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ORIGINAL RESEARCH

Curcumin-cinnamaldehyde hybrids as antiproliferative agents against women's cancer cells

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Abstract

Curcumin and cinnamaldehyde are natural products whose antineoplastic activity has been well explored in biological evaluations. However, their poor chemical stability under physiological conditions has been an obstacle to their use as therapeutic agents. Herein, we designed and synthesized two series of curcumin-cinnamaldehyde hybrids by removing reactive functionalities, including β-diketone and aldoxyl moieties. All compounds were evaluated by the MTT assay to determine their antiproliferative activity against women's cancer cells. Compound 5a (3′-hydroxychalcone) demonstrated potent antiproliferative activity against all cancer cell lines tested, with IC_{50} values ranging from 2.7 to 36.5 μM. Compound 5a was more active and selective than curcumin and cinnamaldehyde (parent compounds) against the CaSki, SiHa, C33, and A431 cell lines, displaying a higher selectivity index (SI = 8.5) than curcumin (SI = 0.8) toward the non-tumorigenic HaCaT cell line. Clonogenic experiments indicated that compound 5a inhibited A431 colony formation in a concentration-dependent manner. In addition, 5a was more stable than its parent compounds in pH 7.4 at 37 °C. In silico investigations suggested that 5a has good drug-likeness properties. In conclusion, our results indicate the use of curcumin and cinnamaldehyde as parent compounds for the design of hybrids with attractive antiproliferative activity and chemical stability.

Keywords Curcumin · Cinnamaldehyde · Antiproliferative · Hybridization · Cancer

Introduction

Cancer is a leading cause of death worldwide [1] and its incidence has increased to about 18.1 million new cases and 9.6 million deaths in 2018 [2]. Women are more likely to die from cancer than men [1]. Breast cancer is the leading cancer in women [3] and the triple-negative type is the most severe because of the lack of targeted pharmacotherapy [4]. Cervical cancer is the fourth most common cancer among women [3] and its main causative agent is human papillomavirus (HPV). The two main types (HPV-16 and HPV-18), which are classified as high risk, are responsible for 70% of cervical cancers [5] and may also be associated with vulvar cancer [6]. In 2018, there were 44,235 cases of vulvar cancer and 15,222 deaths due to this cancer worldwide [7].

Curcumin (from *Curcuma longa*) and cinnamaldehyde (from *Cinnamomum* species) are used as food additives and are classified as safe by the FDA [8, 9]. Curcumin and cinnamaldehyde exert activity against breast [10, 11] and cervical cancers [12, 13]. The mechanisms of action of curcumin include the induction of apoptosis, regulation of the p53 and Rb tumor suppressor genes, and suppression of HPV E6 and E7 oncogene expression [14]. Cinnamaldehyde exerts an antiproliferative effect and decreases the number of breast cancer cells [15]. Although curcumin and cinnamaldehyde have shown to be promising

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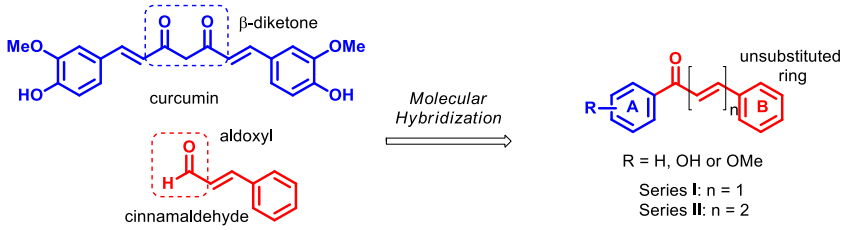


Scheme 1 Design of curcumin-cinnamaldehyde hybrids of series I and II

antineoplastic agents, their poor chemical stability has impeded advances in clinical studies [16, 17]. In previous work, we demonstrated the potent antiproliferative activity of curcumin analogs in which the β-diketone moiety (chelatogenic group) was replaced by a monoketone [18–20]. We also found that the replacement of the aldoxyl group of cinnamaldehyde (reactive functionality) by an acetophenone moiety resulted in more stable antibacterial and antitubercular analogs [21].

In the present study, we selected curcumin and cinnamaldehyde as parent compounds for molecular hybridization (Scheme 1). Two series of curcumin-cinnamaldehyde hybrids were designed by removing the undesirable functionalities of the parent compounds, including the β-diketone and aldoxyl moieties of curcumin and cinnamaldehyde, respectively. We explored the number of double bonds between aromatic rings A and B, as well as the role of hydroxyl and methoxyl substituents on ring A. Series I and II curcumin-cinnamaldehyde hybrids consisted of α,β-unsaturated ketones, and α,β,γ,δ-unsaturated ketones, respectively.

The antiproliferative activity of the curcumin-cinnamaldehyde hybrids was evaluated against a panel of women's cancer cells, including cervical, breast, and vulvar cancers. The most active hybrid (5a) was investigated regarding its toxicity and selectivity toward non-tumorigenic keratinocytes. We also investigated whether 5a inhibits the formation of vulvar cell colonies. The chemical stability of hybrid 5a was evaluated under physiological conditions. In silico analysis using the Molinspiration® explorer, toolkit contributed to predicting the drug-likeness properties of 5a.

Experimental

Synthesis of curcumin-cinnamaldehyde hybrids

Curcumin, cinnamaldehyde, doxorubicin, starting reagents, and solvents were purchased from Merck®. The series I and II curcumin-cinnamaldehyde hybrids were synthesized by base-catalyzed Claisen-Schmidt aldol condensation reactions using ethanol as a protic solvent. Series I hybrids (1a–6a) were synthesized as previously described by our group with minor modifications [22]. Appropriate acetophenone derivatives (5 mmol) and benzaldehyde (5 mmol) were solubilized in ethanol (10 mL). A 5-mL aliquot of NaOH ethanolic solution (1 mol L^{-1}) was added dropwise to the reaction medium, which was submitted to magnetic stirring at room temperature. Series II hybrids (1b–6b) were synthesized as previously described by our group with minor modifications [21]. Cinnamaldehyde (6 mmol) was added to a solution of respective acetophenone (5 mmol) in ethanol (5 mL). The solution was stirred at room temperature for 30 min, followed by dropwise addition of 5 mL NaOH ethanolic solution (1 mol L^{-1}). The progress of the reactions was monitored using thin-layer chromatography plates examined under UV light at 254 and 365 nm. All compounds were purified over a silica gel chromatography column eluted with mixtures of hexane and ethyl acetate. The structure of the compounds was identified by analysis of NMR spectra, which were obtained with a Bruker Avance III 14.095 T (600 MHz) spectrometer.

Cell lines and cell culture

A panel of human cell lines was used: breast cancer cell lines MCF-7 (ATCC® HTB-22 ™) and MDA-MB-231 (ATCC® HTB-26 ™); cervical cancer cell lines C33—HPV-negative (ATCC® HTB-31™), HeLa—positive for HPV-18 (ATCC® CCL-2™), CaSki—positive for HPV-16 (ATCC® CRM-CRL-1550™), and SiHa—positive for HPV-16 (ATCC® HTB-35™); vulvar cancer cell line A431 (SIGMA-85090402); immortalized non-tumorigenic human keratinocyte cells (HaCaT—CLS 300493). The MCF-7 cell lines were maintained in Eagle's minimum essential medium (Gibco Life Technologies). The MDA-MB-231, C33, HeLa, CaSki, SiHa, and A431 cells were maintained in Dulbecco's Modified Eagle Medium (Gibco Life Technologies). All cell cultures were supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Cultilab), 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen) in plastic flasks (Corning) at 37 °C in a humidified 5% CO_2 atmosphere.

MTT assay

Cell viability was analyzed using the protocol described by our group [22, 23]. An aliquot of 1×10^4 cells/well was

seeded into 96-well cell culture plates in a total volume of 100 µL and incubated for 24 h. The cells were treated with the parent compounds and their hybrids at concentrations ranging from 200.0 to 1.56 µM. After 48 h, the medium was removed, 100 µL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (1 mg/mL) was added, and the plates were incubated for 30 min at 37 °C. MTT was removed, 100 µL DMSO was added, and the mixture was maintained under agitation for 5 min at 60 rpm. Absorbance was measured at 570 nm in a FLUOstar Omega microplate reader (BMG LABTECH). DMSO (0.1%) was used as a solvent for the compounds and as vehicle control. Doxorubicin was used as the positive control. The results were plotted as the percentage of cell viability inhibition (CVI) calculated as follows: CVI (%) = [1 − (absorbance of cells treated with compounds/absorbance of cells treated with 0.1% DMSO)] × 100. The IC_{50} values were calculated from this plot by non-linear regression. The experiments were carried out in triplicate and three independent events were used for statistical analysis.

Clonogenic assay

The clonogenic assay using A431 and HaCaT cell lines was performed according to the protocols of Franken et al. [24], with minor modifications [25]. Briefly, 5×10^3 cells/well were seeded into 12-well plates and incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. After incubation, the cells were treated with hybrid 5a at concentrations corresponding to their respective ¼ IC_{50} and IC_{50} values obtained in the MTT assays. After 48 h, the cells were recovered in the compound-free medium for 15 days, allowing cell growth and the formation of colonies. The colonies were counted for qualitative analysis. Images of the colonies were obtained with an iPhone digital camera (Apple). For quantitative analyses, cell viability was measured by the MTT assay. DMSO (0.1%) was used as vehicle control. The experiments were carried out in triplicate and three independent events were used for statistical analysis.

Statistical analyses

The experiments were repeated three times and quantitative data are expressed as the mean ± SD. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's test using the GraphPad Prism 7 (GraphPad Software, Inc.) and Microsoft Office Excel 2007 software. A p value < 0.0001 was considered statistically significant.

Chemical stability assay

The chemical stability of hybrid 5a (at λ_{max} 314 nm), curcumin (at λ_{max} 426 nm), and cinnamaldehyde (at λ_{max} 288 nm) was analyzed by monitoring their UV–visible absorption (Perkin Elmer® Lambda 25 UV/Visible Spectrophotometer) in phosphate buffer (pH 7.4) at 37 °C. Minor modifications in this protocol and the detailed procedures are reported in our previous work [26].

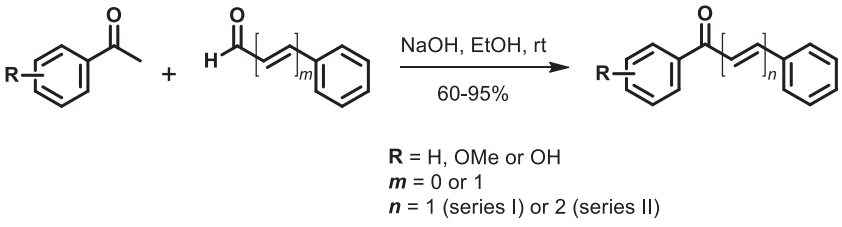
Drug-likeness properties

In silico investigations of the drug-likeness properties of hybrid 5a were performed using the free Molinspiration® toolkit to explore Lipinski's, Veber's, Muegge's, and Ghose's rules [27].

Results and discussion

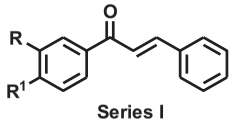
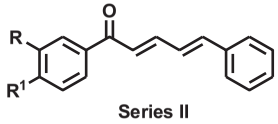
Two series of curcumin-cinnamaldehyde hybrids were synthesized by Claisen-Schmidt condensation reaction, with yields ranging from 60 to 95% (Scheme 2). All NMR parameters, including hydrogen and carbon chemical shifts (δ_H and δc in ppm), integrations, multiplicities, and coupling constants (J in Hz), corresponded to the designed structures and agreed with former literature [21, 22]. NMR spectra and their parameter values are presented in the Supplementary Material.

The antiproliferative effects of curcumin, cinnamaldehyde, and their hybrids were evaluated against human breast (MCF-7 and MDA-MB-231), cervix (C33, HeLa, CaSki, and SiHa), and vulvar (A431) cancer cells (Table 1). All cell lines were selected because of the high incidence of these cancers in women around the world, highlighting breast and cervix [3]. Two breast cancer cell lines were used, MCF-7 and MDA-MB-231, which are estrogen-receptor-positive (ER) and triple-negative (TNBC), respectively [28]. The chemotherapy of breast cancer depends on the type of cellular receptors. The ER-positive cancer is treated with



Scheme 2 Synthesis of curcumin-cinnamaldehyde hybrids of series I and II

Table 1 Antiproliferative activity of parent compounds and curcumin-cinnamaldehyde hybrids expressed as IC_{50} values (in μM)

Compounds/Entry		*R*	R^1	MCF-7(breast)	MDA-MB-231(breast)	HeLa(cervical)	CaSki(cervical)	SiHa(cervical)	C33(cervical)	A431(vulva)	mIC50[a]
Curcumin[b]		–	–	14.9 ± 2.9	31.0 ± 4.3	29.7 ± 3.3	16.6 ± 2.9	54.1 ± 0.6	29.0 ± 2.4	10.4 ± 0.5	26.5
Cinnamaldehyde[b]		–	–	>100	>100	>100	>100	>100	>100	>100	>100
Doxorubicin[c]		–	–	0.02 ± 0.002	1.87 ± 0.25	0.98 ± 0.19	1.52 ± 0.20	1.18 ± 0.26	1.55 ± 0.38	0.05 ± 0.009	1.02
 Series I	1a	OMe	OH	46.8 ± 1.3	66.3 ± 2.2	59.2 ± 7.7	61.4 ± 0.9	62.6 ± 0.2	47.1 ± 3.0	27.1 ± 2.7	53.0
	2a	H	H	17.1 ± 5.6	39.9 ± 3.1	52.9 ± 5.2	25.2 ± 2.4	30.1 ± 3.7	37.1 ± 0.9	4.1 ± 1.1	29.5
	3a	OMe	H	42.0 ± 4.6	42.7 ± 7.4	49.7 ± 4.8	57.3 ± 3.1	51.6 ± 5.1	32.6 ± 5.6	12.5 ± 2.3	45.9
	4a	H	OH	30.1 ± 6.3	50.1 ± 7.0	51.8 ± 5.1	27.4 ± 2.5	44.0 ± 5.1	20.0 ± 2.3	7.7 ± 1.7	33.0
	5a	OH	H	22.3 ± 2.9	35.2 ± 3.5	36.5 ± 3.7	15.5 ± 1.5	11.9 ± 1.5	11.3 ± 1.4	2.7 ± 0.2	19.3
	6a	H	OMe	41.5 ± 6.6	48.8 ± 0.7	67.1 ± 6.0	34.7 ± 6.3	41.0 ± 2.7	34.5 ± 5.3	33.8 ± 5.3	43.1
	1b	OMe	OH	65.5 ± 6.0	60.3 ± 9.8	59.1 ± 2.4	66.9 ± 4.9	65.0 ± 4.9	41.1 ± 1.8	12.3 ± 2.0	52.9
 Series II	2b	H	H	62.3 ± 1.1	94.5 ± 6.6	>100	>100	97.7 ± 0.3	33.6 ± 3.7	29.1 ± 2.8	>100
	3b	OMe	H	> 100	>100	>100	91.5 ± 6.8	39.1 ± 3.2	>100	51.0 ± 0.9	>100
	4b	H	OH	41.5 ± 6.6	35.1 ± 3.7	58.6 ± 7.1	50.8 ± 6.0	81.6 ± 0.7	38.2 ± 2.1	41.8 ± 1.2	49.7
	5b	OH	H	38.8 ± 6.2	51.0 ± 0.7	64.2 ± 0.8	37.8 ± 3.2	31.6 ± 3.8	30.0 ± 2.2	12.0 ± 4.6	37.9
	6b	H	OMe	>100	>100	>100	>100	>100	82.7 ± 1.5	35.4 ± 3.4	>100

[a]Average of the sum of all IC_{50} values

[b]Parent compounds

[c]Positive control

estrogen-receptor modulators, such as tamoxifen or aromatase inhibitors [29]. The TNBC cancer treatment is focused on cytotoxic agents, such as doxorubicin and taxanes [30]. Thus, the investigation using two types of breast cancer cells can contribute to targeted treatments. We also used four types of cervix cancer cells, which were malignantly transformed by the effects of HPV (HeLa, CaSki, and SiHa cells) or the non-viral process (C33 cells). The HeLa cells are positive for HPV-18 and Caski and SiHa cells are positive for HPV-16. In this context, the HPV-16 and HPV-18 are the most aggressive HPV types in the induction of high-risk cervical intraepithelial neoplasia and cervical carcinoma [31].

The antiproliferative potency was expressed as IC_{50} values (the concentration able to inhibit 50% of cell viability), which were obtained by the MTT assay. In addition, we calculated average IC_{50} values (mIC_{50}) to analyze the overall effect of the curcumin-cinnamaldehyde hybrids and their parent compounds on the panel of women's cancer cells. A $mIC_{50} > 100$ μM was attributed to hybrids with IC_{50} values > 100 μM against at least one cancer cell line. Doxorubicin was used as a reference antineoplastic drug, displaying IC_{50} values ranging from 0.02 to 1.87 μM and a mIC_{50} value of 1.02.

Curcumin (parent compound) displayed activity against all cell lines, with IC_{50} values of 10.4–54.1 μM (mIC_{50} = 26.5 μM). Against MCF-7 (breast cancer cell line), curcumin exhibited an IC_{50} value of 14.9 μM after 48 h. These results are similar to those reported by other authors. Srivastava and Srivastava found an IC_{50} value of 18 μM for curcumin against MCF-7 after 72 h of treatment [10]. Wang and collaborators reported an IC_{50} value of 12.1 μM against HeLa after 72 h of curcumin treatment [32]. Paulraj and co-authors described IC_{50} values of 26.7 and 15.8 μM against HeLa and CaSki cells, respectively, after 72 h of treatment [12]. Wu and collaborators showed that curcumin (at 15 μM) inhibited A431 cell viability by 54% after 48 h of treatment [33].

On the other hand, cinnamaldehyde, another parent compound of the hybridization design, exhibited IC_{50} > 100 μM against all cell lines. Similarly, Wani and collaborators reported IC_{50} values of 160 and 80 μM against MCF-7 and MDA-MB-231 cells, respectively, after 24 h [11]. Singh and co-authors described a 53% reduction of A431 cell viability after treatment with cinnamaldehyde at 80 μM for 24 h. Treating SiHa cells with the same concentration, cinnamaldehyde reduced cell viability by 65% [13].

Hybrids 1a (series I) and 1b (series II) were evaluated to investigate the initial efficacy of the molecular hybridization design. Both hybrids contain a guaiacol ring (*meta*-OMe and *para*-OH on a benzene ring), which is also found in curcumin. Guaiacol (ring A) is spaced to unsubstituted ring B in 1a and 1b by α,β-unsaturated and α,β,γ,δ-unsaturated ketones, respectively. Hybrids 1a and 1b displayed activity against all cell lines, with IC_{50} values of 12.3–66.9 and 27.1–66.3 μM, respectively. The mIC_{50} values of hybrids 1a and 1b were 53.0 and 52.9 μM, respectively. The antiproliferative activity of hybrids 1a and 1b encouraged us to investigate other analogs in an attempt to elucidate the role of substituents (hydroxyl and methoxyl) on ring A, deriving structure-activity relationship data.

We compared the activity of 1a and 1b with their unsubstituted analogs 2a and 2b, respectively. Compound 2b ($mIC_{50} > 100$ μM) was less active than 1b (mIC_{50} = 52.9 μM). Among the cancer cell lines, HeLa and CaSki cells were not sensitive to 2b ($IC_{50} > 100$ μM). On the other hand, 1a ($mIC_{50} = 53.0$ μM) was less active than 2a (mIC_{50} = 29.5 μM). Taken together, these results suggest that the OMe and OH substituents at *meta* and *para* positions, respectively, are important for antiproliferative activity.

For a separate analysis of the effects of *meta*-OMe and *para*-OH substituents, we evaluated pairs of compounds 3a/3b and 4a/4b, respectively. In general, hydroxy-hybrids 4a/4b was more potent than methoxy-hybrids 3a/3b, demonstrating mIC_{50} values of 33.0/49.7 μM and 45.9/100 μM, respectively. These results suggest that the hydroxyl group at *para* position is more important for antiproliferative activity than the methoxyl group at *meta* position.

We evaluated the regioisomers of pairs 3a/3b and 4a/4b, which were designed by replacement of positions of OMe and OH groups, providing hybrids 6a/6b and 5a/5b, respectively. Hydroxy-hybrids 5a/5b ($mIC_{50} = 19.3$ and 37.9 μM) displayed more potent antiproliferative activity than methoxy-hybrids 6a/6b ($mIC_{50} = 43.1$ and >100 μM), corroborating the importance of hydroxyl substituents for bioactivity. Compound 5a exhibited the lowest IC_{50} value among the series I and II curcumin-cinnamaldehyde hybrids ($IC_{50} = 2.7$ μM against vulvar cancer cells), as well as a mIC_{50} of 19.3 μM. Both values were lower than those obtained for curcumin ($IC_{50} = 10.4$ μM and mIC_{50} = 26.5 μM).

In brief, series I hybrids ($mIC_{50} = 19.3$–53.0 μM) displayed higher antiproliferative activity than series II hybrids ($mIC_{50} = 37.9$–>100 μM). The comparison of these mIC_{50} values indicated that one double carbon-carbon bond as the spacer is more important for antiproliferative activity than two double carbon-carbon bonds. Within this context, series I hybrids contain an α,β-unsaturated ketone bridge with an electrophilic β carbon, which can act as a Michael acceptor in reactions with biological nucleophiles from cancer cells. This moiety has demonstrated pharmacophoric contribution to the therapeutic effect of antineoplastic drugs such as afatinib, osimertinib, and rociletinib [34].

Taken together, the bioactivity of curcumin-cinnamaldehyde hybrids corroborated the use of curcumin

Table 2 IC_{50} values (in μM) of parent compounds and 5a against A431 and HaCaT cell lines and respective selectivity index (SI)[a]

Compounds	HaCaT	A431	SI
Curcumin[b]	7.6 ± 2.1	10.4 ± 0.5	0.7
Cinnamaldehyde[b]	>100	>100	nc
Doxorubicin[c]	0.88 ± 0.23	0.05 ± 0.009	17.6
5a	23.0 ± 3.9	2.7 ± 0.2	8.5

nc not calculated

[a]SI: the ratio between IC_{50} for HaCaT and IC_{50} for A431

[b]Parent compounds

[c]Positive control

and cinnamaldehyde as parent compounds in molecular hybridization. This tool has been widely used for the discovery of antineoplastic agents. Wang and co-authors used isatin and curcumin as parent compounds to design a series of hybrids with antineoplastic activities. The activity of curcumin-isatin hybrids was attributed to the presence of an α,β-unsaturated ketone side chain as Michael acceptor [35]. Thus, our results indicate the potential of natural products containing Michael acceptor moieties such as zerumbone [36], xanthohumol [37], withaferin A [38], and celastrol [39] for the discovery of novel antineoplastic agents.

Hybrid 5a was the most potent series I and II curcumin-cinnamaldehyde hybrid and was selected for the subsequent assays. To determine the effect of 5a on non-tumorigenic cells, we selected a spontaneously transformed human keratinocyte cell line derived from histologically normal skin (HaCaT) (Table 2). Hybrid 5a exhibited an IC_{50} of 23.0 μM against HaCaT cells, which was three times less toxic than curcumin ($IC_{50} = 7.6$ μM). The effect of hybrid 5a and its parent compounds on tumorigenic and non-tumorigenic cells was compared by determining their SI. This index was calculated as the ratio between the IC_{50} values obtained for the HaCaT and A431 cell lines. Curcumin and hybrid 5a exhibited SIs of 0.7 and 8.5, respectively, suggesting that hybrid 5a was ninefold more selective than curcumin (Table 2). Cinnamaldehyde showed IC_{50} values > 100 μM against A431 and HaCaT cells, which did not allow the calculation of its SI. Mai and collaborators suggested compounds with IC_{50} values ≤ 10 μM against tumorigenic cells and SI values ≥5 to be selective and promising hits for in vivo investigation. Hybrid 5a exhibited IC_{50} and SI values within the range proposed by Mai and collaborators and may thus be a promising antineoplastic hit [40].

The inhibitory effect of hybrid 5a on the colony formation of A431 and HaCaT cells was evaluated by the clonogenic assay (Fig. 1). This assay permits the evaluation of the antiproliferative activity of compounds over long periods of time [24]. A431 and HaCaT cells were treated with

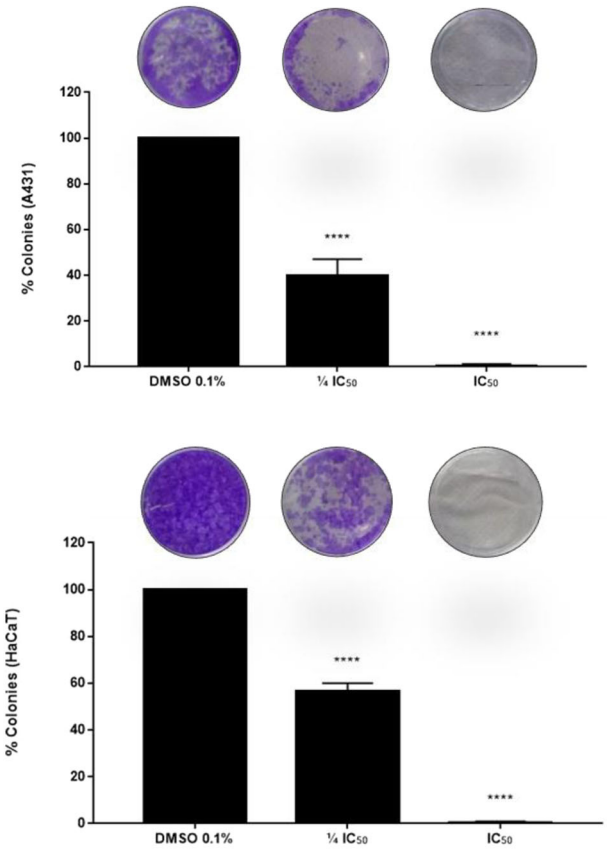


Fig. 1 Qualitative and quantitative data of the inhibitory effect of hybrid 5a against cell colony formation of A431 and HaCaT lines, after 15 days. Cells were treated with hybrid 5a at concentrations corresponding to their IC_{50} (2.7 and 23 μM) and ¼ IC_{50} (0.7 and 6.2 μM) values. Significant differences were determined using ANOVA followed by Tukey test for results ****$p < 0.0001$ vs. vehicle control

hybrid 5a at concentrations corresponding to their IC_{50} (2.7 and 23 μM) and ¼ IC_{50} (0.7 and 6.2 μM) (Fig. 1). A431 colony formation was significantly reduced by hybrid 5a in a concentration-dependent manner when compared to the vehicle control (0.1% DMSO). The number of A431 colonies decreased by 60% in samples treated with 5a (at ¼ IC_{50}), while no colony formation was observed in samples treated with the hybrid at IC_{50}. Hybrid 5a reduced the number of HaCaT colonies by 44% (at ¼ IC_{50}) when compared to the vehicle control. Similar to the inhibition of A431 colonies, HaCaT colonies were completely inhibited by 5a at IC_{50}. Comparison of the inhibitory effect of 5a (at ¼ IC_{50}) against A431 and HaCaT colony formation suggested a higher antiproliferative activity against tumorigenic than non-tumorigenic cells, demonstrating its selectivity over a long period of time.

Compound 5a belongs to the class of chalcones, which are recognized as privileged structures by medicinal chemistry because they have shown numerous interesting bioactivities, including anti-cancer activity [41]. Mahapatra and

co-authors reviewed the inhibitory potential of chalcones against various anti-cancer targets, including 5α-reductase, aromatase, histone deacetylases, sirtuins, proteasome, JAK/STAT signaling pathway, Wnt signaling pathway, cathepsin-K, tubulin, topoisomerases, kinase, and NF-κB. In addition, chalcones are able to act on multidrug resistance channels, inhibiting ABCG2, BCRP, and P-glycoprotein [42]. Moreover, our group has dedicated efforts to describe other anti-cancer targets of chalcones, including protein p53, DNAJB1, Sp1, and CRM1 [43–46].

The chemical stability of hybrid 5a and its parent compounds was evaluated at pH 7.4 and 37 °C by UV–Vis absorption decay (Fig. 2). Curcumin and cinnamaldehyde were rapidly degraded after 24 h (reduction of 60 and 40%, respectively). The values of UV–Vis absorption decay of curcumin were previously described by our group [26]. On the other hand, hybrid 5a was more stable than its parent compounds (reduction of 21%). The enhanced stability of 5a under physiological conditions is key to the development of curcumin-cinnamaldehyde hybrids because their parent compounds have demonstrated poor chemical stability, which has been an obstacle to in vivo assays [16, 17].

Drug-likeness properties may be defined as a set of structural and physicochemical features that indicate whether or not a hit compound is similar to drugs [47], justifying further chemical and biological studies. Several drug-likeness sets to filter hit compounds have been described, including Lipinski's [48], Veber's [49], Muegge's [50, 51], and Ghose's rules [52]. Among these sets, the "rule-of-five" established by Lipinski and collaborators is one of the best-known and widely used filters [48]. Lipinski's rule includes the logarithm values of the compound partition coefficient between *n*-octanol and water (log $P_{o/w} \leq 5.0$), molecular weight (MW ≤ 500 Da), the number of hydrogen bond acceptors (HBA ≤ 10), and the number of hydrogen bond donors (HBD ≤ 5). Compound 5a demonstrated log $P_{o/w} = 3.31$, MW = 224.26 Da, HBD = 1, and HBA = 2. Veber and collaborators [49] reported two main drug-likeness properties: the sum of HBA and HBD (HBA + HBD ≤ 12) and the number of rotatable bonds (NROBT ≤ 10). Hybrid 5a presented HBA + HBD = 3 and NROBT = 3. Muegge and co-authors developed rapid and simple criteria to select pharmacophore points. The central point is related to the number of functionalities and rings on the drug structure, which can display pharmacophoric contributions [50, 51]. Hybrid 5a exhibited two functional groups, a carbonyl group (as part of the enone bridge) and a hydroxyl group (as part of phenolic ring A), which may be pharmacophoric motifs. Moreover, hybrid 5a displayed two rings, a phenol (ring A) and an unsubstituted benzene ring (ring B), which are the result of molecular hybridization between curcumin and cinnamaldehyde. Ghose and collaborators analyzed the Comprehensive Medicinal Chemistry (CMC) database and classified ranges of molecular properties of drugs, including antineoplastic drugs [52]. Among these, drugs displayed a total number of atoms in the range of 20–70 (average number of 48). Compound 5a possesses 29 atoms. Thus, hybrid 5a did not violate the Lipinski, Veber, Muegge, or Ghose rules, demonstrating its drug-likeness properties.

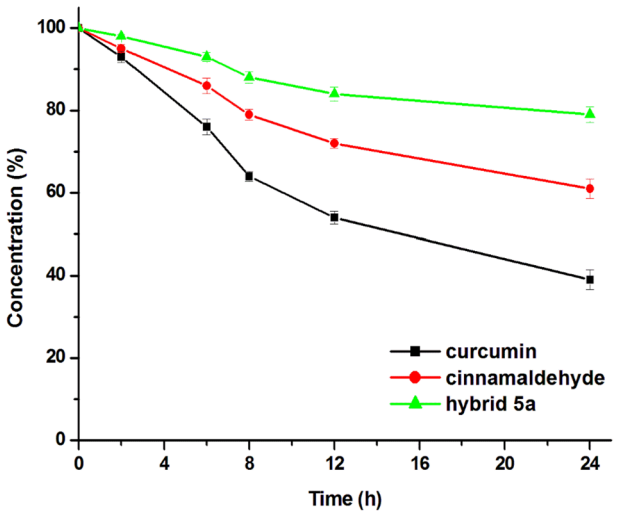


Fig. 2 Chemical stability of hybrid 5a and parent compounds (pH 7.4) by monitoring UV–Vis absorption decay at their respective maximum absorbance and 37 °C for 24 h. Data are represented as means ± SEMs and expressed as a percentage (%)

Conclusions

We evaluated two series of curcumin-cinnamaldehyde hybrids as part of the ongoing search for antineoplastic compounds derived from natural products using molecular hybridization. Series I hybrids were more potent antiproliferative agents than series II hybrids, a finding that might be related to the presence of the electrophilic β carbon. Hybrid 5a was found to be a hit, showing activity against breast, cervical and vulvar cancer cells and inhibiting vulvar colony formation after 15 days. Investigations using non-tumorigenic cells (HaCaT) indicated that 5a was ninefold more toxic to cancer vulvar cells than to normal cells. Chemical stability assays demonstrated 5a to be more stable than curcumin and cinnamaldehyde under physiological conditions. In addition, a brief in silico investigation confirmed the drug-likeness properties of 5a. These results indicate the use of curcumin and cinnamaldehyde as parent compounds for the design of hybrids with attractive antiproliferative activity and chemical stability.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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