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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel 1-methoxyindole- and 2-alkoxyindole-based chalcones: design, synthesis, characterization, antiproliferative activity and DNA, BSA binding interactions

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Abstract

Indole-based chalcones have been identified as interesting compounds with anticancer properties. In the present study, we report the synthesis and evaluation of new 1-methoxyindole and 2-alkoxyindole chalcone hybrids as antiproliferative agents active against colorectal carcinoma cell line. Among the 19 investigated molecules, four inhibit the proliferation of colorectal cancer cells HCT-116 with IC_{50} values <8 μM and display low cytotoxicity to fibroblast cell line 3T3. The UV–visible, CD and fluorescence competitive displacement assays with ethidium bromide and Hoechst 33258 performed with two active chalcones demonstrated that investigated chalcones interact with calf thymus (CT) DNA through the groove binding mode. Likewise, the quenching interaction of chalcones with bovine serum albumin (BSA) was studied in vitro under optimal physiological condition (pH = 7.4). The Stern–Volmer constant for chalcone-BSA system was found in the range of 10^5 M^{-1}.

Keywords Chalcones · 1-methoxyindole · Nucleophilic substitution · Antiproliferative activity · DNA and BSA binding activities

Introduction

Chalcone scaffold [(*E*)-1,3-diphenylprop-2-en-1-one)] retains an attention among the medicinal chemists due to its wide variety of pharmacological activities, namely cancer-preventive and neuroprotective effects, as well as anti-inflammatory, antibacterial, antiviral, antidiabetic, antioxidant, antimalarial and other biological activities [1–3]. Several chalcones have shown significant anticancer activity mediated through inhibitory effect against various targets [4].

The indole moiety is a component of biomolecules and many natural bioactive substances. It is frequently used structure in an anticancer drug design [5]. Considering interesting biological properties of indole-based compounds and their derivatives, several types of hybrid indole-chalcone compounds have been prepared. The indole skeleton may be located at the 1 or 3 position of the prop-2-en-1-one structure, most commonly attached via indole carbon 3 or 5. Many indole-derived chalcones have shown anti-tumor activity. For instance, 1-(3,4,5-trimethoxyphenyl)-3-(1-indol-3-yl)-chalcones **1a**,**b** were identified as selective and potent anticancer agents against pancreatic carcinoma PaCa-2 (Fig. 1) [6]. The cytotoxic indole-chalcones **2**-**4** were synthesized and docking studies that support the

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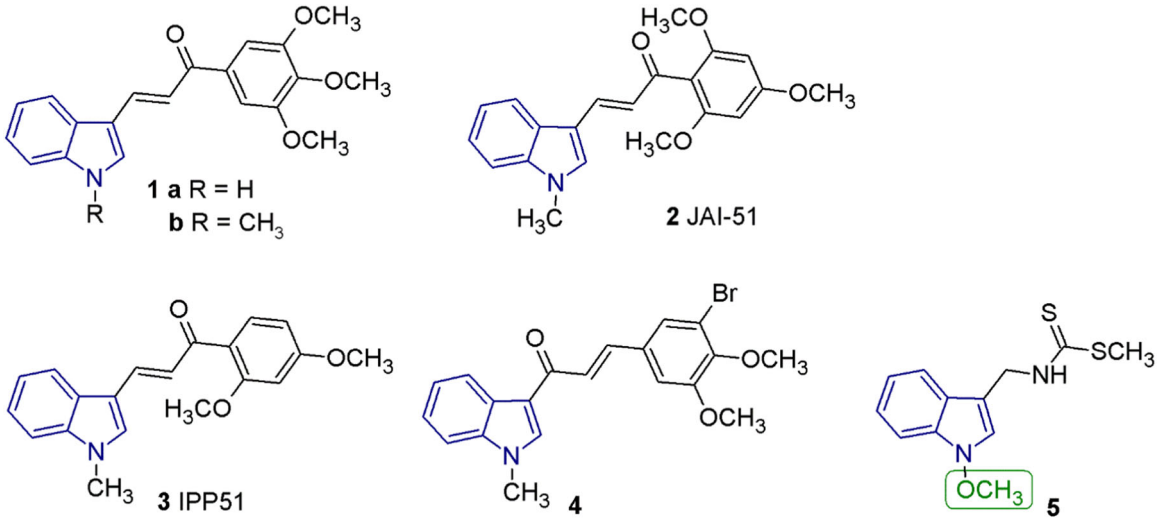


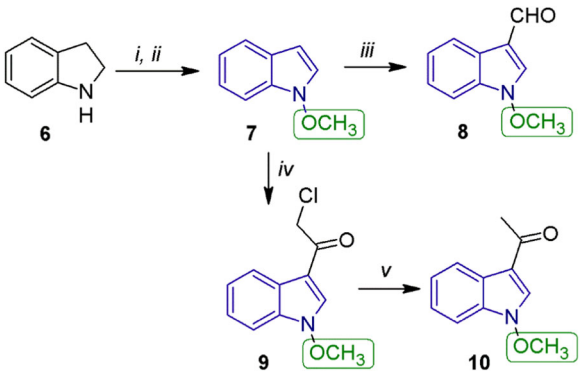
Fig. 1 Structure of biologically active chalcones with indol skeleton and 1-methoxybrassinin

potential of these compounds to bind to the colchicine site of tubulin and inhibit tubulin polymerization were reported [7–9]. In addition, in vitro and in vivo experiments suggest that **2** (JAI-51) can easily cross the brain-blood barrier and therefore the molecule is a potential candidate for a new treatment of the central nervous system tumors [9].

Indole phytoalexins are a specific group of natural compounds produced by worldwide-cultivated plants of the *Cruciferae* family [10]. Besides their antimicrobial activity against different plants' pathogenic species, cruciferous phytoalexins have shown anticancer activities against various human cell lines [11]. Nearly half of indole phytoalexins contain 1-methoxyindole skeleton. Among them, 1-methoxybrassinin (**5**) showed the most effective cytotoxic and apoptosis-inducing activity against human Jurkat cell lines [12]. The study of the antiproliferative activity of brassinin and its twenty derivatives on human cancer cell lines revealed that **5** effectively inhibits cell proliferation and induces apoptosis in colorectal cancer cells. 1-Methoxybrassinin (**5**) also has potential as a radio- or chemosensing agent [13].

Many natural chalcones contain methoxy and hydroxyl groups on the phenyl core. The presence of these groups significantly affects their biological properties and are important structural elements in antioxidant activities of chalcones [14]. The importance of the presence of methoxy groups on the phenyl nucleus was highlighted and investigated in the study of the antitumor activity of indole hybrid chalcones [15, 16]. To our best knowledge, there is only one publication of synthesis of chalcones with a 1-methoxyindole skeleton from 3-formyl-2-substituted 1-methoxyindole-5,6-dicarbonitriles [17].

In the present study, we report the synthesis, characterization and evaluation of biological activity of 19 new

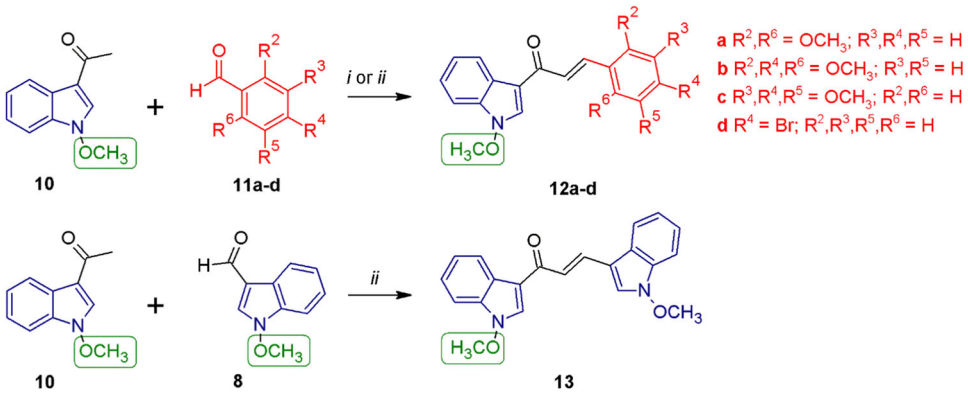


Scheme 1 Synthesis of key synthetic intermediates (i) $Na_2WO_4.2H_2O$, H_2O_2; (ii) K_2CO_3, DMS; (iii) $POCl_3$, DMF; (iv) $ClCH_2COCl$; (v) AIBN, $(n\text{-}Bu)_3SnH$

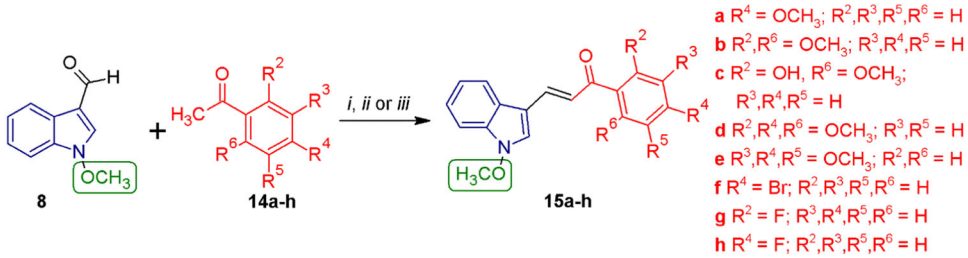
hybrid chalcones with 1-methoxy- or 2-alkoxy- indole moiety and methoxy- or halogen-substituted phenyl ring.

Chemistry

We have synthesized two sets of chalcones with 1-methoxyindole skeleton in position 1 or 3 of prop-2-en-1-one. Third set includes 1-(4-fluorophenyl)-3-(2-alkyloxyindol)prop-2-en-3-ones. Acid or basic catalyzed Claisen–Schmidt condensation was a key step of the synthesis. In the first step, aldehyde **8**, and ketone **10**, were synthetized from indoline (**6**, Scheme 1). Oxidation of indoline (**6**) using Soimei-Wolframan method and subsequent methylation with dimethylsulphate according to the literature [18] resulted in production of 1-metoxyindole (**7**). 1-Methoxyindole-3-carboxaldehyde (**8**) was prepared by the Vilsmeier–Haack reaction [19]



Scheme 2 Preparation of chalcones: Type A Reagents and conditions: (i) 50% aq. KOH, EtOH or iso-ProOH, r.t.; (ii) $SOCl_2$, dry EtOH, r.t



Scheme 3 Preparation of chalcones: Type B Reagents and conditions: (i) 50% aq. KOH, EtOH or iso-ProOH, r.t.; (ii) $SOCl_2$, dry EtOH, r.t.; (iii) piperidine, EtOH, 50 °C

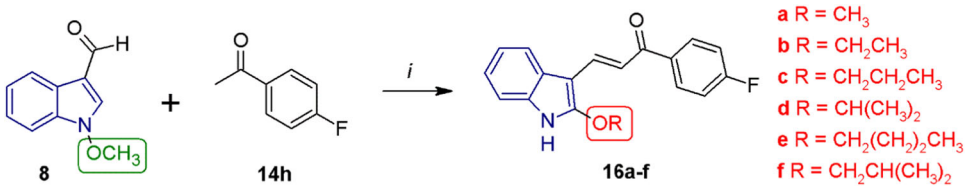
and 3-acetyl-1-methoxyindole (**10**) [20] in two steps from the 1-methoxyindole (**7**).

General method for preparation of chalcones involves acid or base catalyzed Claisen–Schmidt condensation [2]. The synthesis of indole hybrid chalcones was described by Claisen–Schmidt condensation in the presence of LiOH hydrate in EtOH at r.t [8], the 10% aq. NaOH [6, 21], the 50% aq. KOH [15, 16] under refluxing conditions in EtOH or MeOH. The first set of chalcones A was prepared by the condensation of 3-acetyl-1-methoxyindole (**10**) with appropriate aldehydes **11a-d** and **8** (Scheme 2) using the 50% aq. KOH in EtOH. The synthesis of unsubstituted and N-methylated indole hybride chalcones is usually performed under the reflux [15, 16], however, in our case this led to the increased amount of by-products. Therefore, it was advisable to carry out the reaction at r.t. resulting in the lower presence of by-products. In the cases where basic catalysis resulted in the low yield of the desired compound **12a**, **12b**, **13**, an in situ generated HCl by acid catalytic system of $SOCl_2$ in anhydrous EtOH [22] was applied.

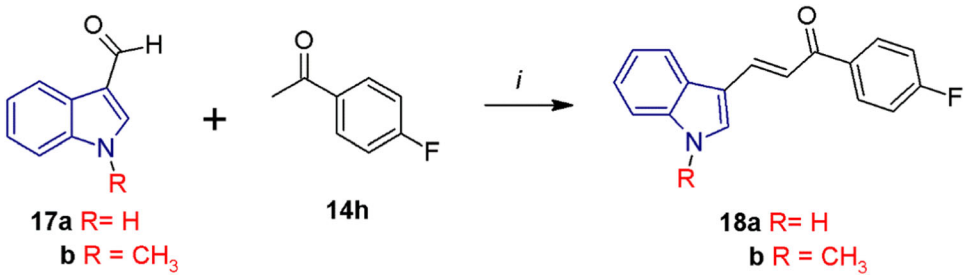
For the synthesis of 3-(1-methoxyindol-3-yl)propenones (set B), the same reaction conditions as for the previous set were used, starting from 1-methoxyindole-3-carboxaldehyde (**8**, Scheme 3) and derivatives of acetophenones **14a-h**. In the case of 2-hydroxy-6-methoxyacetophenone **14c**, the application of piperidine-mediated synthesis [23] prevents cyclisation of 2-hydroxy functional group to the corresponding flavanones that may occur under catalysis with stronger bases.

Interestingly, during the basic catalyzed condensation (with 50% aq. KOH in EtOH) we have observed the formation of a by-product in a significant concentration. Analysis of the by-product of reaction with acetophenone **14 h** showed that the nucleophilic substitution at the 2-position of the indole with simultaneous liberation of 1-methoxy group leading to the formation of **16b** takes place together with condensation reaction. Indole is a well-known electron-rich hetero-aromatic structure and electrophilic substitution reactions of indoles are well studied. Until the discovery of 1-hydroxyindole chemistry by Somei [24] the nucleophilic substitution reaction was not common in the indole chemistry. Since then, it was demonstrated that hydroxy or methoxy group at the indol nitrogen can behave as a good leaving group when electron-withdrawing group is present in the indole skeleton [25]. It was concluded that 1-methoxyindoles having electron-withdrawing group such as acetyl **10** and formyl **8** at the 3-position readily undergo nucleophilic substitution reactions regioselectively at the position 2 with simultaneous release of 1-methoxy group [20, 26].

These findings, as well as the excellent antiproliferative potency of the 4-fluoro derivative **15 h**, led us to the preparation of the last series C (Scheme 4). We examined condensations of aldehyde **8** with acetophenone **14 h** in alcohols with different alkyl chains under basic conditions. The condensation and co-nucleophilic substitution reaction products 3-(2-alkoxyindol-3-yl)-1-(4-fluorophenyl)prop-2-en-4-ones **16a–f** were obtained in 26–32% yield. The last two 4-fluorophenyl chalcones **18a** and **18b** (Scheme 5),



Scheme 4 Preparation of chalcones: Type C Reagents and conditions: (i) 50% aq. KOH, ROH, r.t



Scheme 5 Preparation of chalcones **18a,b** Reagents and conditions: **18a** [6], for **18b** (i) 50% aq. KOH, ROH, r.t., 68%

previously synthesized by [6] supplemented the C series with indole nitrogen substituted derivatives.

All compounds were isolated, purified, and characterized by means of ^{1}H and ^{13}C NMR and HR-MS (Figs. S1–S63 in supplementary). The vinylic protons were seen as doublets at 6.99–8.02 ppm for H-2 and at 7.48–8.25 ppm for H-3. The obtained chalcones were assigned as (*E*)-stereoisomers based on the observed coupling constants between vinylic hydrogens in the range from 15.3 to 15.8 Hz in the ^{1}H NMR spectrum. The signal of carbonyl carbon was between δ 182.3 and 194.6 ppm in ^{13}C NMR spectrum. It was found the C=O stretching vibrations for the enones ranged from 1610 to 1653 cm^{-1} in IR spectrum.

The presence of a methoxy group on the indole nitrogen significantly increases the reactivity of the Claisen–Schmidt condensation starting materials (**8**, **10**), increases the solubility of the products and especially allows the derivatization of chalcones in position 2 of the indole skeleton due to nucleophilic substitution under basic conditions.

Antiproliferative activity

Screening for potential antiproliferative activity of the synthesized hybrid chalcones was performed on the A549 (human alveolar adenocarcinoma), Caco-2 (human epithelial colorectal adenocarcinoma), HCT116 (human colorectal carcinoma), HeLa (human cervical adenocarcinoma), MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human mammary gland adenocarcinoma), and Jurkat (human acute T-lymphoblastic leukemia) cell lines. In order to investigate the selective cytotoxicity against cancer cells over normal cells, all compounds were further tested using the normal fibroblast cell line 3T3 (mouse embryo fibroblast). The determined IC_{50} values of synthesized indole hybrid chalcones in comparison with reference drug cisplatin are listed in Table 1. The data are presented as the mean ± SD of three independent experiments and IC_{50} values less than μM of Cisplatin are typed in bold.

The highest efficacy was demonstrated by newly synthesized chalcones against leukemic Jurkat cell, which are rapidly proliferating and more sensitive cells. The IC_{50} values (excluding three compounds) ranged from 3.9 to 15 μM. In contrast, the lowest sensitivity to test compounds was found for the mammary adenocarcinoma cell line MDA-MB-231. In the first tested series, the presence of three methoxy groups at 2,4,6-positions (compound **12b**) resulted in the best activity with $IC_{50} < 7$ μM on HCT116, MCF-7 and Jurkat cell lines. This finding is consistent with the data reported for its 1-methylindole analog, one of the three most active compounds against bladder cancer cells in the Martel-Frachet study [15]. The regioisomeric analog **15d** showed lower activity on all cell lines than **12b**, as well as the regioisomer in the mentioned study. This indicates the need for a suitable choice of linker to the propenone chain, which will ensure a suitable electronic distribution throughout the molecule. The 3,5-dimethoxy and 3,4,5-trimethoxy substituted chalcones **12a** and **12c** from the first series showed very good specificity toward HCT116 cells. However, the regioisomer, 3,4,5-trimethoxy substituted chalcone **15e** of the second series, exhibits high toxicity against both cancer and non-cancerous 3T3 cell lines. In addition to the impact of methoxy groups, we investigated the effect of the introduction of halogens on the phenyl moiety on the biological efficiency. The 4-bromo derivatives **12d** and **15f** displayed no significant activity, except the one against Jurkat cells. The chalcone **15g** with 2-fluorophenyl ring has displayed significant cytotoxicity against Caco-2 (IC_{50} 8.5 μM), but also considerable toxicity against non-cancer line 3T3. In contrast

Table 1 IC_{50} (μM) ± SD[a] of tested compounds in different cell lines after 72 h incubation

Compounds	Cell line, IC_{50} (μM) ± SD[a]									
	R	A549	Caco-2	HCT116	HeLa	MCF-7	MDA-MB-231	Jurkat	3T3	*c*LogP[b]
12a	3,5-di-MeO	55.3 ± 8.4	62.3 ± 12.5	**5.5 ± 2.2**	25.4 ± 8.2	30.9 ± 4.3	>100	**15.2 ± 3.7**	43.4 ± 5.3	3.43
12b	2,4,6-tri-MeO	11.2 ± 5.9	40.3 ± 12.2	**5.0 ± 1.2**	18.3 ± 5.1	**6.3 ± 1.2**	>100	**6.5 ± 0.6**	>100	3.43
12c	3,4,5-tri-MeO	60.2 ± 10.3	35.2 ± 10.1	**5.4 ± 1.6**	42.2 ± 4.2	>100	>100	>100	>100	2.73
12d	4-Br	>100	>100	63.5 ± 7.9	>100	>100	>100	**12.3 ± 1.8**	>100	4.29
13		>100	70.4 ± 15.4	45.7 ± 5.3	40.2 ± 5.1	39.5 ± 5.1	>100	**8.2 ± 0.6**	>100	3.23
15a[c]	4-MeO	>100	>100	>100	50.0 ± 5	>100	>100	>100	>100	3.65
15b[c]	2,6-di-MeO	80 ± 5.7	33.8 ± 7.3	**15.1 ± 0.8**	50.0 ± 4.3	>100	53.0 ± 10.4	**10.4 ± 3.3**	>100	3.67
15c[c]	2-OH-6-MeO	>100	80.6 ± 10.1	42.9 ± 5.4	35.5 ± 1.9	36.4 ± 4.6	>100	**7.1 ± 1.5**	>100	3.83
15d[c]	2,4,6-tri-MeO	>100	>100	**10.4 ± 4.2**	45.6 ± 5.7	46.3 ± 2.3	>100	35.2 ± 2.8	>100	3.63
15e	3,4,5-tri-MeO	**5.9 ± 2.2**	**5.2 ± 0.8**	**5.7 ± 0.2**	**7.6 ± 0.1**	33.4 ± 6.2	25.3 ± 5.6	**5.5 ± 0.7**	7.2 ± 1.8	2.93
15f	4-Br	>100	69.8 ± 0.2	27.1 ± 1.1	70.7 ± 10.4	59.0 ± 13.4	>100	**8.7 ± 1.6**	72.3 ± 8.3	4.36
15g	2-F	53.9 ± 8.6	**8.5 ± 0.9**	37.9 ± 8.9	19.2 ± 2.9	20.0 ± 8.0	21.1 ± 3.9	**5.9 ± 0.9**	23.4 ± 1.7	3.19
15h	4-F	65.6 ± 4.8	62.6 ± 10.6	**5.8 ± 1.2**	59.9 ± 12.1	72.3 ± 8.7	>100	**10.1 ± 2.2**	>100	3.64
16a	OMe	32.9 ± 8.7	>100	30.2 ± 5.9	22.2 ± 7.6	28.2 ± 6.7	38.8 ± 6.8	**4.7 ± 0.7**	29.3 ± 3.4	3.89
16b	OEt	40.6 ± 8.6	35.1 ± 5.4	**7.5 ± 1.7**	28.1 ± 5.7	60.5 ± 9.6	>100	**4.6 ± 0.6**	>100	4.42
16c	O-*n*Pr	>100	32.7 ± 8.6	26.5 ± 4.6	28.3 ± 5.6	50.4 ± 8.9	34.7 ± 9.3	**5.4 ± 0.1**	36.7 ± 7.4	4.95
16d	O-*iso*Pr	**5.5 ± 0.1**	**5.4 ± 0.2**	**4.4 ± 1.7**	**6.4 ± 1.3**	**5.2 ± 2.0**	**10.3 ± 1.3**	**3.9 ± 0.9**	5.4 ± 3.4	4.73
16e	O-*n*Bu	>100	>100	>100	>100	>100	>100	**8.6 ± 0.9**	>100	5.48
16f	O-*iso*Bu	>100	>100	>100	>100	>100	>100	>100	>100	5.35
18a	H	>100	>100	>100	>100	>100	>100	>100	>100	3.83
18b	Me	27.1 ± 3.5	**8.5 ± 1.3**	>100	>100	>100	>100	>100	>100	4.30
Cisplatin		9.5 ± 0.2	15.2 ± 0.3	15.3 ± 0.5	13.1 ± 0.2	15.6 ± 0.3	17.5 ± 0.5	16.2 ± 0.6	20.9 ± 0.3	–

[a]Standard deviation

[b]Logarithm of calculated octanol-water partition (calculated from ChemBioDrawUltra 12.0.2)

[c]Antiproliferative data previously published in [46]

4-fluorophenyl derivative **15h** has shown very high and selective activity against HCT116 (IC_{50} 5.8 μM). The knowledge of the rapidly expanding role of fluorine in the design and development of new drugs has led us to the synthesis and examination of a third group of chalcones having a substituted indole skeleton and 4-fluorophenyl ring. In recent years, the number of fluorine-containing drugs represents a one-third of all approved small molecule drugs [27]. Fluorine incorporation into the structure of a drug may dramatically change its physiochemical properties such as lipophilicity, solubility, metabolic stability, and partition coefficient [28]. The fluoro-substituted chalcone derivatives with excellent antiproliferative activity were synthesized by Burmaoglu [29]. Roman et al. [30] identified 4-fluoro-3,4,5-trimethoxychalcone as a new anti-invasive agent with cytotoxic effects. The first member of the third series, isostere **16a** having methoxy group at position 2 of indole skeleton has a moderate activity and increased toxicity against non-cancer 3T3 cells. The 2-ethoxyindol-3-yl derivative **16b** has selective activity on the same cancer cell lines HCT116 as lead compound **15h** with IC_{50} 7.5 μM. The *iso*-propyl chain has caused high toxicity to both cancer and non-cancer cells with IC_{50} below 10.3 μM. In contrast, the lipophilic (cLog P > 5) and bulky butoxy group caused a decrease in biological activity. For biological studies, we prepared and investigated 4-fluorophenyl derivatives both without a substituent on the indole nitrogen **18a** and with the methyl group on the indole nitrogen **18b**, previously described by Kumar et al. [6]. Unsubstituted chalcone **18a** showed no activity, while N-methylated chalcone **18b** showed selective activity against Caco-2 cell lines to the same extent as **15g** derivative and moderate activity against A-549 cells. It can be stated that the presence of methoxy group on the indole nitrogen has a positive effect on the antiproliferative activity of hybrid indole chalcones.

DNA binding activities

DNA is one of the central components of cellular machinery and storage unit of genetic information. It plays a key role in

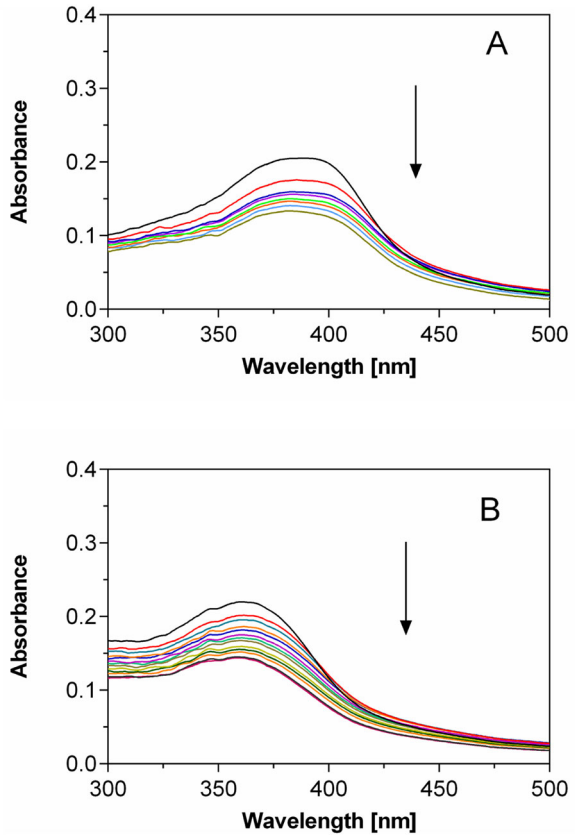


Fig. 2 Absorption spectra of compound **12b** (**A**), **15h** (**B**) (10.4 µM) in 0.01 M Tris buffer (pH 7.3, 24 °C) with increasing concentration of CT DNA. The uppermost black line corresponds to the spectrum of the free chalcone

replication, transcription, protein-coding, and cell integrity [31]. In order to validate the specific binding mode of the new bioactive compounds, the chalcones with significant activity against HCT116 cells **12b** and **15h**, were selected for further biophysical spectroscopic studies.

UV–vis DNA binding studies

The binding of the chalcones to DNA helix is often monitored through spectral changes in the absorbance and wavelength shifting [32]. To clarify the interaction between the chalcones and calf thymus DNA (CT DNA), the absorption spectra of **12b** and **15h** in the absence and presence of DNA are illustrated in Fig. 2. Absorption titration experiments of studied chalcones in Tris buffer were performed using fixed compound concentration to which increments of the CT DNA stock solution were added. With increasing CT DNA concentration, for **12b**, the hypochromism of the band at 378 nm reaches 38.2% with a blue shift of 6 nm. The absorption peak for **15h** at 361 nm showed only slight blue shift (1 nm) and substantial hypochromic effect (about 39.1%) upon addition of CT DNA (Table 2). The hypochromism or decrease in absorption intensity of the studied compounds observed on gradual addition of CT DNA to its fixed concentration (Fig. 2A, B), was attributed to interaction of its π^* molecular orbitals with the π-orbitals of the DNA base pairs, resulting in a decreased π–π^* transition probability and hence hypochromism [33].

Fluorescence binding experiments

Fluorescence displacement titration is based on the quenching of fluorescence using dyes as reporter molecules. The extent of fluorescence decrease is directly related to the binding of the ligand to CT DNA. The displacement assay was carried out with a well-known minor groove binder Hoechst 33258 (HO, Fig. S64 in supplementary) and the intercalator Ethidium bromide (EB, Fig. S65 in supplementary), using protocols reported in the literature [32, 33].

In the case of EB we have noted only negligible changes in fluorescence spectra after the addition of the chalcones to DNA-EB complex. This implies that the tested substances are not intercalators. Subsequently we did the minor groove displacement assay with HO. The addition of the studied chalcones caused a significant decrease in the intensities of the emission maxima at 465 nm, suggesting a noticeable interaction of the drugs with Hoechst 33258 (Fig. 3).

Linear quenching plots were used to estimate the values of the Stern–Volmer constants (K_{SV}), which are 1.71×10^4 M^{-1} for **12b** and 1.54×10^4 M^{-1} for **15h** (Table 2, Figs. S66, S67 in supplementary). Fluorescence binding experiments confirmed that examined chalcones preferred minor groove binding mode. The analogous fluorescence quenching profile was obtained for S009-131 coumarin-chalcone hybrid reported in literature [34].

Plasma protein binding is an important factor to understand the pharmacokinetic and pharmacodynamic properties of drugs, as it strongly influences drug distribution and determines the free fraction, which is available to the target [35]. Fluorescence methods have been widely used also to investigate the interaction between ligands and plasma proteins and can give information about the quenching mechanism, binding constants, and binding sites [36]. We have utilized this technique to study the interaction between **12b, 15h** and bovine serum albumin (BSA). The fluorescence spectra of BSA at increasing concentrations of **12b** (A) and **15h** (B) are shown in Fig. 4.

We have observed that the fluorescence intensity of BSA decreased regularly with an increased concentration of chalcones **12b** and **15h**. The K_{sv} constants for chalcone-BSA systems show values in the order of 10^5 M^{-1} (Table 2, Figs. S68, S69 in supplementary), which suggest the significant affinity of investigated drugs to important plasma protein. The slightly higher value of the K_{sv} constant was found for the chalcone **12b**, which has three methoxy substituents in its structure.

Table 2 Spectral and binding characteristics of chalcones 12b and 15h

Compound	λ_{max} [nm]	λ_{max} [nm]	Hypochromism [%]	K_{sv}^{HO} [M^{-1}]	K_{sv}^{BSA} [M^{-1}]
12b	378	6	38.2	1.71×10^4	2.55×10^5
15h	361	1	39.1	1.54×10^4	2.19×10^5

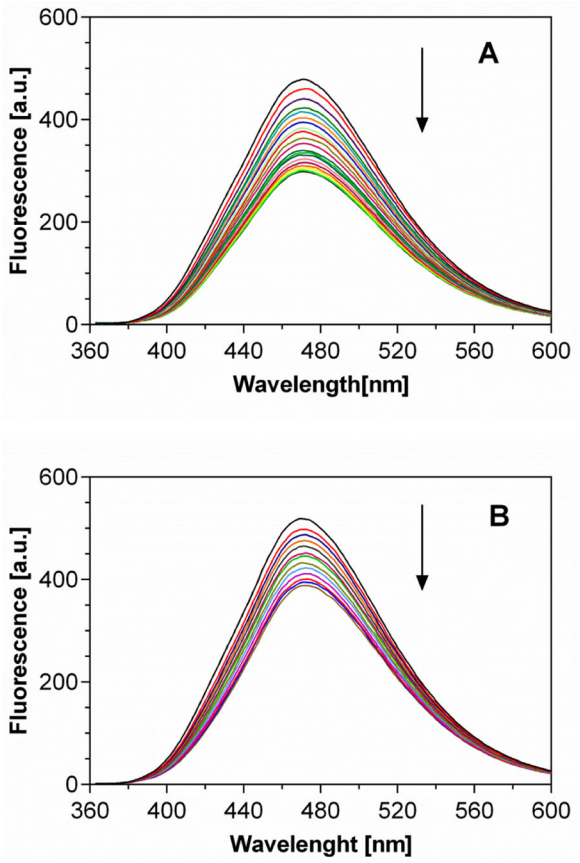


Fig. 3 Fluorescence emission spectra of HO bound to CT DNA in the presence of **12b** (**A**) and **15h** (**B**) in 0.01 M Tris buffer (pH 7.3), λex = 343 nm, 24 °C). The uppermost line corresponds to the spectrum before addition of chalcones

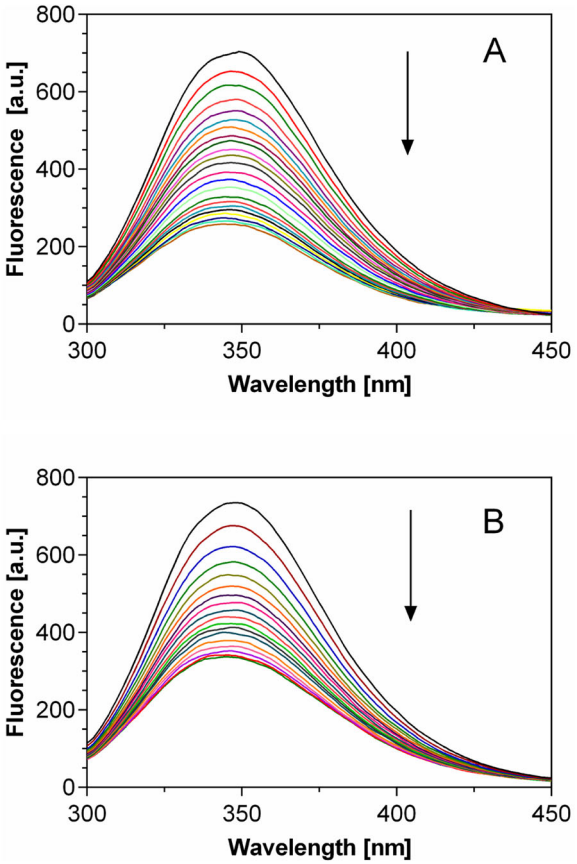


Fig. 4 Fluorescence quenching spectra of BSA–chalcone system with increased concentration of **12b** (**A**) and **15h** (**B**) in PBS buffer pH 7.3, λex = 280 nm at 24 °C. The uppermost black line corresponds to the spectrum of the free BSA

Circular dichroism

In addition, the interaction of chalcones with CT DNA was examined using circular dichroism (CD) measurements. CD measures the difference in the absorption of right and left circularly polarized light. It is a sensitive method for monitoring conformational changes of DNA [37, 38]. Transitions in the DNA bases give rise to a CD spectrum in the 200–350 nm region due to the chiral sugar at N1 or N9 and due to the helical arrangement of the bases [39]. The CD spectrum of free CT DNA in UV region (Fig. 5) has a positive band with maximum at 275 nm attributable to base stacking and a negative band with maximum at 246 nm due to helicity, which is characteristic for right-handed B-form of DNA [40]. These peaks are sensitive to the binding of any small molecule and can therefore be exploited to study the interaction of small molecules with DNA [41]. Simple groove binding and electrostatic interactions of drugs lead either to no changes or to marginal perturbations in the two CD bands of B-DNA. On the other hand, when classical intercalators (e.g., methylene blue or ethidium bromide) are bound to DNA, the positive band shows an increase in molar elipticity at 275 nm and the negative band shows a decrease in intensity at 246 nm with a slight red shift of the band maximum [42]. Figure 5 shows the CD spectra of CT DNA in the absence and presence of the studied chalcones.

Both the positive (275 nm) and negative (246 nm) bands were minimally decreased in intensity with slight red-shift after the addition of the compounds **12b** and **15h**. Furthermore, the incubation of DNA with tested compounds caused perturbation of the negative band, which is indicative of the

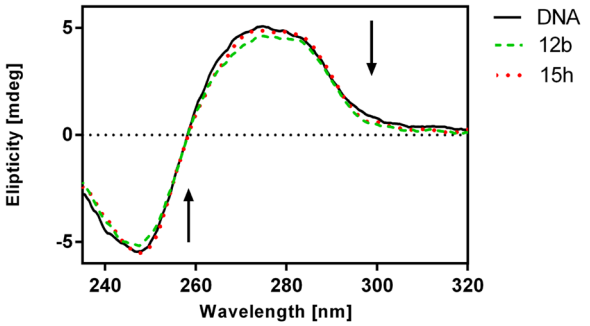


Fig. 5 CD spectra of CT DNA (4.4×10^{-4} M) in the absence (black line) and presence **12b** (green dashed line) and **1h** (red dotted line) in 0.01 M Tris buffer (pH = 7.3, 24 °C)

unwinding of the DNA helix. We have noticed only minimal changes in CD spectra. This fact provides further evidence of groove binding nature of investigated chalcones.

Conclusion

In summary, two series of chalcones containing 1-methoxyindole unit were synthesized and characterized. The nucleophilic substitution reaction on the 1-methoxyindole nucleus accompanying the condensation reaction was used to prepare the third series of compounds: 3-(2-alkoxyindol-3-yl)-1-(4-fluorophenyl)prop-2-en-1-ones. The obtained results of antiproliferative activity confirmed the assumption of suitability of preparation of 1-methoxyindole hybrid chalcones as antiproliferative agents. Most of the tested compounds showed activity against human leukemic T cell lymphoma Jurkat with IC_{50} below 15 μM. Compounds **12b, 12c, 15h, 16b** displayed most prominent antiproliferative activity against human colon cancer cell line HCT116 with $IC_{50} < 8$ μM and weak toxicity against a mouse embryo fibroblast cell line 3T3 ($IC_{50} > 100$ μM). In addition, chalcone **12b** was also effective against other cancer cell lines MCF-7 and Jurkat with $IC_{50} < 7$ μM and A549 with $IC_{50} = 11.2$ μM. Of the series tested, the chalcones **12b** and **15h** provided the best balance between high antiproliferative potency and low cytotoxicity and were selected for biochemical spectroscopic studies. The present study also demonstrated binding interaction of CT DNA and BSA with novel chalcone derivatives using a variety of spectroscopic methods. The results obtained from fluorescence titration experiments suggest that the investigated chalcones interact with CT DNA through minor groove binding mode and that these drugs could strongly quench the intrinsic fluorescence of BSA (K_{sv} constants are in the range from 2.19×10^5 M^{-1} to 2.54×10^5 M^{-1}). The results of this study suggest that the 1-methoxyindolyl chalcone hybrids are potential candidates for future development of therapeutic agents against colon cancer.

Experimental procedures

Chemistry

All reagents used in the synthesis were obtained commercially and were used without further purification, unless otherwise specified. The reactions were monitored by thin-layer chromatography (TLC) using TLC-sheets ALUGRAM-SIL G/UV254 (Macherey Nagel, Germany). Purification by flash chromatography was performed using Silica gel 60 Å (0.0040–0.063 mm, Merck, Germany) with the indicated eluent. Melting points of all the synthesized derivatives were determined by open-capillary tube method on digital melting point apparatus (Electrothermal). NMR spectra were recorded at r.t. on a VNMRS spectrometer (Varian) operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C at 299.15 K and on Varian Mercury Plus (Varian) 400 MHz operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts (δ in ppm) are given from internal solvent, DMSO-d_6 or $CDCl_3$. The infrared spectra were recorded in the range 4000–500 cm^{-1} on IRAffinity-1 FTIR Spectrophotometer (Shimadzu) using the KBr method or on FTIR Spectrometer Tensor 27 (Bruker Optik GmbH, Germany). High-resolution mass spectra were determined using a the Synapt G2-Si Mass Spectrometer (Waters, Manchester, UK), according the procedure described in literature [43] with the following modifications: the samples were dissolved in chloroform or MeOH (1 mg mL^{-1}) and diluted 1000-fold. The atmospheric solid analysis probe was dipped into the sample solution, placed into the ion source and analyzed in full scan mode. The probe was kept at a constant temperature of 450 °C for 2 min.

General procedure for 1-(1-metoxyindol-3-yl)prop-2-en-1-ones (Set A)

(A) Base-catalyzed Claisen–Schmidt condensation: To a stirred solution of 3-acetyl-1-methoxyindole (**10**, 0.2 mmol) in EtOH or *iso*-PrOH (2 ml) was added KOH (0.2 ml, 50% solution in H_2O) and benzaldehyde derivative **11** (0.2 mmol). The mixture was stirred at r.t. After completion of the reaction, reaction mixture was acidified with 1 M HCl to pH 4, extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The product was purified by column chromathography on SiO_2 and then crystalized to yield the chalcones.

(B) Acid-catalyzed Claisen–Schmidt condensation: To a stirred solution of the 3-acetyl-1-methoxyindole (**10**, 0.2 mmol) and substituted benzaldehyde (**11**, 0.2 mmol) in anhydrous EtOH (2 ml) thionyl chloride (0.4 mmol) was added. The solution turned deep red immediately. After completion of the reaction, reaction mixture was poured into H_2O, extracted with EtOAc, organic layer washed with brine and after drying over Na_2SO_4 evaporated. Chalcone

was separated by column chromathography on SiO_2 and then crystalized.

(2*E*)-3-(3″,5″-dimethoxyphenyl)-1-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (12a)

Procedure B: 22 h; 52%. $R_f = 0.43$ (hexane/EtOAc 2:1); light-yellow crystals; mp 141–142 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 400 MHz): 8.53–8.51 (m, 1H, H-4′), 8.06 (s, 1H, H-2′), 7.73 (d, 1H, *J* 15.6 Hz, H-3), 7.50–7.49 (m, 1H, H-7′), 7.38 (td, 1H, *J* 7.2, 1.4 Hz, H-6′), 7.34 (td, 1H, *J* 7.2, 1.3 Hz, H-5′), 7.29 (d, 1H, *J* 15.5 Hz, H-2), 6.79 (d, 2H, *J* 2.3 Hz, H-2″,H-6″), 6.52 (t, 1H, *J* 2.2 Hz, H-4″), 4.21 (s, 3H, N-OCH_3), 3.85 (s, 6H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 184.2 (C, C-1), 161.2 (C, C-3″, C-5″), 141.7 (CH, C-3), 137.3 (C, C-1″), 132.6 (C, C-7′a), 128.5 (CH, C-2′), 124.4 (CH, C-6′), 124.2 (CH, C-5′), 123.4 (CH, C-2), 123.3 (CH, C-4′), 123.3 (C, C-3a′), 113.9 (CH, C-3′), 108.6 (CH, C-7′), 106.3 (2CH, C-2″,6″), 102.3 (CH, C-4″), 66.9 (CH_3, N-OCH_3), 55.6 (CH_3, OCH_3). IR (KBr) ν_{max} 3139, 2942, 2833, 1648, 1596, 1507, 1455, 1379, 1331, 1240, 1205, 1160, 1070, 972, 823, 740 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 338.1395 for $C_{20}H_{20}NO_4$ (calcd. 338.1392).

(2*E*)-1-(1′-methoxy-1′*H*-indol-3′-yl)-3-(2″,4″,6″-trimethoxyphenyl)prop-2-en-1-one (12b)

Procedure A: EtOH; 24 h; 16%. **Procedure B**: 10 min; 63%. R_f 0.32 (hexane/EtOAc 3:2); light-yellow crystals; mp 172–173 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 400 MHz,): 8.55–8.53 (m, 1H, H-4′), 8.25 (d, 1H, *J* 15.8 Hz, H-3), 7.99 (s, 1H, H-2′), 7.72 (d, 1H, *J* 15.8 Hz, H-2), 7.48-7.46 (m 1H, H-7′), 7.34 (td, 1H, *J* 7.3, 1.22 Hz, H-6′), 7.31 (td, 1H, *J* 7.3, 1.4 Hz, H-5′), 6.16 (s, 2H, H-3″, H-5″), 4.18 (3H, s, N-OCH_3), 3.93 (6H, s, OCH_3), 3.86 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 186.5 (C, C-1), 162.7 (C, C-4″), 161.6 (2 C, C-2″,C-6″), 132.9 (CH, C-3), 132.5 (C, C-7′a), 128.1 (CH, C-2′), 123.9 (CH, C-6′), 123.9 (CH, C-2), 123.3 (C, 3′ a), 123.3 (CH, C-4′), 122.9 (CH, C-5′), 114.5 (C, C-3′), 108.5 (CH, C-7′), 106.8 (C, C-1″), 90.8 (2CH, C-3″, C-5″), 66.8 (CH_3, N-OCH_3), 56.0 ($2CH_3$, OCH_3), 55.5 (CH_3, OCH_3). IR (KBr) ν_{max} 2942, 2836, 1638, 1602, 1571, 1508, 1460, 1376, 1327, 1212, 1155, 1121, 1064, 977, 743 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 368.1498 for $C_{21}H_{22}NO_5$ (calcd. 368.1498).

(2*E*)-1-(1′-methoxy-1′*H*-indol-3′-yl)-3-(3″,4″,5″-trimethoxyphenyl)prop-2-en-1-one (12c)

Procedure A: *iso*-PrOH; 24 h; 60%. Procedure B: 48 h; 42%. $R_f = 0.53$ (hexane/EtOAc 1:1); mp 196–197 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 400 MHz): 8.54–8.51 (m, 1H, H-4′), 8.07 (s, 1H, H-2′), 7.74 (d, 1H, *J* 15.5 Hz, H-3), 7.50–7.48 (m, 1H, H-7′), 7.38 (td, 1H, *J* 7.2, 1.4 Hz, H-6′), 7.35 (td, 1H, *J* 7.2, 1.3 Hz, H-5′), 7.22 (d, 1H, *J* 15.5 Hz, H-2), 6.87 (s, 2H, H-2″, H-6″), 4.21 (s, 3H, N-OCH_3), 3.94 (s, 6H, OCH_3), 3.90 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 184.2 (C, C-1), 153.6 (2 C, C-3″,5″), 141.9 (CH, C-3), 140.2 (C, C-4″), 132.6 (C, C-7′a), 130.9 (C, C-1″), 128.4 (CH, C-2′), 124.5 (CH, C-6′), 123.4 (CH, C-5′), 123.3 (CH, C-4′), 123.2 (C, C-3′a), 123.0 (CH, C-2), 113.9 (C, C-3′), 108.6 (CH, C-7′), 105.6 (2CH, C-2″,6″), 66.9 (CH_3, N-OCH_3), 61.2 (CH_3, OCH_3), 56.4 ($2CH_3$, OCH_3). IR (KBr) ν_{max} 3127, 2945, 1638, 1582, 1506, 1450, 1420, 1339, 1268, 1243, 1125, 829, 756 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 368.1498 for $C_{21}H_{22}NO_5$ (calcd. 368.1498).

(2*E*)-3-(4″-bromophenyl)-1-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (12d)

Procedure A: *iso*-PrOH; 7 h; 58%. $R_f = 0.649$ (hexane/acetone 1:1); light-yellow crystals; mp 158–159 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 400 MHz): 8.53–8.50 (m, 1H, H-4′), 8.06 (s, 1H, H-2′), 7.75 (d, 1H, *J* 15.6 Hz, H-3), 7.55 (d, 2H, *J* 8.6, H-3″, H-5″), 7.51 (d, 2H, *J* 8.6, Hz, H-2″, H-6″), 7.50-4.48 (m, 1H, H-7′), 7.38 (1H, td, *J* 7.2, 1.5 Hz, H-6′), 7.35 (1H, td, *J* 7.2, 1.4 Hz, H-5′), 7.32 (d, 1H, *J* 15.6 Hz, H-2), 4.21 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 183.9 (C, C-1), 140.3 (CH, C-3), 134.3 (C, C-1″), 132.6 (C, C-7′a), 132.3 (2CH, C-3″,5″), 129.7 (2CH, C-2″,6″), 128.5 (CH, C-2′), 124.5 (CH, C-6’), 124.3 (C, C-4″), 124.2 (CH, C-2), 123.5 (CH, C-5’), 123.3 (CH, C-4′), 123.2 (C, C-3′a), 113.9 (C, C-3′), 108.7 (CH, C-7′), 67.0 (CH_3, OCH_3). IR (KBr) ν_{max} 3046, 2982, 2942, 1653, 1595, 1516, 1487, 1401, 1380, 1330, 1059, 1006, 943, 819, 731 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 356.0287 for $C_{18}H_{15}NO_2Br$ (calcd. 356.0286).

(2*E*)-1,3-bis(1-methoxy-1*H*-indol-3-yl)prop-2-en-1-one (13)

Procedure B: 1-Methoxyindol-3-carbaldehyde (0.2 mmol) was used as the aldehyde; 4 h; 52%. R_f 0.20 (hexane/acetone 4:1); yellow solid; mp 75–76 °C. ^1H ($CDCl_3$, 400 MHz): 8.57–8.55 (m, 1H, H-4′), 8.08 (s, 1H, H-2′), 8.03 (d, 1H, *J* 15.6 Hz, H-3), 8.03–8.00 (m, 1H, H-4″), 7.67 (s, 1H, H-2″), 7.51–7.48 (m, 2H, H-7″, H-7′), 7.38 (d, 1H, *J* 15.4 Hz, H-2), 7.39–7.29 (m, 4H, H-5′, H-6′, H-5″, H-6″), 4.22 (s, 3H, OCH_3), 4.16 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 184.8 (C, C-1), 134.7 (CH, C-3), 133.1 (C, C-7″ a), 132.6 (C, C-7′a), 127.9 (CH, C-2′), 126.3 (CH, C-2″), 124.2 (CH, C-6′), 123.7 (CH, C-6″), 123.3 (CH, C-4′), 123.2 (C, C-3′a), 123.1 (CH, C-5′) 122.6 (C, C-3″a), 121.9 (CH, C-5″), 120.8 (CH, C-4″), 120.1 (CH, C-2), 114.2 (C, C-3′), 109.7 (C, C-3″), 109.1 (CH, C-7″), 108.6 (CH, C-7′), 66.9 (CH_3, O$\underline{C}H_3$), 66.6 (CH_3, O$\underline{C}H_3$). IR: HRMS: *m/z* $[M + H]^+$: 347.1398 for $C_{21}H_{19}N_2O_3$ (calcd. 347.1396).

General procedure for 3-(1-metoxyindol-3-yl)prop-2-en-1-ones (Set B)

(A) Base-catalyzed Claisen–Schmidt condensation: To a stirred solution of substituted acetophenone **14** (0.2 mmol) in EtOH or *iso*-PrOH (2 mL) KOH (0.2 mL, 50% solution in H_2O) and 1-methoxyindol-3-carbaldehyde (**8**, 0.2 mmol) were added. The mixture was stirred at r.t. After completion of the reaction, reaction mixture was acidified with 1 M HCl to pH 4, extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The product was purified by column chromathography on SiO_2 and then crystalized to yield the chalcones.

(B) Acid-catalyzed Claisen-Smidt condensation: To a stirred solution of substituted acetophenone **14** (0.2 mmol) and 1-methoxyindol-3-carbaldehyde (**8**, 0.2 mmol) in anhydrous EtOH (2 mL) with molecule sieves (3 Å) thionyl chloride (0.4 mmol) was added. The solution turned deep red immediately. After completion of the reaction, reaction mixture was poured into water, extracted with EtOAc, the organic layer was washed with brine and after drying over anhydrous Na_2SO_4, filtered and evaporated. Product was separated by column chromathography on SiO_2 and then crystalized.

(2*E*)-3-(1′-methoxy-1′*H*-indol-3′-yl)-1-(4″-methoxyphenyl)prop-2-en-1-one (15a)

Procedure B: There was used 2 eq. of 4-methoxyacetophenone because of close Rf values of product and unreacted aldehyde **6**; 40 min; 65%. R_f 0.41 (hexane/EtOAc 2:1); light-yellow crystals; mp 94–96 °C (CH_2Cl_2/hexane). ^{1}H ($CDCl_3$, 400 MHz): 8.07 (d, 2H, *J* 8.9, H-2″, H-6″), 8.02 (d, 1H, *J* 15.5 Hz, H-3), 8.00 (dd, 1H, *J* 7.8, 1.0 Hz, H-4′), 7.68 (s, 1H, H-2′), 7.56 (d, 1H, *J* 15.5 Hz, H-2), 7.50 (dd, 1H, *J* 7.9, 1.0 Hz, H-7′), 7.36 (ddd, 1H, *J* 8.1, 7.3, 1.1 Hz, H-6′), 7.31 (ddd, 1H, *J* 8.1, 7.1, 1.2 Hz, H-5′), 7.00 (d, 2H, *J* 8.8 Hz, H-3″, 5″), 4.16 (s, 3H, N-OCH_3), 3.90 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 189.0 (C, C-1), 163.2 (C, C-4″), 137.2 (CH, C-3), 133.1 (C, C-7′a), 131.8 (C, C-1″), 130.7 (2CH, C-2″, C-6″), 126.9 (CH, C-2′), 123.8 (CH, C-6′), 122.6 (C, C-3′a), 122.1 (CH, C-5′), 120.9 (CH, C-4′), 118.1 (CH, C-2), 113.9 (2CH, C-3″, C-5″), 109.8 (C, C-3′), 109.1 (CH, C-7′), 66.6 (CH_3, N-OCH_3), 55.6 (CH_3, OCH_3). IR ν_{max} 3103, 3005, 2935, 1652, 1584, 1564, 1506, 1365, 1277, 1167, 978, 948, 820, 737 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 308.1289 for $C_{19}H_{18}NO_3$ (calcd. 308.1287).

(2*E*)-1-(2″,6″-Dimethoxyphenyl)-3-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (15b)

Procedure A: EtOH; 14 h; 34%. R_f 0.21 (hexane/acetone 2:1); yellow crystals; mp 146–148 °C (EtOAc/hexane). ^{1}H ($CDCl_3$, 400 MHz): 7.89 (d, 1H, *J* 8.0 Hz, H-4′), 7.54 (s, 1H, H-2′), 7.48 (d, 1H, *J* 16.1 Hz, H-3), 7.45 (d, 1H, *J* 8.0 Hz, H-7′), 7.33 (t, 1H, *J* 8.4 Hz, H-4″), 7.32 (ddd, 1H, *J* 8.0, 7.1, 1.1 Hz, H-6′), 7.24 (ddd, 1H, *J* 8.0, 7.1, 1.1 Hz, H-5′), 6.99 (d, 1H, *J* 16.1 Hz, H-2), 6.63 (d, 2H, *J* 8.4, H-3″, H-5″), 4.11 (s, 3H, N-OCH_3), 3.79 (s, 6H OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 182.3 (C, C-1), 157.7 (2 C, C-2″, C-6″), 138.6 (CH, C-3), 133.1 (C, C-7′a), 130.5 (CH, C-4″), 126.6 (CH, C-2′), 125.6 (CH, C-2), 123.8 (CH, C-6′), 122.6 (C, C-3′a), 122.1 (CH, C-5′), 121.0 (CH, C-4′), 119.1 (C, C-1″), 109.4 (C, C-3′), 109.0 (CH, C-7′), 104.3 (2CH, C-3″, C-5″), 66.6 (CH_3, N-OCH_3), 56.2 (2CH_3, OCH_3). IR ν_{max} 3088, 2949, 2842, 1610, 1589, 1472, 1433, 1240, 1103, 1059, 953, 741 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 338.1393 for $C_{20}H_{20}NO_4$ (calcd. 338.1392).

(2*E*)-1-(2″-Hydroxy-6″-methoxyphenyl)-3-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (15c)

To a solution of 1-methoxyindol-3-carboxaldehyde (**8**; 0.070 g; 0.4 mmol) and 2-hydroxy-6-methoxyacetofenone (**14c**; 0.066 g; 0.4 mmol) in EtOH (2 ml) was added dropwise piperidine (0.04 ml; 0.4 mmol). Reaction mixture was heated at 50 °C for 21 h. After evaporation of solvent product was isolated by column chromathography on SiO_2 (eluent hexane/EtOAc 2:1) with the yield 28%. R_f 0.66 (hexane/EtOAc 2:1); colourless powder; mp 118–121 °C (EtOAc /hexane). ^{1}H ($CDCl_3$, 400 MHz): 13.58 (s, 1H, OH), 8.08 (d, 1H, *J* 15.6 Hz, H-3), 8.02 (d, 1H, *J* 15.6 Hz, H-2), 8.01 (d, 1H, *J* 8.1 Hz, H-4′), 7.66 (s, 1H, H-2′), 7.51 (d, 1H, *J* 8.1 Hz, H-7′), 7.37 (ddd, 1H, *J* 8.1, 7.1, 1.1 Hz, H-6′), 7.35 (t, 1H, *J* 8.2 Hz, H-4″), 7.31 (ddd, 1H, *J* 8.1, 7.2, 1.1 Hz, H-5′), 6.63 (dd, 1H, *J* 8.4, 0.9 Hz, H-3″), 6.45 (dd, 1H, *J* 8.3, *J* 0.8 Hz, H-5″), 4.16 (s, 3H, N-OCH_3), 4.01 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 194.1 (C, C-1), 165.2 (C, C-2″), 161.0 (C, C-6″), 137.1 (CH, C-3), 135.4 (CH, C-4″), 133.3 (C, C-7′a), 127.7 (CH, C-2′), 123.9 (CH, C-6′), 123.8 (CH, C-2), 122.5 (C, C-3′a), 122.2 (CH, C-5′), 121.0 (CH, C-4′), 112.2 (C, C-1″), 111.2 (CH, C-3″), 110.3 (C, C-3′), 109.2 (CH, C-7′), 101.7 (CH, C-5″), 66.7 (CH_3, N-OCH_3), 56.1 (CH_3, OCH_3). IR ν_{max} 3103, 2947, 1622, 1577, 1548, 1456, 1371, 1234, 1085, 1041, 858, 735 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 324.1238 for $C_{19}H_{18}NO_4$ (calcd.324.1236).

(2*E*)-3-(1′-Methoxy-1′*H*-indol-3′-yl)-1-(2″,4″,6″-trimethoxyphenyl)prop-2-en-1-one (15d)

Procedure B: 6 h; 33%. R_f 0.42 (hexane/acetone 2:1); red crystals; mp 47–49 °C (acetone/hexane). ^{1}H ($CDCl_3$, 600 MHz): 7.89 (d, 1H, *J* 8.0 Hz, H-4′), 7.54 (s, 1H, H-2′), 7.53 (d, 1H, *J* 16.2 Hz, H-3), 7.45 (d, 1H, *J* 8.1 Hz, H-7′), 7.32 (t, 1H, *J* 7.6 Hz, H-6′), 7.23 (t, 1H, *J* 7.2 Hz, H-5′),

6.99 (d, 1H, *J* 16.0 Hz, H-2), 6.18 (s, 2H, H-3″, H-5″), 4.11 (s, 3H, N-OCH_3), 3.87 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3). ^{13}C ($CDCl_3$, 150 MHz): 194.6 (C, C-1), 162.2 (C, C-4″), 158.9 (2 C, C-2″, C-6″), 137.8 (CH, C-3), 133.0 (C, C-7′a), 126.4 (CH, C-2′), 126.0 (CH, C-2), 123.8 (CH, C-6′), 122.6 (C, C-3′a), 122.0 (CH, C-5′), 120.9 (CH, C-4′), 112.3 (C, C-1″), 109.5 (C, C-3′), 108.9 (CH, C-7′), 90.9 (2CH, C-3″, C-5″), 66.6 (CH_3, N-OCH_3), 56.1 ($2CH_3$, OCH_3), 55.6 (CH_3, OCH_3). IR ν_{max} 2927, 2853, 1599, 1585, 1454, 1414, 1226, 1203, 1155, 1122, 1026, 740 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 368.1496 for $C_{21}H_{22}NO_5$ (calcd. 368.1498).

(2*E*)-3-(1′-Methoxy-1′*H*-indol-3′-yl)-1-(3″,4″,5″-trimethoxyphenyl)prop-2-en-1-one (15e)

Procedure A: *iso*-PrOH; 45 min; 26%. Procedure B: 3 h; 27%. R_f 0.3 (hexane/acetone 2:1); yellow crystals; mp 120–122 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 400 MHz): 8.02 (d, 1H, *J* 15.5 Hz, H-3), 7.97 (dt, 1H, *J* 7.8, 0.9 Hz, H-4′), 7.71 (s, 1H, H-2′), 7.51 (dd, 1H, *J* 8.1, 1.0 Hz, H-7′), 7.47 (d, 1H, *J* 15.5 Hz, H-2), 7.37 (ddd, 1H, *J* 8.1, 7.3, 1.2 Hz, H-6′), 7.31 (ddd, *J* 7.9, 7.2, 1.2 Hz, H-5′), 7.30 (s, 2H, H-2″, H-6″), 4.17 (s, 3H, N-OCH_3), 3.97 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 100 MHz): 189.6 (C, C-1), 153.3 (2 C, C-3″, C-5″), 142.2 (C, C-4″), 138.0 (CH, C-3), 134.5 (C, C-1″), 133.1 (C, C-7′a), 127.0 (CH, C-2′), 124.0 (CH, C-6′), 122.7 (C, C-3′a), 122.2 (CH, C-5′), 120.8 (CH, C-4′), 118.2 (CH, C-2), 109.7 (C, C-3′), 109.2 (CH, C-7′), 106.1 (2CH, C-2″, C-6″), 66.7 (CH_3, N-OCH_3), 61.1 (CH_3, OCH_3), 56.6 ($2CH_3$, OCH_3). IR (KBr) ν_{max} 3215, 2991, 2938, 1646, 1588, 1559, 1503, 1466, 1455, 1415, 1343, 1275, 1234, 1160, 1130, 810, 732 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 368.1500 for $C_{21}H_{22}NO_5$ (calcd.368.1498).

(2*E*)-1-(4″-Bromophenyl)-3-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (15f)

Procedure A: *iso*-PrOH; 1 h; 27%. **Procedure B**: 5 h; 35%. R_f 0.58 (hexane/acetone 2:1); yellow crystals; mp 105–107 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 400 MHz): 8.03 (d, 1H, *J* 15.5 Hz, H-3), 7.97 (d, 1H, *J* 7.9 Hz, H-4′), 7.91 (d, 2H, *J* 8.5 Hz, H-2″, H-6″), 7.70 (s, 1H, H-2′), 7.65 (d, 2H, *J* 8.6 Hz, H-3″, H-5″), 7,50 (d, 1H, *J* 8.1 Hz, H-7′), 7.47 (d, 1H, *J* 15.5 Hz, H-2), 7.37 (ddd, 1H, *J* 8.1, 7.2, 0.9 Hz, H-6′), 7.32 (ddd, 1H, *J* 7.9, 7.1, 1.0 Hz, H-5′), 4.16 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 101 MHz): 189.5 (C, C-1), 138.7 (CH, C-3), 137.8 (C, C-1″), 133.2 (C, C-7′a), 131.9 (2CH, C-3″, C-5″), 130.0 (2CH, C-2″, C-6″), 127.4 (CH, C-2′), 127.3 (C, C-4″), 124.0 (CH, C-6′), 122.6 (C, C-3′a), 122.3 (CH, C-5′), 120.9 (CH, C-4′), 117.6 (CH, C-2), 109.7 (C, C-3′), 109.2 (CH, C-7′), 66.6 (CH_3, N-OCH_3). IR ν_{max} 3099, 2947, 1647, 1591, 1581, 1560, 1375, 1278, 1244, 1007, 950, 804, 727 cm^{-1} HRMS: *m/z* $[M + H]^+$: 356.0284 for $C_{18}H_{15}NO_2Br$ (calcd. 356.0286).

(2*E*)-1-(2″-Fluorophenyl)-3-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (15g)

Procedure A: *iso*-PrOH; 1 h; 50%. R_f 0.48 (hexane/acetone 2:1); yellow crystals; mp 110–112 °C (CH_2Cl_2/hexane). ^1H (DMSO-d_6, 600 MHz): 8.47 (s, 1H, H-2′), 7.98 (d, 1H, *J* 8.0, H-4′), 7.84 (dd, 1H, *J* 15.7, 0.9 Hz, H-3), 7.76 (dd, 1H, *J* 7.6, 1.7 Hz, H-6″), 7.66-7.62 (m, 1H, H-4″), 7.58 d (d, 1H, *J* 8.1, H-7′), 7.35 – 7.40 (m, 3H, H-6′, H-3″,H-5″), 7.33 (dd, 1H, *J* 15.8, 2.2 Hz, H-2), 7.29 (ddd, 1H, *J* 8.1, 7.2, 1.0 Hz, H-5′), 4.15 (s, 3H, OCH_3). ^{13}C (DMSO-d_6,151 MHz): 188.3 (C, d, J_{CF}.2.0 Hz, C-1), 160.1 (CH, J_{CF}.250.1 Hz, C-2″), 138.5 (CH, C-3), 133.6 (C, d, J_{CF}.8.7 Hz, C-4″), 132.5 (C, C-7′a), 130.4 (CH, d, J_{CF}.2.9 Hz, C-6″), 129.8 (CH, C-2′), 127.5 (C, d, J_{CF}.13.9 Hz, C-1″), 124.8 (CH, d, J_{CF}.3.3 Hz, C-5″), 123.8 (CH, C-6′), 122.2 (CH, C-5′), 121.7 (C, C-3′a), 120.9 (CH, d, J_{CF}.4.7, C-2), 120.4 (CH, C-4′), 116.5 (CH, d, J_{CF}.22.6 Hz, C-3″), 109.2 (CH, C-7′), 108.2 (C, C-3′), 66.7 (CH_3, OCH_3). IR (KBr) ν_{max} 3098, 1629, 1613, 1607, 1570, 1505, 1448, 1376, 1313, 1292, 1244, 1234, 1108, 1081, 976, 951, 768, 741 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 296.1088 for $C_{18}H_{15}NO_2F$ (calcd. 296.1087).

(2*E*)-1-(4″-Fluorophenyl)-3-(1′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (15h)

Procedure A: *iso*-PrOH; 1.5 h; 37%. **Procedure B**: 4 h; 52%. R_f 0.58 (hexane/acetone 2:1); yellow crystals; mp 78–80 °C (CH_2Cl_2/hexane). ^1H ($CDCl_3$, 600 MHz): 8.08 (dd, *J* 8.8, 5.4 Hz, H-2″, H-6″), 8.03 (d, 1H, *J* 15.5 Hz, H-3), 7.98 (dt, 1H, *J* 8.0, 1.1 Hz, H-4′), 7.69 (s, 1H, H-2′), 7.51 (d, 1H, *J* 15.5 Hz, H-2), 7,51 (dt, 1H, *J* 8.2, 1.1 Hz, H-7′), 7.37 (ddd, 1H, *J* 8.2, 7.1, 1.1 Hz, H-6′), 7.32 (ddd, 1H, *J* 8.1, 7.2, 1.1 Hz, H-5′), 7.18 (t, 2H, *J* 8.6 Hz, H-3″, H-5″), 4.17 (s, 3H, OCH_3). ^{13}C ($CDCl_3$, 150 MHz): 189.0 (C, C-1), 165.5 (C, d, J_{CF}.253.4 Hz, C-4″), 138.3 (CH, C-3), 135.4 (C, d, J_{CF}.3.0 Hz, C-1″), 133.2 (C, C-7′ a), 131.0 (2CH, d, J_{CF}.9.1 Hz, C-2″, C-6″), 127.3 (CH, C-2′), 124.0 (CH, C-6′), 122.6 (C, C-3′a), 122.3 (CH, C-5′), 120.9 (CH, C-4′), 117.9 (CH, C-2), 115.7 (2CH, d, J_{CF}.21.8 Hz, C-3″, C-5″), 109.7 (C, C-3′), 109.2 (CH, C-7′), 66.7 (CH_3, OCH_3). IR (KBr) ν_{max} 3094, 1652, 1601, 1584, 1506, 1375, 1280, 1211, 1158, 957, 809, 725 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 296.1085 for $C_{18}H_{15}NO_2F$ (calcd. 296.1087).

General procedure for 3-(2′-alkoxy-1′H-indol-3′-yl)-1-(4″-fluorophenyl)prop-2-en-1-ones

To a stirred solution of 4-fluoroacetophenone (**14h**, 0.5 mmol) in alcohol (5 ml) was added 50% KOH in H_2O (0.5 ml from a) and 1-methoxyindol-3-carbaldehyde

(**8**, 0.5 mmol). The reaction mixture was stirred at r.t. After completion of the reaction, reaction mixture was cooled to 0–10 °C, acidified with 1 M HCl to pH 4. Then the precipitated product was filtered, washed with H_2O, dried and crystalized. When the product was not precipitated, the mixture was extracted with EtOAc and the extract was washed with brine. After drying over anhydrous Na_2SO_4 filtrate was evaporated to dryness and product was purified by column chromathography on SiO_2 and then crystalized to yield the chalcones.

(2*E*)-1-(4″-Fluorophenyl)-3-(2′-methoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (16a)

Reaction time: 5 days; yield: 32%. R_f 0.43 (hexane/acetone 3:2); orange crystals; mp 177–179 °C (acetone/hexane). ^{1}H (DMSO-d_6, 600 MHz): 12.11 (s, NH), 8.14 (dd, 2H, *J* 8.8, 5.6 Hz, H-2″, H-6″), 8.02 (d, 1H, *J* 15.2 Hz, H-3), 7.86 (d, 1H, *J* 7.8 Hz, H-4′), 7,34 (d, 1H, *J* 7.7 Hz, H-7′), 7.34 (t, *J* 8.8 Hz, 2H, H-3″, H-5″), 7.33 (d, 1H, *J* 15.1 Hz, H-2), 7.19 (td, 1H, *J* 7.7, 1.1 Hz, H-5′), 7.13 (td, 1H, *J* 7.8, 1.1 Hz, H-6′), 4.17 (s, 3H, OCH_3). ^{13}C (DMSO-d_6, 150 MHz): 186.7 (C, C-1), 164.3 (C, d, J_{CF}.249.8 Hz, C-4″), 157.5 (C, C-2′), 136.2 (CH, C-3), 135.6 (C, d, J_{CF}.2.7 Hz, C-1″), 131.9 (C, C-7′a), 130.6 (2CH, d, J_{CF}.9.1 Hz, C-2″, C-6″), 125.1 (C, C-3′a), 121.7 (CH, C-5′), 121.0 (CH, C-6′), 119.0 (CH, C-4′), 115.4 (2CH, d, J_{CF} 21.6 Hz, C-3″, C-5″), 111.4 (CH, C-7′), 110.9 (CH, C-2), 93.7 (C, C-3′), 58.8 (CH_3, OCH_3). IR (KBr) ν_{max} 3215, 2937, 1638, 1595, 1560, 1533, 1506, 1489, 1344, 1219, 1065, 1017, 814, 737 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 296.1086 for $C_{18}H_{15}NO_2F$ (calcd. 296.1087).

(2*E*)-3-(2′-ethoxy-1′*H*-indol-3′-yl)-1-(4″-fluorophenyl) prop-2-en-1-one (16b)

Reaction time: 22 h; yield: 30%. R_f 0.34 (hexane/acetone 2:1); orange crystals; mp 174–175 °C (acetone/hexane). ^{1}H (DMSO-d_6, 600 MHz): 12.06 (s, NH), 8.14 (dd, 2H, *J* 8.8, 5.6 Hz, H-2″, H-6″), 8.03 (d, 1H, *J* 15.2 Hz, H-3), 7.87 (d, 1H, *J* 7.8 Hz, H-4′), 7.35 (d, 1H, *J* 15.3 Hz, H-2), 7.35 (t, *J* 8.7 Hz, 2H, H-3″, H-5″), 7,33 (d, 1H, *J* 7.7 Hz, H-7′), 7.18 (td, 1H, *J* 7.6, 1.1 Hz, H-6′), 7.13 (td, 1H, *J* 7.8, 1.1 Hz, H-5′), 4.47 (q, 2H, *J* 7.0 Hz, $OC\underline{H}_2CH_3$), 1.47 (t, 3H, $OCH_2C\underline{H}_3$). ^{13}C (DMSO-d_6, 150 MHz): 186.7 (C, C-1), 164.3 (C, d, J_{CF}.250 Hz, C-4″), 156.8 (C, C-2′), 136.4 (CH, C-3), 135.6 (C, d, J_{CF}.2.7 Hz, C-1″), 132 (C, C-7′a), 130.6 (2CH, d, J_{CF}.9.1 Hz, C-2″, C-6″), 124.9 (C, C-3′a), 121.7 (CH, C-5′), 121.1 (CH, C-6′), 119.1 (CH, C-4′), 115.5 (2CH, d, J_{CF}.21.6 Hz, C-3″, C-5″), 111.3 (CH, C-7′), 110.8 (CH, C-2), 94.2 (C, C-3′), 67.6 (CH_2, $O\underline{C}H_2CH_3$), 14.7 (CH_3, $OCH_2\underline{C}H_3$). IR (KBr) ν_{max} 3079, 2981, 1627, 1601, 1560, 1507, 1481, 1339, 1220, 1160, 1055, 815, 736 cm^{-1}. HR-MS: *m/z* $[M + H]^+$: 310.1243 for $C_{19}H_{17}NO_2F$ (calcd. 310.1243).

(2*E*)-1-(4″-Fluorophenyl)-3-(2′-propoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (16c)

Reaction time: 25 h; yield: 32%, R_f 0.32 (hexane/acetone 2:1); orange crystals; mp 177–179 °C (acetone/hexane). ^{1}H (DMSO-d_6, 400 MHz): 8.13 (dd, 2H, *J* 8.4, 5.8 Hz, H-2″, H-6″), 8.03 (d, 1H, *J* 15.2 Hz, H-3), 7.85 (d, 1H, *J* 7.8 Hz, H-4′), 7.34 (t, *J* 8.8 Hz, 2H, H-3″, H-5″), 7.33 (d, 1H, *J* 15.3 Hz, H-2), 7,32 (d, 1H, *J* 7.8 Hz, H-7′), 7.17 (t, 1H, *J* 7.5 Hz, H-6′), 7.11 (t, 1H, *J* 7.5 Hz, H-5′), 4.37 (t, 2H, *J* 6.5 Hz, $OC\underline{H}_2CH_2CH_3$), 1.86 (sextet, 2H, *J* 6.9 Hz, $OCH_2C\underline{H}_2CH_3$), 1.05 (t, 3H, *J* 7.4 Hz, $OCH_2CH_2C\underline{H}_3$). ^{13}C (DMSO-$d_6$, 100 MHz): 186.8 (C, C-1), 164.4 (C, d, J_{CF}.251.8 Hz, C-4″), 157.1 (C, C-2′), 136.5 (CH, C-3), 135.7 (C, d, J_{CF}.2.8 Hz, C-1″), 132,4 (C, C-7′a), 130.6 (2CH, d, J_{CF}.9.1 Hz, C-2″, C-6″), 125.1 (C, C-3′a), 121.7 (CH, C-5′), 121.2 (CH, C-6′), 119.1 (CH, C-4′), 115.5 (CH, d, J_{CF}.21.8 Hz, C-3″,C-5″), 111.5 (CH, C-7′), 110.8 (CH, C-2), 94.3 (C, C-3′), 73.0 (CH_2, $O\underline{C}H_2CH_2CH_3$), 22.2 ($CH_2$, $OCH_2\underline{C}H_2CH_3$), 10.2 ($CH_3$, $OCH_2CH_2\underline{C}H_3$). IR (KBr) ν_{max} 3071, 2974, 1624, 1591, 1570, 1506, 1489, 1337, 1285, 1217, 1153, 1044, 1011, 817, 744 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 324.1403 for $C_{20}H_{19}NO_2F$ (calcd. 324.1400).

(2*E*)-1-(4″-Fluorophenyl)-3-(2′-isopropoxy-1′*H*-indol-3′-yl)prop-2-en-1-one (16d)

Reaction time: 25 h; yield: 26%. R_f 0.35 (hexane/acetone 2:1); orange crystals; mp 167–169 °C (acetone/hexane). ^{1}H ($CDCl_3$, 600 MHz): 8.82 (s, NH), 8.19 (d, 1H, *J* 15.4 Hz, H-3), 8.08 (dd, 2H, *J* 8.6, 5.6 Hz, H-2″, H-6″), 7.81 (d, 1H, *J* 7.9 Hz, H-4′), 7.44 (d, 1H, *J* 15.4 Hz, H-2), 7.29 (d, 1H, *J* 8.0 Hz, H-7′), 7.25 (t, 1H, *J* 7.7 Hz, H-6′), 7.18 (t, 1H, *J* 7.6 Hz, H-5′), 7.17 (t, 2H, *J* 8.7 Hz, H-3″, H-5″), 4.76 (t, 1H, *J* 6.1 Hz, $OC\underline{H}(CH_3)_2$), 1.43 (d, 6H, *J* 6.1 Hz, $OCH(C\underline{H}_3)_2$). ^{13}C ($CDCl_3$, 150 MHz): 188.8 (C, C-1), 165.3 (C, d, J_{CF}.252.7 Hz, C-4″), 155.1 (C, C-2′), 137.7 (CH, C-3), 135.7 (C, d, J_{CF}.2.8 Hz, C-1″), 131.9 (C, C-7′a), 130.8 (CH, d, J_{CF}.9.0 Hz, C-2″, C-6″), 125.6 (C, C-3′a), 122.3 (2CH, C-5′, C-6′), 119.7 (CH, C-4′), 115.6 (2CH, d, J_{CF}.21.7 Hz, C-3″, C-5″), 114.0 (CH, C-2), 111.3 (CH, C-7′), 98.5 (C, C-3′), 77.6 (CH, $O\underline{C}H(CH_3)_2$), 22.6 (2xCH_3, $OCH(\underline{C}H_3)_2$). IR (KBr) ν_{max} 3210, 2978, 2926, 1630, 1597, 1522, 1506, 1481, 1339, 1277, 1217, 1153, 1101, 1051, 978, 812, 739 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 324.1399 for $C_{20}H_{19}NO_2F$ (calcd. 324.1400).

(2*E*)-3-(2′-butoxy-1′*H*-indol-3′-yl)-1-(4″-fluorophenyl) prop-2-en-1-one (16e)

Reaction time: 29 h; yield: 31%. R_f 0.35 (hexane/EtOAc 2:1); red crystals; mp 151–153 °C (acetone/hexane). ^{1}H (DMSO-d_6, 600 MHz): 8.13 (dd, 2H, *J* 8.8, 5.6 Hz, H-2″,

H-6″), 8.02 (d, 1H, *J* 15.2 Hz, H-3), 7.86 (d, 1H, *J* 7.8 Hz, H-4′), 7.35 (t, 2H, *J* 8.8 Hz, H-3″, H-5″), 7.35 (d, 1H, *J* 15.3 Hz, H-2), 7.33 (d, 1H, *J* 7.8 Hz, H-7′), 7.18 (td, 1H, *J* 7.6, 1.0 Hz, H-6′), 7.12 (td, 1H, *J* 7.8, 1.0 Hz, H-5′), 4.42 (t, 2H, *J* 6.4 Hz, $OC\underline{H}_2CH_2CH_2CH_3$), 1.83 (tt, 2H, *J* 7.6, 6.4 Hz, $OCH_2C\underline{H}_2CH_2CH_3$), 1.52 (qt 2H, *J* 7.4, 7.6 Hz, $OCH_2CH_2C\underline{H}_2CH_3$), 0.98 (t, 3H, *J* 7.4, $OCH_2CH_2CH_2C\underline{H}_3$). ^{13}C (DMSO-$d_6$, 150 MHz): 186.8 (C, C-1), 164.4 (C, d, J_{CF}.250.0 Hz, C-4″), 156.9 (C, C-2′), 136.5 (CH, C-3), 135.7 (C, d, J_{CF}.2.8 Hz, C-1″), 132.2 (C, C-7′a), 130.7 (2CH, d, J_{CF}.9.2 Hz, C-2″,C-6″), 125.0 (C, C-3′a), 121.7 (CH, C-5′), 121.1 (CH, C-6′), 119.1 (CH, C-4′), 115.5 (2CH, d, J_{CF}.21.6 Hz, C-3″,C-5″), 111.3 (CH, C-7'), 110.9 (CH, C-2), 94.2 (C, C-3′), 71.4 (CH_2, $O\underline{C}H_2CH_2CH_2CH_3$), 30.7 ($CH_2$, $OCH_2\underline{C}H_2CH_2CH_3$), 18.6 ($CH_2$, $OCH_2CH_2\underline{C}H_2CH_3$), 13.6 ($CH_3$, $OCH_2CH_2CH_2\underline{C}H_3$). IR (KBr) ν_{max} 3177, 2962, 2873, 1632, 1599, 1526, 1477, 1450, 1377, 1261, 1225, 1155, 827, 741 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 338.1557 for $C_{21}H_{21}NO_2F$ (calcd. 338.1556).

(2*E*)-3-(2′-isobutoxy-1′*H*-indol-3′-yl)-1-(4″-fluorophenyl)prop-2-en-1-one (16f)

Reaction time: 24 h; yield: 27%. R_f 0.43 (hexane/EtOAc 2:1); orange crystals; mp 171–172 °C (acetone/hexane). ^{1}H (DMSO-d_6, 600 MHz): 12.08 (s, NH), 8.13 (dd, 2H, *J* 8.8, 5.5 Hz, H-2″, H-6″), 8.04 (d, 1H, *J* 15.2 Hz, H-3), 7.86 (d, 1H, *J* 7.8 Hz, H-4′), 7.35 (t, 2H, *J* 8.6 Hz, H-3″, H-5″), 7.35 (d, 1H, *J* 15.3 Hz, H-2), 7.32 (d, 1H, *J* 7.9 Hz, H-7′), 7.18 (td, 1H, *J* 7.6, 1.1 Hz, H-6′), 7.12 (t, 1H, *J* 7.7, 1.0 Hz, H-5′), 4.21 (d, 2H, *J* 6.6 Hz, $OC\underline{H}_2CH(CH_3)_2$), 2.17 (nonet, 1H, *J* 6.6, Hz, $OCH_2C\underline{H}(CH_3)_2$), 1.06 (d 6H, *J* 6.7 Hz, $OCH_2C\underline{H}(C\underline{H}_3)_2$). ^{13}C (DMSO-$d_6$, 150 MHz): 186.7 (C, C-1), 164.3 (C, d, J_{CF}.250.0 Hz, C-4″), 156.9 (C, C-2′), 136.3 (CH, C-3), 135.7 (C, d, J_{CF}.2.7 Hz, C-1"), 132.0 (C, C-7′a), 130.6 (2CH, d, J_{CF}.9.1 Hz, C-2″, C-6″), 125.0 (C, C-3′a), 121.7 (CH, C-5′), 121.1 (CH, C-6′), 119.0 (CH, C-4′), 115.5 (2CH, d, J_{CF}.21.6 Hz, C-3″, C-5″), 111.4 (CH, C-7′), 110.9 (CH, C-2), 94.0 (C, C-3′), 77.3 (CH_2, $O\underline{C}H_2CH(CH_3)_2$), 27.9 (CH, $OCH_2\underline{C}H(CH_3)_2$), 18.7 (2x$CH_3$, $OCH_2CH(\underline{C}H_3)_2$). IR (KBr) ν_{max} 3167, 2959, 1634, 1599, 1526, 1479, 1373, 1357, 1265, 1224, 1151, 1015, 823, 737 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 338.1556 for $C_{21}H_{21}NO_2F$ (calcd. 338.1556).

Data for 1-(4″-Fluorophenyl)-3-(1′H and 1-metyl-indol-3′-yl)prop-2-en-1-ones

(2*E*)-1-(4″-Fluorophenyl)-3-(1′*H*-indol-3′-yl)prop-2-en-1-one (18a)

mp 185–188 °C [6], yellow crystals; mp 185–187 °C (acetone/hexane). ^{1}H (DMSO-d6, 600 MHz): 11.92 (s, NH), 8.21 (dd, *J* 8.8, 5.6 Hz, H-2″, H-6″), 8.12 (s, 1H, H-2′), 8.11–8.09 (m, 1H, H-4′), 8.06 (d, 1H, *J* 15.4 Hz, H-3), 7.64 (d, 1H, *J* 15.4 Hz, H-2), 7.50–7.49 (m, 1H, H-7′), 7.38 (t, 2H, *J* 8.8 Hz, H-3″, H-5″), 7.25 (ddd, 1H, *J* 7.6, 7.1, 1.5 Hz, H-6′), 7.23 (ddd, 1H, *J* 7.5, 7.1, 1.5 Hz, H-5′). ^{13}C (DMSO-d6, 150 MHz): 187.3 (C, C-1), 164.6 (C, d, J_{CF} 250.6 Hz, C-4″), 139.2 (CH, C-3), 137.5 (C, C-7′a), 135.1 (C, d, J_{CF}.2.8 Hz, C-1″), 133.4 (CH, C-2′), 131.0 (2CH, d, J_{CF}.9.2 Hz, C-2″, C-6″), 125.1 (C, C-3′a), 122.7 (CH, C-6′), 121.2 (CH, C-5′), 120.4 (CH, C-4′), 115.6 (2CH, d, J_{CF}.21.6 Hz, C-3″, C-5″), 115.0 (CH, C-2), 112.8 (C, C-3′), 112.4 (CH, C-7′). IR (KBr) ν_{max} 3257, 1648, 1630, 1596, 1561, 1536, 1432, 1360, 1226, 1046, 817, 747 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 266.0981 for $C_{17}H_{13}NOF$ (calcd.266.0981).

(2*E*)-1-(4″-Fluorophenyl)-3-(1′-metyl-1′*H*-indol-3′-yl)prop-2-en-1-one (18b)

Procedure A: EtOH; 8 h; 68%. R_f 0.38 (hexane/ethylacetate 2:1); yellow crystals; mp 154–156 °C (acetone/hexane). ^{1}H (DMSO-d6, 400 MHz): 8.21 (dd, *J* 8.8, 5.8 Hz, H-2″, H-6″), 8.12 (d, 1H, *J* 8.2 Hz, H-4′), 8.11 (s, H-2′), 8.02 (d, 1H, *J* 15.4 Hz, H-3), 7.63 (d, 1H, *J* 15.4 Hz, H-2), 7.57 (d, 1H, *J* 7.8 Hz, H-7′), 7.38 (t, 2H, *J* 8.7 Hz, H-3″, H-5″), 7.32 (ddd, 1H, *J* 7.6 Hz, H-6′), 7.28 (t, 1H, *J* 7.6 Hz, H-5′), 3.86 (s, 3H, N-CH_3). ^{13}C (DMSO-d6, 100 MHz): 187.2 (C, C-1), 164.6 (C, d, J_{CF} 249.3 Hz, C-4″), 138.6 (CH, C-3), 138.0 (C, C-7′a), 136.8 (CH, C-2′), 135.1 (C, d, J_{CF}.2.8 Hz, C-1″), 131.0 (2CH, d, J_{CF}.9.2 Hz, C-2″, C-6″), 125.6 (C, C-3′a), 122.8 (CH, C-6′), 121.4 (CH, C-5′), 120.6 (CH, C-4′), 115.0 (CH, C-2), 115.6 (2CH, d, J_{CF}.21.6 Hz, C-3″, C-5″), 111.7 (C, C-3′), 110.9 (CH, C-7′), 33.1 (CH_3, N-CH_3). IR (KBr) ν_{max} 3094, 1652, 1601, 1584, 1506, 1375, 1280, 1211, 1158, 957, 809, 725 cm^{-1}. HRMS: *m/z* $[M + H]^+$: 280.1136 for $C_{18}H_{15}NOF$ (calcd. 280.1138).

Antiproliferative activity

Hela cells (human cervical carcinoma), HCT116 (human colorectal carcinoma), and Jurkat (human leukemic T cell lymphoma) were cultured in RPMI 1640 medium (Biosera, Kansas City, MO, United States). MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human mammary gland adenocarcinoma), A-549 (human lung adenocarcinoma), Caco-2 (cervical adenocarcinoma) and 3T3 (murine fibroblasts) cell lines were maintained in a growth medium consisting of high glucose Dulbecco's Modified Eagle Medium with sodium pyruvate (GE Healthcare, Piscataway, NJ, United States). The growth medium was supplemented with a 10% fetal bovine serum, 1X HyClone™ Antibiotic/Antimycotic Solution (GE Healthcare, Little Chalfont, UK). Cells were cultured in an atmosphere containing 5% CO_2 in humidified air at 37 °C.

Cell viability, estimated by trypan exclusion, was >95% before each experiment.

The effect of synthetic chalcone derivatives on the viability and proliferation of seven cancer cell lines and one non-cancer cell line was evaluated using 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 5×10^3 cells were plated per well in 96-well polystyrene microplates (SARSTEDT, Nümbrecht, Germany). Twenty four hours after cell seeding, various concentrations (100, 50, 10, 5 and 1 µmol/L,) of tested compounds were added. After 72 h of incubation, 10 µl of MTT (5 mg/ml) were added in each well and incubated for an additional 4 h at 37 °C in 5% CO_2 during which insoluble formazan was produced, followed by addition of 100 µL of 10% sodium dodecyl sulfate in each well and another 12 h were allowed for the formazan to be dissolved. The absorbance was measured at 540 nm using the automated Cytation™ 3 Cell Imaging Multi-Mode Reader (Biotek, Winooski, VT, United States). Three independent experiments were performed for each test. The measured values were shown as percent metabolic activity of the cells as compared to unaffected control, which amounting 100%.

DNA binding activity

Chemicals for biochemical study

For DNA binding experiments Dimethyl Sulfoxide (DMSO), Calf Thymus DNA (CT DNA), Ethidium Bromide (EB), Hoechst 33 258 (HO) and Tris(hydroxymethyl) aminomethane (Tris) were purchased from Sigma-Aldrich Chemie.

UV–Vis absorption measurements

All the spectra were measured in 0.01 M Tris buffer (pH 7.3) by using a Varian Cary 100 Bio spectrophotometer in the range of 300–500 nm and recorded at r.t. (24 °C) using quartz cuvette of 1 cm path length. The concentration of investigated chalcones was 10.4×10^{-6} M. During the titration, an aliquot of buffered CT DNA solution was added to each cuvette (sample and reference) to eliminate the absorbance of DNA itself. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm.

Fluorescence binding study

The fluorescence experiments for competitive binding studies of chalcones with Hoechst 33 258 (HO) were performed on a Varian Cary Eclipse spectrophotometer. The emission spectra of DNA-HO system were measured in the range of 360–600 nm using excitation wavelength of 343 nm, with a slit width of 5.5 nm for the excitation and emission beams. The DNA-HO complex was prepared by adding 1.2×10^{-6} M of dye and 39.2×10^{-6} M of CT DNA in 10 mM Tris-HCl buffer (pH 7.3, 24 °C). K_{SV} quenching constant were determined according to the classical Stern–Volmer Eq. (1) in literature [44, 45].

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

where I_0 and I are the fluorescence intensities in the absence and the presence of the quencher at 470 nm. K_{SV} is the Stern–Volmer quenching constant and $[Q]$ is the concentration of quencher.

Circular dichroism measurements

The CD spectra of DNA in the presence of chalcones recorded on a JASCO (J-810) spectropolarimeter in the wavelength range of 235–320 nm, in 10 mM Tris-HCl buffer (pH 7.3) at 24 °C. The concentration of CT DNA and chalcones was 4.4×10^{-4} M and 5.2×10^{-4} M, respectively.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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