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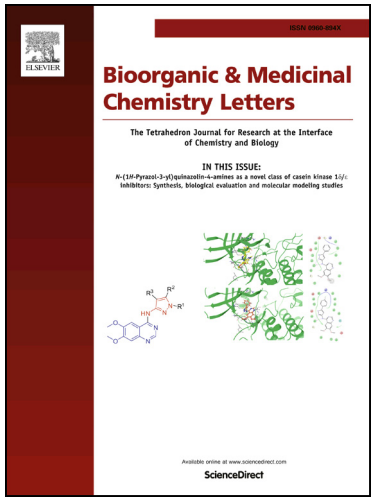

Design and multi-step synthesis of chalcone-polyamine conjugates as potent antiproliferative agents

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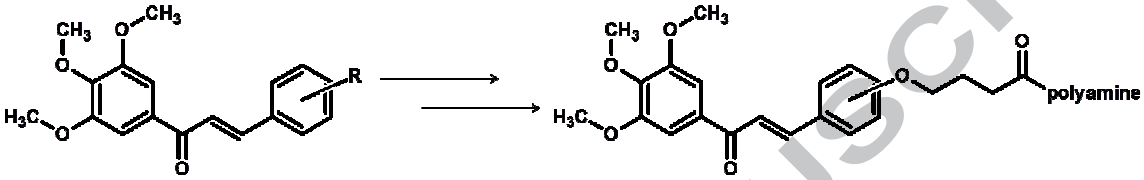

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Design and multi-step synthesis of chalcone-polyamine conjugates as potent antiproliferative agents.

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ABSTRACT

The aim of this study is to synthesize chalcone-polyamine conjugates in order to enhance bioavailability and selectivity of chalcone core towards cancer cells, using polyamine-based vectors. 3-hydroxy-3',4,4',5'-tetramethoxychalcone (**1**) and 3',4,4',5'-tetramethoxychalcone (**2**) were selected as parent chalcones since they were found to be efficient anti-proliferative agents on various cancer cells. A series of ten chalcone-polyamine conjugates was obtained by reacting carboxychalcones with different polyamine tails. Chalcones **1** and **2** showed a strong cytotoxic activity against two prostatic cancer (PC-3 and DU-145) and two colorectal cancer (HT-29 and HCT-116) cell lines. Then, chalcone-spermine conjugates **7d** and **8d** were shown to be the most active of the series and could be considered as promising compounds for colon and prostatic cancer adjuvant therapy.

Chalcones constitute an important group of natural products belonging to the flavonoid family.[1] They are open-chain molecules in which two aromatic rings are joined by a three-carbon enone fragment. Chalcones and derivatives received significant attention due to their wide range of pharmacological activities, including anti-inflammatory,[2] anti-bacterial,[3] and anti-cancer properties.[4-6] More particularly, some of these compounds have been found to induce apoptosis in a variety of cell lines.[7-10] Considering structure-activity relationship, a trimethoxyphenyl ring is thought to be of great interest for anticancer activity of chalcones.[11-13] As reported by Ducki et al., 3-hydroxy-3',4,4',5'-tetramethoxychalcone (chalcone **1** in our study, Figure 1), have shown an important antiproliferative activity against K562 cell line.[12] This chalcone, bearing the same aromatic substitution pattern as combretastatin A4 (CA-4) (Figure 1), a highly cytotoxic natural product, also demonstrated an antiproliferative effect against various cancer cells and was further used as a lead compound of a series of anti-mitotic and pro-apoptotic agents.[14] Besides, Qi et al. reported a series of CA-4 related chalcones;[15] among them, 3',4,4',5'-tetramethoxychalcone (chalcone **2** in our study, Figure 1) was found to inhibit the proliferation of ovarian cancer cells. Considering these findings, we became interested in performing structural modifications of the chalcone core to improve both bioavailability and selectivity towards cancer cells. Our study focused on the derivatization of chalcones **1** and **2**.

An interesting approach for enhanced drug delivery is the use of polyamine-based vectors. Polyamines such as putrescine, spermidine or spermine, play an essential role as regulators of cell proliferation and differentiation.[16-17] It has been shown that many tumor cell lines are highly dependent on these growth factors.[18] Cancer cells are unable to biosynthesize enough polyamines to sustain their rapid growth rate. They consequently rely on exogenous polyamines, imported by the polyamine transport system (PTS) which is actually hyperactive in cancer cells. This system has been shown to display a relatively loose specificity;[19] accordingly, some polyamine-drug conjugates have been reported to be delivered into tumor cells expressing the PTS.[20-25]

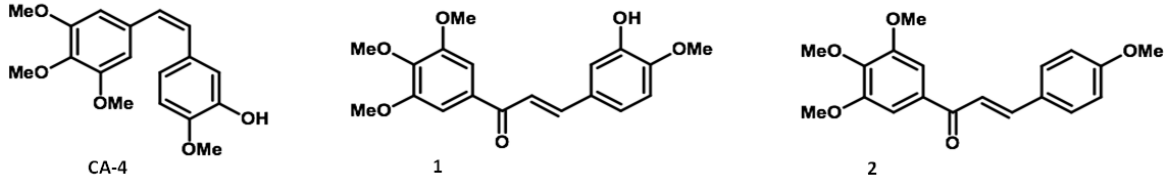


Figure 1 Chemical structures of combretastatin A4 (CA-4), 3-hydroxy-3',4,4',5'-tetramethoxychalcone (**1**) and 3',4,4',5'-tetramethoxychalcone (**2**).

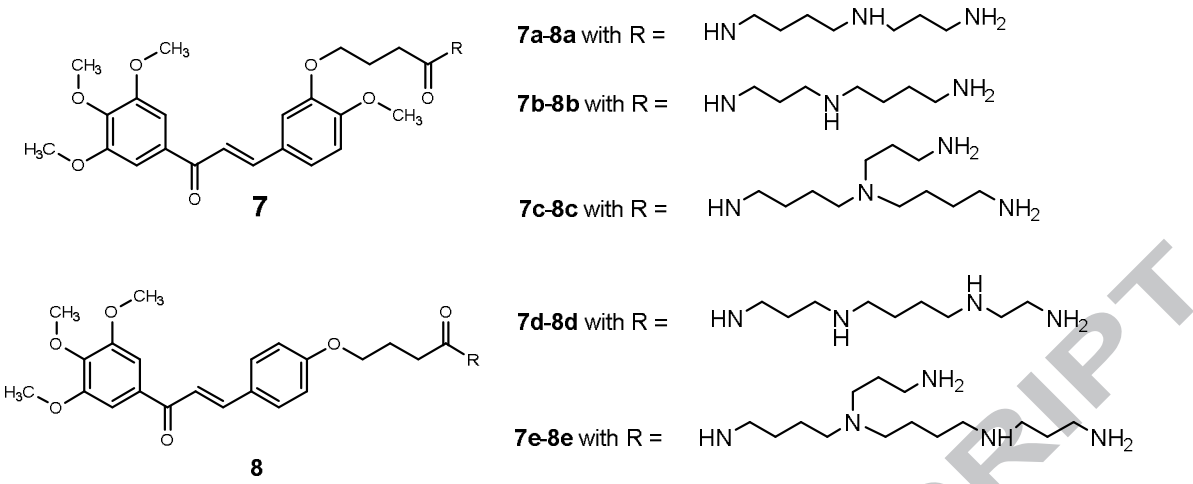


Figure 2 Chemical structures of chalcone-polyamine conjugates

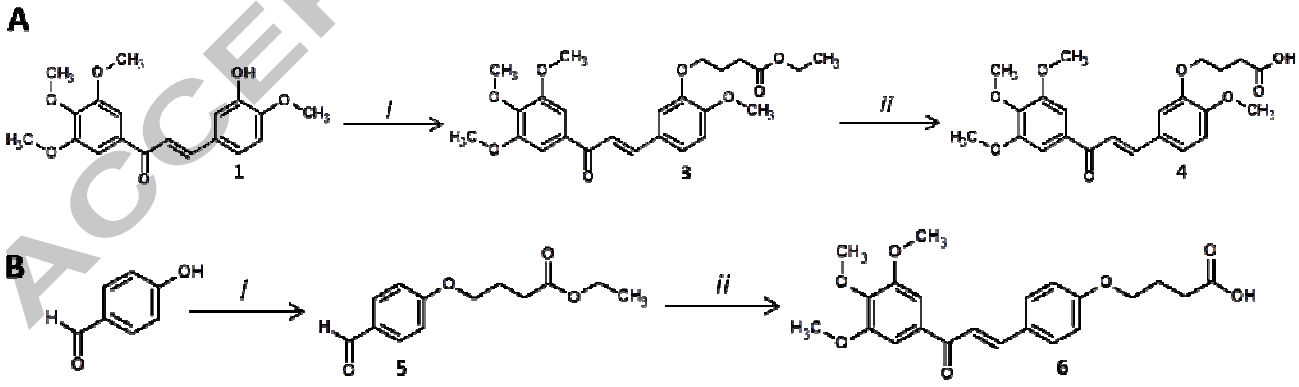
The present work deals with the synthesis of chalcone-polyamine conjugates **7a-e**, **8a-e** (Figure 2). Then, antiproliferative activities of these derivatives were evaluated against two colorectal (HT-29 and HCT-116) and two prostatic (PC-3 and DU-145) cancer cell lines, and compared with those of parent chalcones **1** and **2**.

Chalcones **1** and **2** were prepared using the Claisen-Schmidt condensation from building-blocks, namely 3,4,5-trimethoxyacetophenone and appropriate benzaldehydes.[10, 15] ^{1}H NMR spectra showed that the (*E*)-stereoisomers were specifically generated since the coupling constant between the two ethylenic protons was about 15-16 Hz. Chalcone **2** was obtained from 4-methoxybenzaldehyde in 51% yield while chalcone **1** was prepared in a good yield (69%) from 3-hydroxy-4-methoxybenzaldehyde.

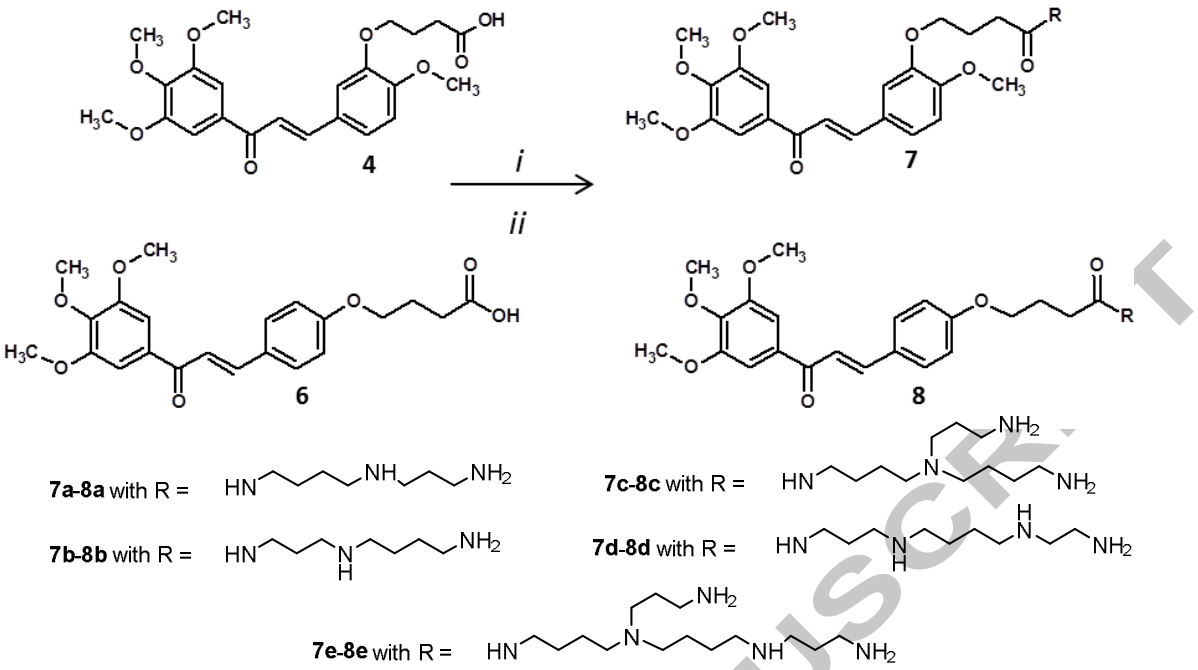
Ten polyamine-chalcone conjugates (**7a-e**, **8a-e**) were synthesized, following a multistep strategy where chalcone core and polyamine tails were fused through an amide bond. The synthetic pathway involved the preparation of carboxy-chalcones **4** and **6** for the synthesis of the series **7** and **8** respectively. The preparation of carboxy-chalcone **4** first started with alkylation of the phenolic function of chalcone **1** by ethyl-4-bromobutyrate, to give **3** in a quantitative yield (Scheme 1A). Finally, the ester function was turned into a carboxylic acid in quantitative yield too, through the action of lithium hydroxide.

The synthesis of carboxy-chalcone **6** used a different synthetic pathway in order to avoid the multistep synthesis of the 4-hydroxy-3',4',5'-trimethoxychalcone as an intermediate. Indeed, the preparation of 4-hydroxychalcones involved a preliminary protection step of 4-hydroxy-benzaldehyde prior to the Claisen-Schmidt condensation. A new pathway consisted in the *O*-substitution of 4-hydroxy-benzaldehyde, by reaction with ethyl-4-bromobutyrate, followed by the Claisen-Schmidt condensation with 3',4',5'-trimethoxyacetophenone (Scheme 1B). Due to the alkaline conditions, condensation and saponification occurred during the same step, leading directly to compound **6**. This strategy only involved two steps with a 57% global yield.

Protected linear polyamines were purchased whereas ramified polyamines were prepared following the strategy developed in our laboratory and previously described.[23] Many ways for the selective protection of polyamines have been reported.[26] In our study, we chose the BOC-protective group that can be selectively removed with TFA or HCl.



Scheme 1 A: Reagents and conditions for synthesis of compound **4**: (i) K_2CO_3 (20 eq), ethyl-4-bromobutyrate (10 eq), DMF, rt, 2h, 98%; (ii) LiOH (5 eq), THF/H_2O (8/2), rt, 3h, 94%. **B**: Reagents and conditions for synthesis of **6**: (*i*) K_2CO_3 (20 eq), ethyl-4-bromobutyrate (10 eq), DMF, rt, 1h, 98%; (*ii*) 3,4,5-trimethoxyacetophenone (0.84 eq), NaOH (4.17 eq), MeOH, reflux, 1h, 58%.



Scheme 2 (i) Corresponding polyamine tail (1.1 eq), DCC (1.1 eq), HOBt (1.1 eq), CH_2Cl_2/MeOH (10/1), rt, 4h; (ii) HCl (12 eq), EtOH, reflux, 1h30.

The synthetic pathway of polyamine-chalcone conjugates through the formation of an amide bond was described in Scheme 2 ; it involved the coupling of the carboxylic acid function of chalcones **4** and **6**, with the primary amine function of the polyamine tails, in presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) as coupling agents.
Removal of the *tert*-butoxy-carbonyl (BOC) was performed using aqueous hydrochloric acid in ethanol at reflux. All the yields are reported in Table 1.

Table 1: synthesis conditions of polyamine-chalcone conjugates **7** & **8**.

Compounds	Coupling yield (%) (*i*)	BOC removing yield (%) (*ii*)	Global yield (%)
7a	77	74	57
7b	88	95	84
7c	66	82	55
7d	96	54	52
7e	87	49	43
8a	68	44	30
8b	64	51	33
8c	64	38	25
8d	62	45	28
8e	68	33	23

The coupling reaction between the carboxy-chalcones and polyamine tails, using DCC and HOBt as coupling agents, was really efficient since all yields were higher than 60%. On the other hand, removal of the BOC groups was more difficult. Usual conditions with trifluoroacetic acid (TFA) in dichloromethane (CH_2Cl_2) led to the degradation of the reaction mixture. Improved results were obtained in presence of hydrochloric acid (HCl) in ethanol (EtOH) although with variable and sometimes moderate yields, due to the formation of by-products. Synthetic procedures and characterizations (FTIR, ^1H and ^{13}C NMR, MS) are detailed in supplementary data.
In medicinal chemistry, balance between hydrophilicity and lipophilicity has proven to be an important molecular descriptor that often is well-correlated with the bioactivity of drugs. Lipophilicity could be indicated, for example, by the logarithm of a partition coefficient, log *P*, which reflects the equilibrium partitioning of a molecule between a nonpolar and a polar phase, such as the *1*-octanol/water system.[27] In this work, we determined log *P* of parent chalcones **1, 2** and chalcone-polyamine conjugates **7a**, **8b**, **7d** and **8d** as log ($[Chalcone]_{1\text{-octanol}}$ /$[Chalcone]_{water}$) (see supplementary data). Results indicated that the addition of polyamines tails strongly increased the hydrophilic character of chalcones which is a very interesting property for biological applications. (Table 2).

Table 2: partition coefficients of chalcone **1**, **2** and chalcone polyamine conjugates **7a**, **8b**, **7d** and **8d**.

Derivatives	1	2	7a	7d	8b	8d
log *P*	2.99	3.10	-1.28	-1.57	-1.43	-1.98

Parent chalcones **1** and **2**, considered as reference standards, as well as the synthesized polyamine-chalcone conjugates were evaluated for their antiproliferative effect against two colorectal (HT-29 and HCT-116) and two prostatic (PC-3 and DU-145) cancer cell lines using a MTT-based assay (supplementary data).
Chalcones **1** and **2** are known to possess promising anticancer activity. Thus, chalcone **1** was identified for antiproliferative activity on leukemia (K562) and lung (A549) cancer cell lines,[11-14] while chalcone **2** proved antiproliferative activity against ovarian cancer cell lines.[15] Our work is focused for a long time on colorectal and prostatic cancers.[28-31] Thus, a previous study was performed to explore the cell death pathway induced by chalcone **1** in colorectal cancer cell lines.[32]

In the present work, chalcones **1** and **2** were found to be active on all the cancer cell lines studied. The results are outlined in Table 2. Chalcone **1** showed a higher activity than chalcone **2** except against HT-29 cell line, which is COX-2 sufficient. Meanwhile, it has been demonstrated that COX-2 plays a critical role in resistance to apoptosis.[28,29,32] Antiproliferative effects of chalcone-polyamine conjugates were moderate in comparison with those of parent chalcones **1** and **2**. Comparison of the IC_{50}

values between the two series **7** and **8** shows that compounds bearing the same substituent pattern do not display significant differences in their respective cytotoxicities, with the exception of experiments on HCT-116 cell line. Indeed, in this case, IC_{50} of series **8** conjugates are generally lower than IC_{50} of series **7** ones. Considering the four cell lines, HT-29 appeared as the most resistant. Indeed, all the IC_{50} were higher than 70 µM. On the contrary, HCT-116 which is COX-2 deficient, seemed to be the most sensitive cell line. Among the ten conjugates, half of them possessed IC_{50} lower than 40 µM. Thus, **8a** conjugate was the most active compound against HCT-116 cells, with an IC_{50} of 20 µM. These results highlighted the potential of the N^8 spermidine tail and the total lack of interest of the N^1 spermidine tail, and were in agreement with those obtained against PC-3 and DU-145 cell lines. The **7b** and **8b** conjugates were devoid of activity against these prostatic cancer cells whereas **7a** and **8a** exhibited a weak, but measurable, activity. Therefore, the architecture of the spermidine tail clearly affected the bioactivity. As described for artemisinin spermidine conjugates,[25] coupling at the N^8-centre of the spermidine tail provided compounds more active than the N^1-conjugates (Figure 2).
Concerning PC-3 and DU-145 cell lines, interesting results were obtained with derivatives built with the linear spermine pattern as shown in **7d** and **8d**. Indeed, in both series **7** and **8**, this polyamine moiety provided compounds with IC_{50} values around 30 µM whereas all other conjugates gave IC_{50} higher than 50 µM.

In a global manner, spermine conjugates **7d** and **8d** appeared as the most interesting compounds. These two compounds were not only the most active against PC-3 and DU-145 cell lines but they also presented an antiproliferative effect against HCT-116 cells. These results are in agreement with published studies showing that the spermine tail is able to increase the water solubility of the spermine-drug conjugate, to serve as cell delivery vector by using the PTS for tumor cell delivery as well as DNA anchor.[20, 33,34] In our case, and even if these conjugates are less active *in vitro* than their parent chalcone **1** and **2**, the water solubility is really increased and, the lower cytotoxicity of **7d** and **8d**, can be explained by the higher affinity of spermine for DNA which are not the key intracellular target of our chalcone derivatives.

Table 2: IC_{50}* values (µM) from MTT assay with colorectal (HT-29 and HCT-116) and prostatic (PC-3 and DU-145) cancer cell lines.

Compounds	HT-29	HCT-116	PC-3	DU-145
7a	>100	38.0±3.1	51.0±8.5	99.3±5.6
7b	>100	>100	81.0±9.3	>100
7c	74.7±2.9	73.6±4.2	60.6±9.0	82.3±5.3
7d	>100	55.8±5.4	31.8±4.8	28.5±1.5
7e	89.1±6.4	29.5±9.7	48.1±2.9	84.9±1.3
8a	>100	20.0±1.2	79.3±4.1	54.8±3.3
8b	>100	>100	>100	>100
8c	87.2±3.2	29.2±6.4	55.4±6.4	86.8±0.2
8d	98.0±4.8	33.8±3.3	34.0±4.5	35.4±5.2
8e	91.7±5.3	75.4± 2.1	81.6±9.9	92.5±5.7
1	23.1±1.7	0.85±0.11	0.09±0.002	6.3±1.2
2	7.4±0.6	10.7±0.6	7.8±0.5	13.8±1.7

*IC_{50} values are the average of three determinations at least.

In summary, this study allowed to confirm the great interest of chalcones **1** and **2** for anticancer activity since they were found to be active on two prostatic (PC-3 and DU-145) and two colorectal (HT-29 and HCT-116) cancer cell lines. Polyamine conjugates (compounds **7a-e** and **8a-e**) were designed, based on an amide linkage, from these two chalcones. Contrary to our expectations, the parent chalcones remained more active than their polyamine counterparts. Nevertheless, some conjugates could be efficient therapeutics since, on the one hand, their polyamine tail is intended to increase their hydrophilic character and improve drug delivery and selectivity toward cancer cells and, on the other hand, their *in vitro* antiproliferative effect is significant.[35] Thus, N^8-spermidine conjugate **8a** could be a promising compound for colon cancer adjuvant therapy since it demonstrated a great antiproliferative activity against HCT-116 cells. Besides, spermine conjugates also gave good results: **8d** displayed an interesting effect on three cancer cell lines (HCT-116, PC-3 and DU-145) and **7d** even showed a slightly better activity than **8d** on prostatic cancer cell lines. These latter molecules may be considered as potent compounds for prostatic cancer therapy. Further studies are necessary to more closely examine the underlying molecular mechanisms of these compounds but also to design new polyamine-chalcone conjugates with a view to studying the influence of the linker and the length of the spacer on cytotoxic properties.

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Supplementary data

Supplementary data (experimental procedure for the synthesis, spectral data, and experimental information concerning partition coefficient measurement) associated with this article can be found in the online version, at

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