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Coumarin–dihydropyrimidinone hybrids as promising agents against ovarian cancer: synthesis, SAR, and *in silico* evaluation

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ABSTRACT

The coumarin scaffold is widely recognized for its broad range of biological activities, including antibacterial, anti-HIV, and anticancer properties. In parallel, molecular hybridization has emerged as an effective strategy to enhance biological activity and expand chemical diversity within drug discovery libraries. In this study, a series of coumarin–dihydropyrimidinone (DHPM) hybrids were synthesized *via* a Biginelli multicomponent reaction. The *in vitro* cytotoxic activity of these compounds was evaluated against a panel of cancer cell lines. A structure–activity relationship (SAR) analysis was carried out to elucidate the influence of different substituents and physicochemical properties on anticancer activity. Among the synthesized compounds, **LSPN925** emerged as a good anticancer candidate and was further investigated for its effects on cell morphology and colony formation. In addition, *in silico* pharmacokinetic and toxicological evaluations were performed for the most active compounds to predict their drug-likeness and safety profiles. The preliminary SAR analysis revealed that lipophilicity and molecular volume of the compounds play a critical role in modulating their cytotoxic activity, highlighting these parameters as key considerations in the rational design of new coumarin-based anticancer agents. Overall, these findings support the potential of coumarin–DHPM hybrids as promising scaffolds for further anticancer drug development.

Cancer is characterized by the uncontrolled growth and spread of abnormal cells in the body. More than 100 distinct types of cancer have been identified, including ovarian cancer (OC), melanoma, and breast cancer.[1] OC is currently the eighth most frequently diagnosed cancer and the eighth leading cause of cancer-related mortality worldwide.[2] This high mortality rate is largely attributed to the fact that both type I and type II OC are often diagnosed at advanced stages (III–IV), making OC the fifth and sixth most common cause of cancer-related death in the United States and the United Kingdom, respectively.[3]

3,4-Dihydropyrimidin-2(1*H*)-one (DHPM) is a structural motif found in several natural products and pharmaceutical agents. DHPM derivatives have attracted considerable attention due to their broad range of biological activities, including antitumor properties.[4] In this context, Monastrol (**1**) (Fig. 1) stands out as one of the most extensively studied DHPM-based compounds, exhibiting pronounced antiproliferative activity against cancer cells.[5,6]

The coumarin (2*H*-1-benzopyran-2-one) scaffold is also well known for its diverse biological activities,[7] such as antibacterial,[8] anti-HIV,[9] and anticancer effects.[10] Numerous coumarin derivatives of natural origin have demonstrated promising *in vitro* and *in vivo* anticancer activity.[11] Coumarin-based hybrid molecules have been developed to address tumor cell resistance mechanisms and to interact with distinct targets.[12] Coumarin **2** (Fig. 1), for example, inhibits angiogenesis through a decrease in CCL2 chemokine levels.[13]

Molecular hybridization, is a valuable strategy to enhance biological activity and expand chemical diversity.[14] In this context, Chavan et al. described the synthesis and antibacterial and anti-inflammatory activities of coumarin-DHPM hybrids.[15] The anti-inflammatory activity by egg albumin denaturation method showed compound **3** with good inhibition of protein denaturation (75%).[15]

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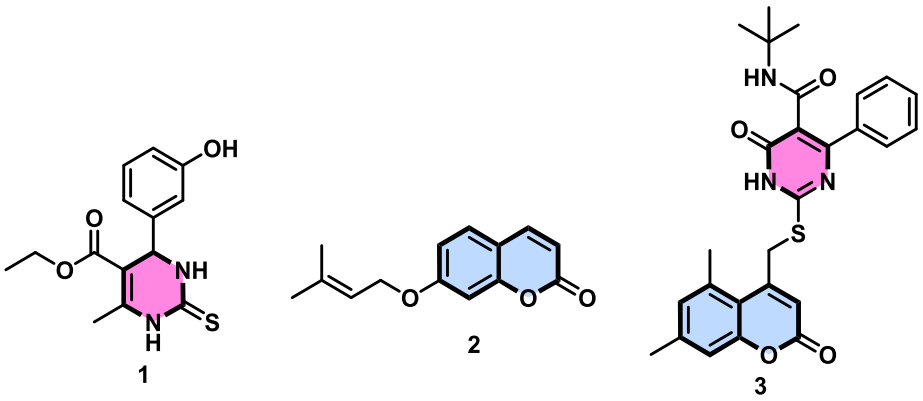
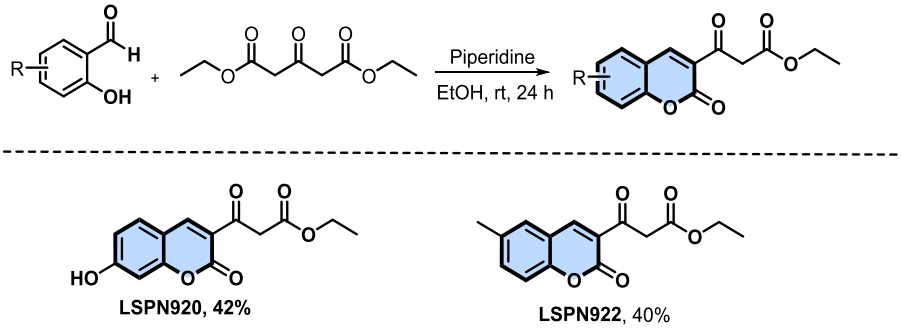
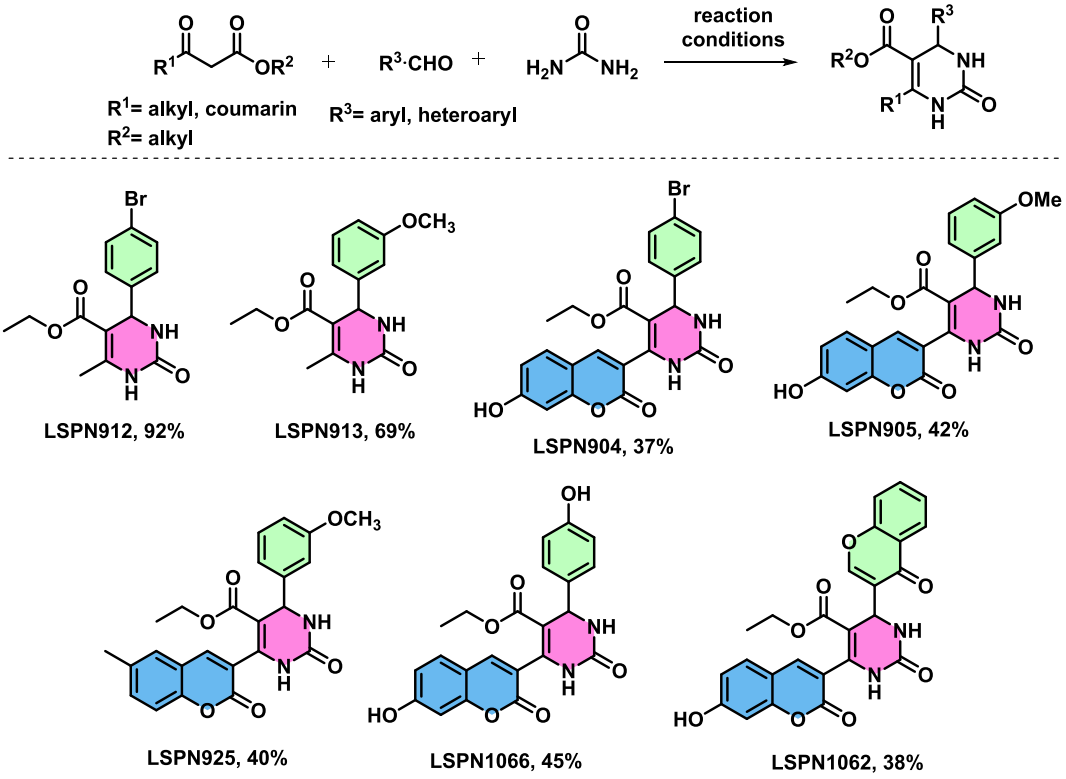


Fig. 1. Examples of biologically active DHPM, coumarin and their hybrid.



Scheme 1. Synthesis of C-3-functionalized coumarins with β-ketoesters.



Scheme 2. Synthesis of dihydropyrimidinones (DHPMs) and their hybrids.

In this work, coumarin–DHPM hybrids were synthesized *via* a Biginelli multicomponent reaction.[16] The *in vitro* cytotoxicity of these derivatives was evaluated across different cell lines. A structure–activity relationship (SAR) analysis was conducted to gain deeper insight into

Table 1
In vitro anticancer activity and molecular descriptors as molecular volume (MV, A^3) and LogP of coumarins derivatives.

Compound	IC_{50} (μM)				MV ($Å^3$)	cLog P
	A2780	A375	MCF-7	MRC-5		
LSPN904	46.65 ± 3.55	77.62 ± 1.05	92.04 ± 1.86	>100	406.73	2.02
LPSN905	>100	>100	>100	>100	416.99	1.07
LSPN912	>100	>100	>100	>100	285.62	1.36
LPSN913	88.80 ± 5.12	>100	>100	71.25 ± 5.73	295.15	0.41
LSPN920	>100	>100	>100	>100	262.16	1.69
LSPN922	70.65 ± 8.45	>100	>100	>100	273.09	2.57
LPSN925	29.59 ± 0.60	65.15 ± 2.17	81.20 ± 7.54	>100	427.89	1.94
LSPN1062	>100	>100	>100	>100	439.47	−0.5
LSPN1066	>100	>100	>100	>100	396.83	0.8
Doxorubicin	1.63 ± 0.12	3.90 ± 0.39	>50	20.59 ± 2.00	nd	nd

The cytotoxicity was obtained on A2780 (ovarian cancer), A375 (melanona) and MCF-7 (breast cancer) cells, and non-cancerous MRC-5 (lung) cells after 48 h of incubation. Data are presented as mean ± SD of three independent replicates. nd = not determined.

the roles of specific moieties and functional groups in modulating anticancer activity of a specific compound. The effects of **LSPN925**, identified as the most promising candidate, on cell morphology and colony-forming capacity were further investigated. Also, albumin interaction studies were conducted. In addition, *in silico* pharmacokinetic and toxicological evaluations were performed for the most potent compounds.

Therefore, a series of coumarin derivatives was synthesized through the Knoevenagel condensation, providing *β*-ketoester components for the subsequent Biginelli multicomponent reaction.[19] Two coumarins bearing different substituents were obtained: one with a hydroxyl group at the C-7 position (**LSPN920**) and another with a methyl group at the C-6 position (**LSPN922**). These compounds were obtained in yields of up to 42% (Scheme 1).

Using **LSPN920** and **LSPN922** as starting materials, the next step involved the synthesis of hybrid compounds. The DHPM derivatives were first obtained *via* the Biginelli multicomponent reaction using *β*-ketoesters, aromatic aldehydes, and urea, affording the desired products in yields of up to 92%. The targeted coumarin–DHPM hybrids were synthesized by incorporating the previously prepared coumarin derivatives into the *β*-ketoester component and reacting them with various aldehydes. These hybrids were obtained in yields of up to 45% (Scheme 2). With these compounds in hand, exploratory cytotoxicity studies were conducted against different cancer cell lines.

The cytotoxicity activity of the coumarin-DHPM hybrids, as well as their precursors, was investigated against A2780 (ovarian cancer), A375 (human melanoma) and MCF-7 (breast cancer), and non-cancerous MRC-5 (lung) cells. As presented in Table 1, compounds **LSPN904** and **LSPN925** were cytotoxic in all cancer cells lines tested, with IC_{50} values of 46.65 μM and 29.59 μM, respectively, against A2780 cells. For these compounds, the dose-response curves can be found in Fig. 2 and the IC_{50} statistical parameters in **Table S1** (Supporting Information file). Interestingly, compounds **LSPN905**, **LSPN1062** and **LSPN1066** had no effect at the highest concentration tested (IC_{50} > 100 μM), suggesting that the presence of hydroxyl substituent does not contribute for the anticancer activity of the compound. As these coumarin hybrids are more potent than the precursors (**LSPN912**, **LSPN913**, **LSPN920**, **LSPN922**), our results clearly indicate that hybridization approach is a good strategy to

Table 2
SI values obtained for the coumarin–DHPM hybrids in different cells.

Compound	SI^1	SI^2	SI^3
LSPN904	>2.1	>1.3	>1.1
LPSN905	nd	nd	nd
LSPN912	nd	nd	nd
LPSN913	<1.0	nd	nd
LSPN920	nd	nd	nd
LSPN922	1.4	nd	nd
LPSN925	>3.4	>1.5	>1.2
LSPN1062	nd	nd	nd
LSPN1066	nd	nd	nd
Doxorubicin	12.6	5.3	<1.0

SI^1 = IC_{50}(MRC-5)/IC_{50}(A2780), SI^2 = IC_{50}(MRC-5)/IC_{50}(A375), and SI^3 = IC_{50}(MRC-5)/IC_{50}(MCF-7). nd = not determined.

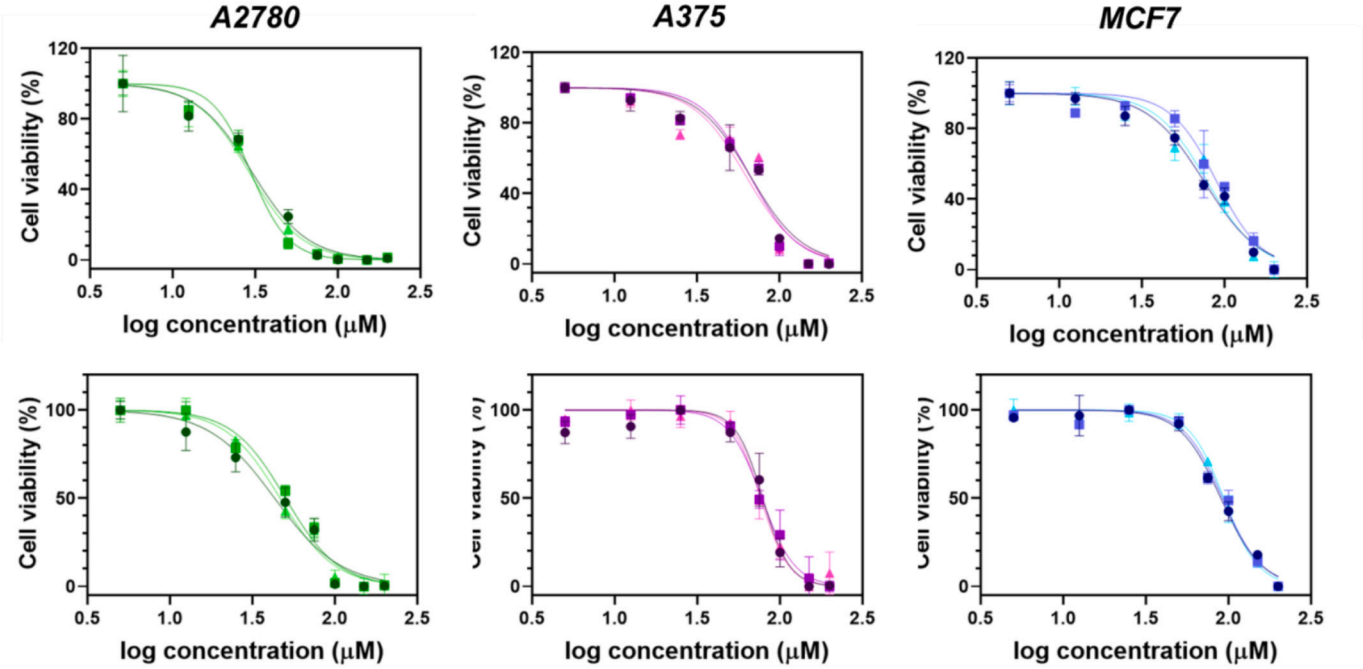


Fig. 2. Dose-response curve obtained for **LSPN925** (top) and **LSPN904** (bottom) toward A2780 (ovarian cancer), A375 (melanoma) and MCF-7 (breast cancer) cells.

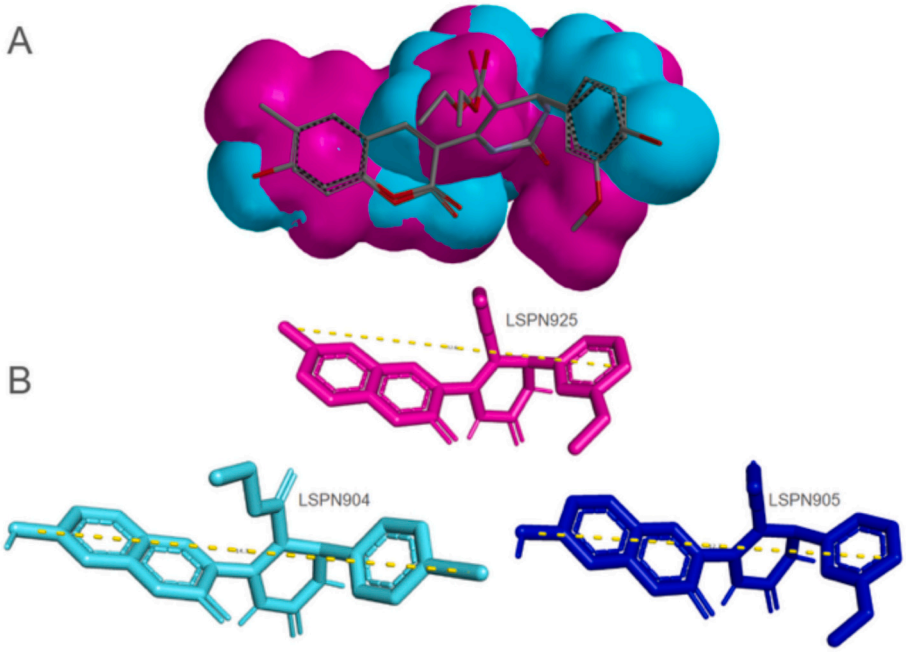


Fig. 3. Steric parameters of the most active coumarin-DHPM hybrids **LSPN925** (pink) and **LSPN904** (cyan). (A) Structural alignment with Van der Waals volume; (B) Distances (yellow dashed lines) between the most distal atoms of the coumarin and aryl moieties for compounds **LSPN905** (dark blue) **LSPN925** and **LSPN904**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

obtain new entities with increased anticancer activity.[17]

Considering the cytotoxicity of the compounds against the non-cancerous MRC-5 cell line, no significant effects were observed for most compounds, except for **LSPN913** ($IC_{50} = 71.25$ μM). The selectivity index (SI) was calculated for these compounds ($SI = IC_{50\ MRC5}/IC_{50\ cancer\ cell}$), revealing **LSPN925** as the most promising compound, with a SI >3.4 for A2780 cells. Although this compound is less selective than doxorubicin, its selectivity is comparable to that obtained for cisplatin drug (SI = 3.3) (Table 2).[18]

In an attempt to obtain a correlation between the chemical structures of coumarin derivatives and their *in vitro* activity against A2780 ovarian cancer cell line, a preliminary structure-activity relationship (SAR) study was performed. To show anticancer activity, the compound must pass through the cell membrane and then interact with its therapeutic target. Since molecular volume and lipophilicity are parameters directly related to cell permeability, an overall analysis suggests that all molecules were able to penetrate the cancer cell, as these parameters were not discriminatory for activity (Table 1).

Despite the therapeutic target remains unknown, we may infer that steric features play a critical role related to anticancer activity of the compound. Since the coumarin–DHPM hybrids **LSPN904** and **LSPN925** exhibited greater activity in comparison to their precursors, showing $IC_{50} < 50$ μM, it is noteworthy that although with similar volume and shape, the substituent and also its position in the coumarin and DHPM moieties appear to be relevant for the activity (Fig. 3). The preference observed for a 6-methyl substituent over a 7-hydroxyl group on the coumarin ring cannot be attributed solely to the increase in lipophilicity conferred by the methyl group but also to the topological arrangement of the substitution. The naturally occurring 7-hydroxyl group, common in biosynthetic pathways of coumarins[19] does not appear to enhance the anticancer activity in this series, whereas introduction of a methyl group at an appropriate position improves both steric accommodation and hydrophobic interactions within the binding pocket. Notably, compound **LSPN905**, which is structurally analogous to **LSPN925** and also bears an *m*-OCH_3 substituent on the aryl ring, exhibited markedly lower activity, likely due to the increased hydrophilicity associated with the 7-OH group, which counteracts the beneficial electronic and spatial contributions provided by the *meta* substituent.

The substitution pattern on the aryl ring also seems to be critical for biological activity. Although the compounds display similar molecular volumes, the end-to-end distance measurement indicates that molecular length is a discriminating feature (Fig. 3A). Compounds **LSPN905** and **LSPN925**, both bearing *meta* substituents, exhibit shorter distances (12.7 and 12.6 Å, respectively). In contrast, compound **LSPN904**, substituted at the *para* position (*p*-Br), shows an extended molecular length (14.3 Å), supporting a preference for *meta* substitution to maintain a more compact geometry (Fig. 3B). Due the best results obtained for **LSPN925**, this compound was selected for further biological investigations.

The cellular morphology of A2780 cells was studied in the absence and presence of **LSPN925** (Fig. 4). As presented, after 48 h these cells have a well-defined morphology. On the other hand, significant changes are clearly observed in the presence of **LSPN925** at concentrations higher than IC_{50}, including non-adherent and round cells. Furthermore, fluorescence images were taken after staining with Hoechst and propidium iodide (PI) dyes. Whereas Hoechst is nuclear DNA staining, PI is able to detect cells with injured membrane.

As observed in Fig. 4A, a cell density reduction and an increase in cell damaged population are observed in the presence of **LSPN925**. Next, we perform a colony formation assay to investigate the anti-proliferative potential of **LSPN925**. For this, A2780 cells were treated with **LSPN925** at different concentrations (1/8 IC_{50}, ¼ IC_{50}, ½ IC_{50} and IC_{50}). The compound was removed after 48 h of treatment and RPMI culture medium was replaced by a fresh medium. The colonies formed after 7 days were stained with crystal violet and counted. The results presented in Fig. 4B, revealed that **LSPN925** is able to reduce the number of the colonies, suggesting a cytostatic effect.

Albumin is an important protein responsible for the transport of different endogenous and exogenous compounds in the body, driving pharmacokinetic profile. Interaction studies with albumin are essential to determine whether the protein may to bind to a drug, which is pivotal for modulating bioavailability and distribution through the body.[20] In our study, the BSA (Bovine Serum Albumin) binding properties of compound **LSPN925** were investigated *via* competition fluorescence

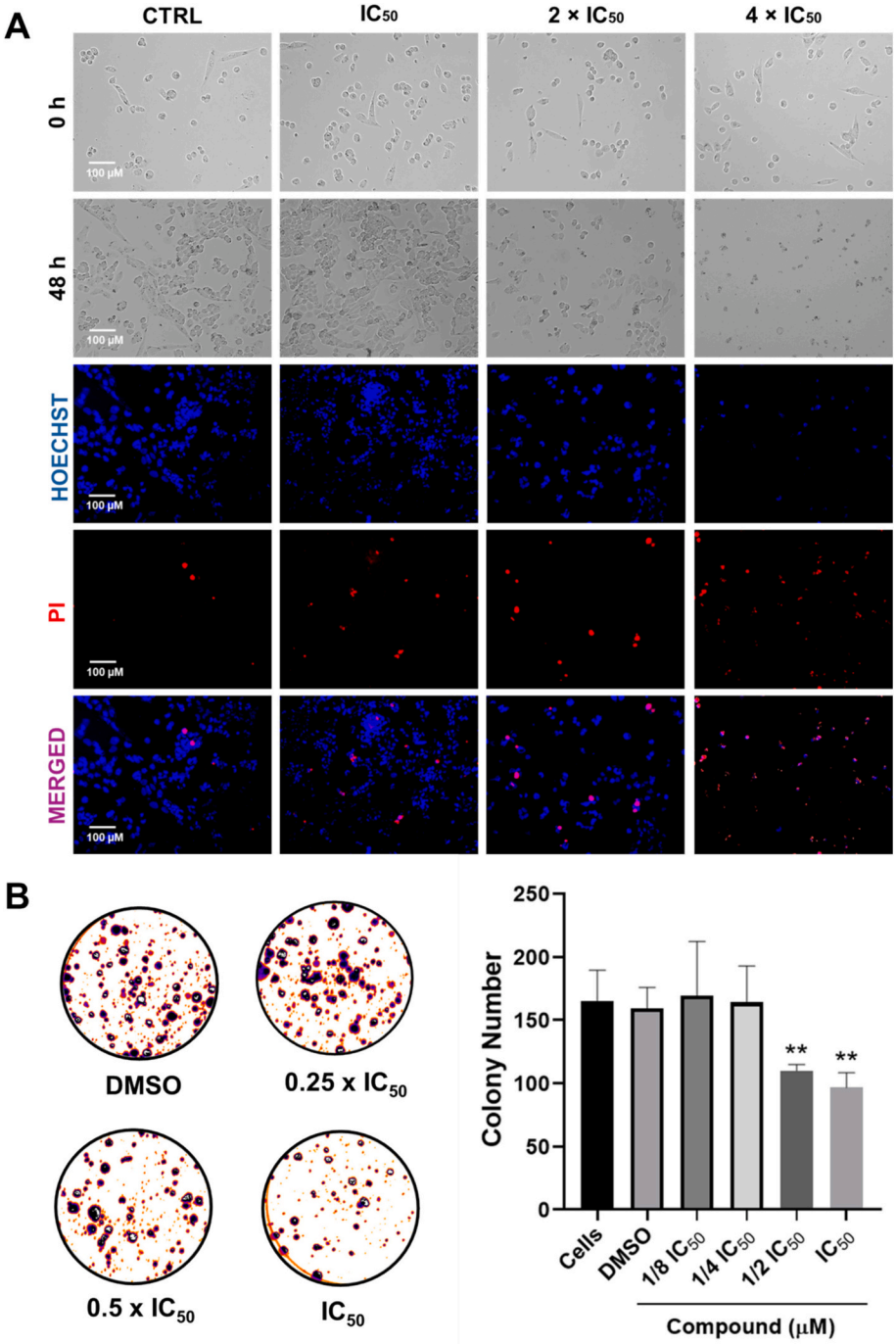


Fig. 4. A) Cell morphology changes of A2780 ovarian cancer cells and double Hoechst/PI staining. Data were obtained after treatment with **LSPN925** for 48 h at different concentrations. The images were taken using a CELENA® S Digital Imaging System (Logos Biosystems) at 10 × zoom. B) Representative colony formation images (left), and quantitative data representing the colony number (right) of A2780 cells in the presence of **LSPN925** at different concentrations. The images were taken using an Invitrogen iBright 1500 Imaging System (Thermofisher). DMSO vehicle (0.5% *v*/v) was used in the negative control. Data are expressed as mean ± SD of three independent measurements. The statistical analysis was performed with one-way ANOVA followed by Dunnett's test (**$p < 0.01$).

assay. This interaction was studied *via* fluorescence quenching of the albumin in the presence of **LSPN925**.

The Stern–Volmer quenching constant (K_{SV}) obtained for the BSA–**LSPN925** system was equal to $6.95 \times 10^3\ M^{-1}$ (Fig. 5). This value is in the range for similar coumarin compounds reported in the literature. The bimolecular quenching rate constant (k_q) is $3.48 \times 10^{13}\ M^{-1}\ s^{-1}$, suggesting a quenching *via* dynamic mechanism. Finally, the binding constant (K_b), $2.33 \times 10^3\ M^{-1}$, indicates a moderate affinity between the compound[21,22] and the protein.[23,24]

Early evaluation of pharmacokinetics and toxicological parameters is of great importance for the optimization of new compounds, being crucial for the successful drug discovery in R&D.[25] We performed these predictions for the most active compounds (**LSPN904**, **LSPN913**, **LSPN922** and **LSPN925**) (Table 3). First, we evaluated the physico-chemical parameters according to Lipinski's rule of five (Ro5). It could be seen that they did not violate any rules, indicating good oral bioavailability. Since high P-gp expression is often observed in various types of cancer cells, we evaluated the potential of compounds to act as

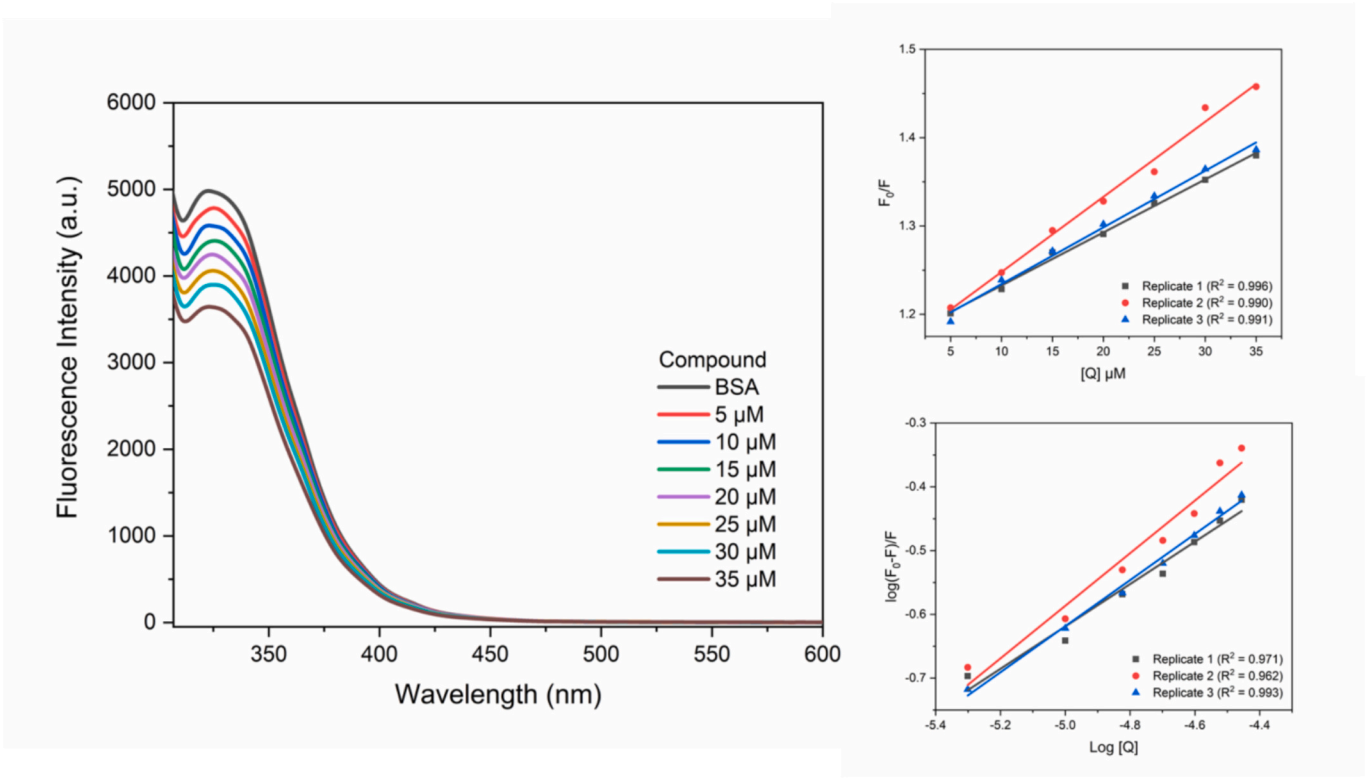


Fig. 5. Fluorescence spectra of BSA (5 μM, λex = 280 nm) in the absence and presence of **LSPN925** at different concentrations, and a Stern–Volmer and plot of log [(F_0 − F)/F] *versus* log[Q], at 298 K.

Table 3
In silico pharmacokinetic and toxicity screening of the most active coumarin derivatives: Lipinski's "rules of five" (Ro5), P-pg substrate, CYP450 enzymes inhibition and substrate, hepatotoxicity (HEP), cardiotoxicity (hERG) and acute toxicity in rodents (LD_{50}, (mg/kg).

Compound	Ro5	P-gp substrate	CYP450 inh/subs	HEP[a]	hERG[b]	LD_{50} (mg/kg)
LSPN904	0	yes	yes/yes	1	no	708.44
LSPN913	0	yes	no/yes	2	no	1947.67
LSPN922	0	no	yes/yes	0	no	2372.39
LSPN925	0	yes	yes/yes	0	no	760.79
Doxorubicin	0	yes	no/no	0	no	608.02

[a] based on the increase of alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH).
[b] the possibility that a compound will block the hERG channel.

substrates of this efflux pump. All the compounds, with the exception of **LSPN922**, were predicted as substrates of the transporter.

All evaluated molecules were predicted to be substrates of the all cytochrome P450 isoforms investigated, except for compound **LSPN922**, which may be metabolized primarily by CYP2C9 and CYP2C19, consistent with its acidic nature.[26] Additionally, an inhibitory profile toward hepatic enzymes was observed, indicating a potential risk of drug–drug interactions, as most of the compounds were predicted to inhibit CYP3A4, CYP2C9 and CYP2C19 (Table 3).

The liver is a critical target organ for anticancer toxicity. Drug-induced liver injury (DILI) includes manifestations ranging from an asymptomatic elevation of liver enzymes to outright acute liver failure.[27,28,29] Compounds are considered hepatotoxic when a simultaneous increase in serum enzymes commonly used to diagnose hepatic adverse drug events (ADR) – AST, ALT, and LDH - is observed.[30] All compounds here studied showed low hepatotoxicity risk (Table 3).

Likewise, no acute toxicity was predicted, since the compounds did not fall below the toxicological range of 200–300 mg/kg for induced toxicity in rats.[28] In addition, no cardiotoxicity risks were observed for all compounds.

Despite its efficacy against several types of cancer, including breast cancer and solid tumors in children, the use of anthracyclines (such as doxorubicin and daunorubicin) is limited by the risk of cardiotoxicity.[31] Anthracycline-induced cardiotoxicity may manifest as acute or chronic cardiomyopathy and can progress to congestive heart failure, being mediated by the interaction of doxorubicin with cardiolipin, a cardiac lipid that is essential for myocardial energy metabolism.[32] In contrast, the hit compounds identified in this study were not predicted to interact with the hERG channel, which plays a critical role in cardiomyocyte repolarization and whose inhibition is also associated with cardiotoxic effects.[33]

Based on the *in silico* ADMET results, compounds **LSPN922** and **LSPN925** appear to be the safest candidates to advance for oral formulation development, as they exhibit lower induction of hepatic serum markers and CYP enzymes inhibition.

It is important to emphasize that, although our predictions reveal a good profile for the compounds, they do not replace the experimental approach, and should be used in conjunction whenever possible.

In conclusion, in this work, two coumarins, two DHPMs and five hybrids were obtained in yields of up to 92%. Among the synthesized compounds, coumarin **LSPN904** and the coumarin-DHPM hybrid **LSPN925** exhibited cytotoxic activity against different cancer cell lines, with a pronounced effect against A2780 ovarian cancer cells. **LSPN925** significantly altered cell morphology and inhibited colony formation in these cells, and it displayed moderate affinity for serum albumin. Although our study indicated that lipophilicity and molecular volume are key physicochemical parameters related to the cytotoxicity, the SAR is limited due the reduced number of molecule in the investigated group. The *in silico* ADMET predictions suggested a favourable pharmacokinetic and safety profile for the most promising coumarin–DHPM hybrids

LSPN922 and **LSPN925** due their combined cytotoxicity, good oral bioavailability, low toxicity predictions. Overall, these preliminary results encourage us to investigate the mechanism of action/possible targets of these molecules in the future, which is still unclear.

CRediT authorship contribution statement

Jhonathan R.N. dos Santos: Writing – original draft, Methodology, Investigation, Formal analysis. **Alice K.A. Martinez:** Investigation. **Saulo H. Mendes Abe:** Investigation, Formal analysis. **Marcos V. Palmeira-Mello:** Writing – original draft, Methodology, Investigation, Formal analysis. **João Pedro Araujo dos Santos:** Methodology, Investigation, Formal analysis. **Carlos Rangel Rodrigues:** Investigation, Formal analysis. **Alessandra M.T. de Souza:** Methodology, Investigation. **Alzir A. Batista:** Writing – review & editing, Funding acquisition. **Arlene G. Corrêa:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Chat Generative Pre-Trained Transformer (ChatGPT; OpenAI, San Francisco, CA, USA) to enhance readability and language, aiding in formulating and structuring content. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2026.130646.

Data availability

Data will be made available on request.

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