H19), 10.36 (1H, s, H7), 7.33 (2H, s, H4), 6.94–6.98 (2H, m, H2, H6), 6.67 (1H, d, J = 7.92 Hz, H3), 2.21 (3H, s, Me); 

13C-NMR (101 MHz, DMSO-d6): δC 177.7 (s, C8), 161.4 (s, C18), 158.2 (s, C12), 153.3 (s, C13), 149.3 (s, C16), 133.6 (s, C5), 130.6 (s, C1), 128.7 (s, C6), 124.3 (s, C2), 117.0 (s, C13), 87.0 (s, C7), 58.1 (s, C13), 46.7 (s, C9), 20.6 (s, Me); ESI (-) FT-ICR MS: [C16H19N2O4]− exp = 336.07381 m/z, calc = 336.07383 m/z (err = 0.06 ppm); [C32H20N10O4Na]− exp = 695.13716 m/z, calc = 695.13688 m/z (err = −0.04 ppm).
126.3 (s, C1, C2), 126.0 (s, C10), 122.5 (s, C15), 62.1 (s, C13), 38.5 (s, C8); ESI (+) FT-ICR MS: [C_{16}H_{20}N_{10}O_{5}]^{+} \text{ exp } = 335.04216 \text{ m/z, calc } = 335.04219 \text{ m/z (err = 0.09 ppm)}, [C_{22}H_{17}N_{5}S_{2}O_{4}]^{+} \text{ exp } = 671.09176 \text{ m/z, calc } = 671.09166 \text{ m/z (err = −0.15 ppm)}.

Ethyl 7′-amino-2,2′,4′,4′-tetroxido-1,1′,2′,3′,4′,4′-tetrahydrospiro[indoline-3,5′-pyra[2,3-dipyrimidine-6′-carboxylate (1k); mp: 208–210 °C; UV max (cm\(^{-1}\), ATR): 3192 (v NH), 1372 (v C=O), 1116 (v C-O); 1^1H-NMR (500 MHz, DMSO-d\(_6\)): δ_H 12.15 (1H, br s, H7), 10.95 (1H, s, H19), 10.22 (1H, s, H7), 7.93 (2H, s, H14), 7.06 (1H, dt, J = 7.6, 1.3 Hz, H2), 6.94 (1H, br d, J = 7.2 Hz, H6), 6.78 (1H, dt, J = 7.4, 0.9 Hz, H1), 6.67 (1H, br d, J = 7.6 Hz, H3), 3.78 (2H, m, H20 e H2O), 0.78 (3H, t, J = 7.1 Hz, H21); 13^1C-NMR (126 MHz, DMSO-d\(_6\)): δ_C 179.3 (s, C8), 167.4 (s, C5), 161.2 (s, C16), 158.6 (S, C12), 152.2 (s, C11), 149.1 (s, C18), 144.0 (s, C4), 135.3 (s, C5), 127.3 (s, C2), 122.7 (s, C6), 120.6 (s, C1), 108.2 (s, C3), 89.2 (s, C10), 76.3 (s, C13), 59.1 (s, C20), 46.2 (s, C9), 13.0 (s, C21); ESI (+) FT-ICR MS: [C_{17}H_{13}N_{4}O_{8}]^{+} \text{ exp } = 369.08404 \text{ m/z, calc } = 369.08406 \text{ m/z (err = 0.05 ppm)}.

2-amino-7,7-dimethyl-2′,5′-dioxy-3,6,7,8-tetrahydrospiro[chromene-4,3′-indoline]-3-carbonitrile (II); mp: 300 °C; IR max (cm\(^{-1}\), ATR): 3306 (v NH), 2960 (v CH\(_2\)), 2192 (v nitrite), 1653 (v C=O), 1346 (v C-N), 1221 (v C-O); 1^1H-NMR (400 MHz, DMSO-d\(_6\)): δ_H 10.39 (1H, s, H7), 7.21 (2H, s, H14), 7.14 (1H, dt, J = 7.6, 1.3 Hz, H2), 6.97 (1H, br d, J = 7.4 Hz, H6), 6.89 (1H, br d, J = 7.5, 0.9 Hz, H1), 6.79 (1H, br d, J = 7.6 Hz, H3), 2.58 (1H, d, J = 17.5 Hz, H19), 2.53 (1, d, J = 17.5 Hz, H19′), 2.17 (1H, d, J = 16.0 Hz, H17′), 2.09 (1H, d, J = 16.0 Hz, H17), 1.03 (3H, s, 20), 1.00 (3H, s, 21); 13^1C-NMR (101 MHz, DMSO-d\(_6\)): δ_C 194.8 (s, C16), 178.0 (s, C8), 164.1 (s, C11), 158.8 (s, C12), 142.0 (s, C4), 134.4 (s, C5), 128.1 (s, C2), 126.3 (s, C6), 121.7 (s, C1), 117.3 (s, C15), 110.8 (S, C10), 109.2 (s, C3), 57.5 (s, C13), 50.0 (s, C17), 46.8 (s, C9), 40.0 (s, C19), 31.9 (s, C18), 27.6 (s, C20), 27.0 (s, C21); ESI (+) FT-ICR MS: [C_{12}H_{12}N_{3}O_{6}]^{+} \text{ exp } = 358.11626 \text{ m/z, calc } = 358.11621 \text{ m/z (err = −0.12 ppm)}, [C_{13}H_{13}N_{4}O_{8}]^{+} \text{ exp } = 693.24332 \text{ m/z, calc } = 693.24320 \text{ m/z (err = −0.17 ppm)}, [C_{14}H_{13}N_{4}O_{8}]^{+} \text{ exp } = 1028.37086 \text{ m/z, calc } = 1028.37020 \text{ m/z (err = −0.65 ppm)}.

5-(2-oxindolin-3-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (6); mp: 220 °C (with decomposition); IR max (cm\(^{-1}\), ATR): 3260 (v NH), 1681 (ν C=O), 1618 (ν C=O, C=O conjugated), 1331 (ν C-N); 1^1H-NMR (400 MHz, DMSO-d\(_6\)): δ_H 11.17 (2H, s, NH), 10.55 (1H, s, NH), 7.17–7.09 (2H, m, H aromatic), 6.89 (1H, t, J = 7.6 Hz, H aromatic), 6.67 (1H, d, J = 7.6 Hz, H aromatic), 5.14 (1H, td, J = 7.8, 0.9 Hz, H aromatic), 7.10 (1H, td, J = 7.8, 0.9 Hz, H aromatic), 6.91 (1H, d, J = 7.8 Hz, H aromatic).

3.3. X-ray Diffraction Analysis

Colorless single crystals were successfully grown from methanol by the slow solvent-evaporation method at room temperature. Single-crystal X-ray data for 1b were collected on a Bruker D8 Venture diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) using graphite-monochromate Mo Kα radiation (λ = 0.7073 Å) at 293K. Data collection, cell refinement, and data reduction were performed with Bruker Instrument Service v6.2.6 (Bruker AXS GmbH, Karlsruhe, Germany), APEX4, and SAINT5, respectively. Absorption correction using equivalent reflections was performed with the SADABS program.5 The structure solutions and full-matrix least-squares refinements based on F^2 were performed with the SHELX package5,5 and were refined with fixed individual displacement parameters (Uiso(H) = 1.2 Ueq(Csp2) and Cα) or 1.5 Ueq(Csp3) using a riding model. All nonhydrogen atoms were refined anisotropically. Crystallographic tables were constructed using Olex2.6 X-ray crystallographic data in the cif format available at CCDC 2213864 can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, accessed on 26 October 2022.

Crystallographic Data: C_{15}H_{14}BrN_{5}O_{7} (M = 456.22 g/mol): triclinic, space group P-1, a = 8.8166(4) Å, b = 9.7965(5) Å, c = 11.7129(5) Å, α = 85.978(2)°, β = 89.864(2)°, γ = 63.810(2)°, V = 905.11(7) Å\(^3\), Z = 2, T = 273.15 K, μ(Mo Kα) = 2.322 mm\(^{-1}\), F(000) = 460.0,
crystal size = 0.32 × 0.212 × 0.116 mm³, ρcalc = 1.674 g/cm³; of the 59,143 reflections measured (4.648° ≤ 2Θ ≤ 51.358°), 3425 were unique (Rint = 0.0760, Rsigma = 0.0281) and were used in all calculations. The final R1 was 0.0417 (I > 2σ(I)), and wR2 was 0.1061 (all data).

3.4. In Vitro Cytotoxicity Assays

Antiproliferative assays were performed against tumor lines, SNB-19 (astrocytoma), HCT-116 (colon carcinoma—human), PC3 (prostate carcinoma), and HL60 (promyelocytic leukemia), provided by the National Cancer Institute (USA), having been grown in RPMI 1640 medium, supplemented with 10% fetal bovine serum and 1% antibiotics, kept in an oven at 37 °C and an atmosphere containing 5% of CO₂. The molecular hybrid samples were weighed and diluted in DMSO to final stock concentrations of 40 mM. Cytotoxicity analysis was performed using the MTT method, cells were plated at concentrations of 0.7 × 10⁵ cells/mL (HCT-116), 0.1 × 10⁶ cells/mL (SNB19 and PC3), and 0.3 × 10⁶ cells/mL (HL60). The samples were tested after serial dilution in concentrations from 0.20 to 200 µM, in duplicate in three different experiments. From these solutions, serial dilutions were performed until obtaining a minimum concentration of 0.20 µM for the evaluation of the inhibitory concentration mean (IC₅₀). The plates were incubated for 72 h in an oven at 5% CO₂ at 37 °C. At the end of this, the plates were centrifuged, and the supernatant was removed. Then, 100 µL of the MTT solution (tetrazolium salt) was added, and the plates were incubated for 3 h. The absorbance was read after dissolving the precipitate with 100 µL of pure DMSO in a plate spectrophotometer, at a wavelength of 595 nm. The experiments were analyzed by linear regression using the GraphPad Prism program, version 6.01.

4. Conclusions

Spiro compounds 1a–j, 1k, and 1l were synthesized with good yields (43–98%) through a microwave-assisted Knoevenagel/Michael/cyclization multicomponent domino methodology, using ethanol as solvent and 1-methylimidazolium chloride ionic liquid as organocatalyst. The reaction conditions considered ideal were determined from a methodological study varying solvent, catalyst, amount of catalyst, temperature, and heating mode. X-ray structure of compounds 1b was obtained. Unfortunately, the antiproliferative activity of the tested compounds was only moderate and spiro compound 1c inhibited the four cell lines tested with the lowest IC₅₀ values: 52.81 µM for HCT116; 74.40 µM for PC3; 101 µM for SNB19, and 49.72 µM for HL60.

Supplementary Materials: The characterization data for all products can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27228051/s1. These data include: Figure S1. Infrared spectrum of compound 1a; Figure S2. 1H NMR spectrum of compound 1a; Figure S3. 13C NMR spectrum of compound 1a; Figure S4. 1H-1H COSY NMR spectrum of compound 1a; Figure S5. 1H-13C HSQC NMR spectrum of compound 1a; Figure S6. 1H-13C HMBC NMR spectrum of compound 1a (cnst13 = 3 Hz); Figure S7. 1H-13C HMBC NMR spectrum of compound 1a (cnst13 = 8 Hz); Figure S8. Infrared spectrum of compound 1b; Figure S9. 1H NMR spectrum of compound 1b; Figure S10. 13C NMR spectrum of compound 1b; Figure S11. 1H-1H COSY NMR spectrum of compound 1b; Figure S12. 1H-13C HSQC NMR spectrum of compound 1b; Figure S13. 1H-13C HMBC NMR spectrum of compound 1b (cnst13 = 3 Hz); Figure S14. 1H-13C HMBC NMR spectrum of compound 1b (cnst13 = 8 Hz); Figure S15. Mass spectrum of compound 1b; Figure S16. Infrared spectrum of compound 1c; Figure S17. 1H NMR spectrum of compound 1c; Figure S18. 13C NMR spectrum of compound 1c; Figure S19. 1H-1H COSY NMR spectrum of compound 1c; Figure S20. 1H-13C HSQC NMR spectrum of compound 1c; Figure S21. 1H-13C HMBC NMR spectrum of compound 1c (cnst13 = 3 Hz); Figure S22. 1H-13C HMBC NMR spectrum of compound 1c (cnst13 = 8 Hz); Figure S23. Mass spectrum of compound 1c; Figure S24. Infrared spectrum of compound 1d; Figure S25. 1H NMR spectrum of compound 1d; Figure S26. 13C NMR spectrum of compound 1d; Figure S27. 1H-1H COSY NMR spectrum of compound 1d; Figure S28. 1H-13C HSQC NMR spectrum of compound 1d; Figure S29. 1H-13C HMBC NMR spectrum of compound 1d (cnst13 = 3 Hz); Figure S30. 1H-13C HMBC NMR spectrum of compound 1d (cnst13 = 8 Hz); Figure S31. Mass spectrum of compound 1d; Figure S32. Infrared spectrum of compound 1e; Figure S33. 1H NMR spectrum of compound 1e; Figure S34. 13C NMR spectrum of compound 1e; Figure S35.
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