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Structural requirement of arylindolylpropenones as anti-bladder carcinoma cells agents

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ABSTRACT

Chalcones have been identified as interesting compounds with cytotoxicity, anti-inflammatory and antioxidant properties. In the present study, we report the synthesis and evaluation of new 1-(*N*-methylindolyl)-3-phenylpropenones as anti-cancer agents acting on bladder carcinoma cell line. Among the 15 investigated molecules, three of them inhibit the growth of bladder cancer cells with IC_{50} values less than 4 μM after 48 h of treatment. To investigate their mode of action, cell cycle analyses were performed. The most active compounds induce high accumulation at the G2+M phase as assessed by flow cytometry. The structure–activity relationship drawn from the present study highlights the importance of the substitution pattern of the phenyl ring and provides valuable information for further development of this class of compounds as novel anti-cancer chemotherapeutic agents.

1. Introduction

According to recent estimations, around 3,86,000 new cases and 1,50,000 deaths from bladder cancer occurred in 2008, worldwide.[1] Approximately 70–80% of newly diagnosed bladder cancer cases, occur with nonmuscle-invasive disease. Despite endoscopic and intravesical treatments, 50–70% will recur, whereas 10–30% will progress to muscle-invasive disease.[2] Treatment of invasive bladder carcinoma is based on three major treatments: surgical resection, intravesical immunotherapy and chemotherapy.[3–5] Unfortunately, in the last case, there is no efficient drug able to cure bladder carcinoma and most of the deaths from bladder cancer are due to advanced unresectable disease, which is resistant to chemotherapy.[6,7] Therefore, there is an urgent need for finding new drugs to treat bladder carcinoma.

Chalcones are open chain flavonoids that are widely biosynthesized in plants. They are important for the pigmentation of flowers and, hence, act as attractants to the pollinators. Owing to their biological activity in different pharmacological domains, naturally occurring chalcones and their synthetic derivatives have drawn considerable attention over the last two decades.[8] The cytotoxic activity of chalcones was largely investigated and different mechanisms of action were reported. Chalcones act as antimitotic agents by interfering with tubulin polymerization into microtubules.[9,10] Some chalcone derivatives are able to dissociate the p53/MDM2 complexes abolishing constitutive inhibition of p53 in tumors with elevated levels of MDM2.[11] Chalcones induce cell cycle perturbations and lead to G1 or G2+M arrest with alteration in the level of the cyclin proteins family.[12–14]

Most of the early SAR relationship studies on chalcones have concerned the variation of the substituent nature and its position at the two phenyl rings of chalcones.[12,15] As part of our ongoing studies aimed at developing new chalcone derivatives as anticancer agents, we were interested by analogs in which a phenyl ring of chalcone is replaced by an indolyl (Fig. 1). In this context, we recently reported the anticancer activity of one chalcone-like derivative with promising cytotoxic effect on bladder carcinoma cell lines.[16] With the aim to find more active derivatives, we underwent the synthesis of substituted 1-indolyl-3-phenylproenones and the investigation of their cytotoxicity on bladder carcinoma cell lines. The ultimate goal was the optimization of the substitution pattern of the phenyl ring and the investigation of the most active derivatives on the cell cycle.

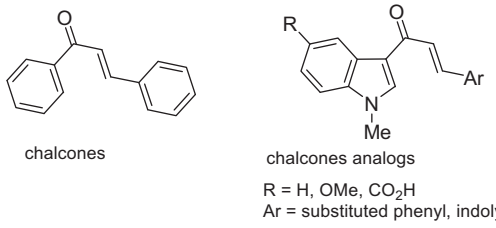

Figure 1. General structure of investigated arylindolylpropenones.

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2. Results and discussion

2.1. Chemistry

The synthesis of the investigated compounds was conducted according to Scheme 1. A solution of 3-acetyl-1-methylindol was treated with an aqueous solution of KOH in methanol and mixed with a benzaldehyde derivative or 1-methylindol-3-carboxaldehyde. After 12 h of refluxing, the desired 1,3-diarylpropenone was isolated, purified and characterized by means of NMR, mass spectrometry and elemental analysis.

2.2. Antiproliferative activity

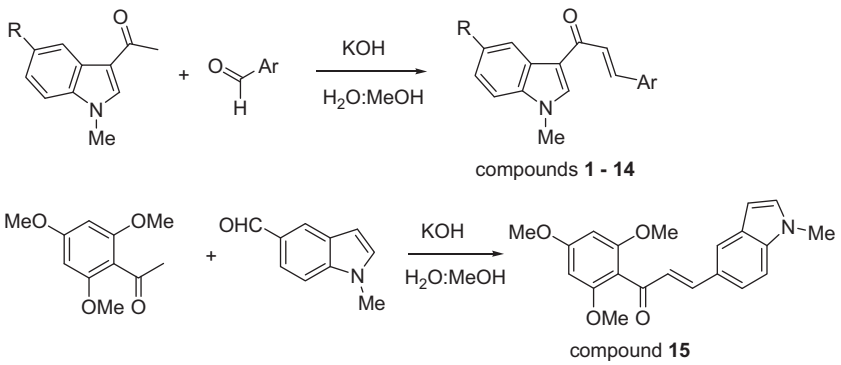
Screening of the 1,3-diarylpropenone derivatives library was performed on the RT112 cell line. This is a human bladder cancer cell line derived from a papillary, non-metastatic transitional cell carcinoma.[17,18] RT112 cells were incubated with various concentrations of the tested compound and their proliferation was determined by a MTT assay.[19] Our results indicated that it was necessary to treat cells for at least 48 h to observe a massive cell death. The antiproliferative activities of tested compounds after 48 h of treatment, expressed as IC_{50} values (the concentration resulting in 50% loss of cell viability relative to untreated cells) are summarized in Table 1. Cisplatin, which is currently used in the management of advanced bladder cancer, was applied as a test referential agent.[20] Our strategy was to investigate 1-(*N*-methylindolyl)-3-phenyl analogs, bearing different substituents at the phenyl ring. The substituent groups were selected among those previously identified as important elements for the anticancer activity of chalcones.[12] The unsubstituted derivative **1** showed moderate cytotoxic effect. The introduction of a fluorine at C-2′ (compound **2**), identified as an important element for the antimitotic activity of chalcones was not relevant.[21] The replacement of the phenyl ring by a 2,6-dichlorophenyl led to a significant increase activity (compound **3**). The introduction of a dimethylamino group at C-4′ (compound **4**) was found to be highly beneficial which is in agreement with previous studies from Peyrot's group.[22] The replacement of the dimethylamino group by a close structure, *N*-methyl piperazinyl (compound **5**) led to a slight decrease activity. Moving the later substituent to the 2′-position (compound **6**) was disadvantageous.

Next, we examined the methoxylation effect. This structural element was found to be determinant for the antitumor activity of chalcones.[23–27] In this line, the introduction of two methoxy groups either at 2′,4′-positions or 2′,6′-positions induced a significant decrease activity (compounds **7** and **8**). However, the presence of three methoxy groups at 2′,4′,6′-positions (compound **9**) led to one of the most active compounds. This data is an agreement with previously reported results on chalcones.[28] In order to confirm the importance of the 2′,4′,6′-positions toward methoxylation, we checked the activity of the 3′,4′,6′-trimethoxy derivative (compound **10**). As expected, the activity of the latter was dramatically dropped. Knowing the importance of the methoxylation effect, we tested derivative **11**, which bears an additional methoxy group at the indolyl moiety and found that it was the most active compound of the series (IC_{50} = 3.1 μM). Replacement of the indolyl methoxy group by a removable element such as a carboxy group (derivative **12**) led to a dramatic drop of activity.

Because the *N*-methylindolyl system was essential for the activity, we aimed to replace the phenyl ring in our general structure by an *N*-methylindolyl, (derivatives **13** and **14**). As shown in Table 1, this modification turns out to be highly beneficial and informative. In one hand, this approach provides compound (**14**) which is one of the three most active compounds and in the other hand it shows the importance of the linkage position of the second *N*-methylindolyl moiety to the propenone chain. In order to check the impact of interchanging the *N*-methylindolyl and phenyl rings, we selected one of the most active derivatives (compound **9**) and synthesized its regioisomeric analog (compound **15**). The results shown in Table 1 clearly indicate that this interchange is not permitted. This point out that the substitution pattern is not the only important element since the electronic distribution throughout the molecule is also essential. Interestingly, the activity of some compounds was higher or comparable to that of the referential cisplatin which is an effective drug used in the clinic.

2.3. Effect on cell cycle progression

From these cytotoxic studies, it appeared that compounds **9**, **11** and **14**, were the most potent cytotoxic agents against bladder cancer cells. It was worth proceeding to further characterization of these molecules to determine their mechanism of action. Cell cycle analysis was performed with the aim to examine the influence of these derivatives on the progression of the cell cycle. Cells were exposed to the vehicle solvent (DMSO) as a control or to IC_{50} concentrations of compounds **9**, **11** and **14**. After 12 or 24 h of incubation, cells in suspension in the medium and attached cells were analyzed by flow cytometry (Fig. 2). The majority of control cells (69%) were in the G1 phase, whereas the rest of the cells were equally distributed between the S phase (17%) and the G2+M phase (14%). As shown in Figure 2A, a 12 h treatment with 3 μM of compounds **9** and **11** resulted in a significant accumulation of RT112 cells in the G2+M phase (74% and 91%, respectively) with concomitant loss from the G1 phase (16% and 5%, respectively). No major change of the S phase was observed. This G2+M accumulation remained stable at 24 h of treatment (Fig. 2B). The results obtained with the compound **14** were slightly different. After 12 h of



Scheme 1. Synthetic pathway for the synthesis of the arylindolylpropenones compounds.

Table 1
Antiproliferative activities of the newly synthesized arylindolylpropenones derivatives. The concentration resulting in 50% loss of cell viability relative to untreated cells (IC_{50}) was determined from dose-response curves. Results represent means ± SD of three independent experiments

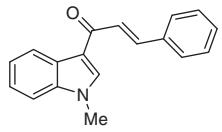
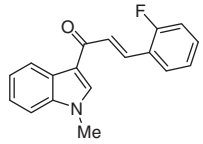
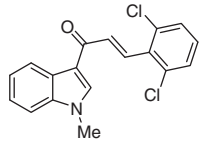
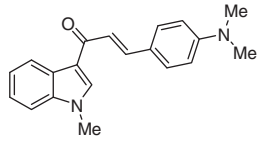
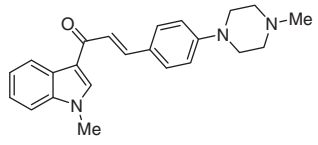
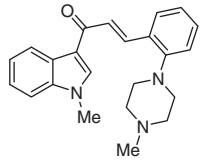
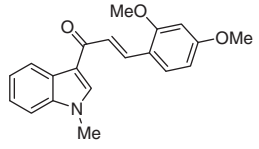
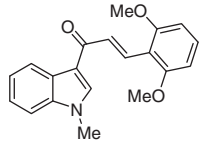
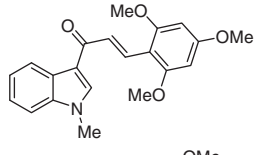
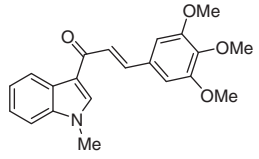
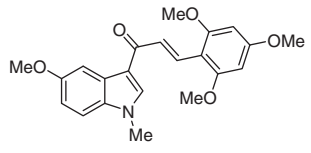
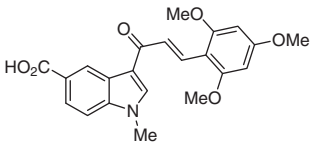
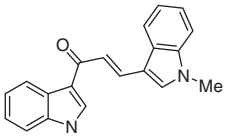
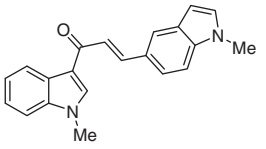
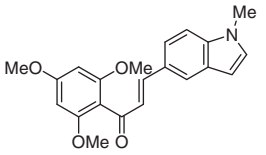
Name	Structure	IC_{50} (μM)
Compound **1**		30.6 ± 0.1
Compound **2**		28.7 ± 3.1
Compound **3**		7.8 ± 0.7
Compound **4**		7.5 ± 0.5
Compound **5**		11.7 ± 3.3
Compound **6**		26.4 ± 1.9
Compound **7**		57.7 ± 6.7
Compound **8**		47.4 ± 3.8
Compound **9**		3.2 ± 0.2
Compound **10**		42.4 ± 0.9
Compound **11**		3.1 ± 0.3

Table 1 (*continued*)

Name	Structure	IC_{50} (μM)
Compound **12**		86.1 ± 10.1
Compound **13**		63 ± 8.3
Compound **14**		4 ± 0.5
Compound **15**		54.3 ± 6
Cisplatin	—	6.7 ± 0.5

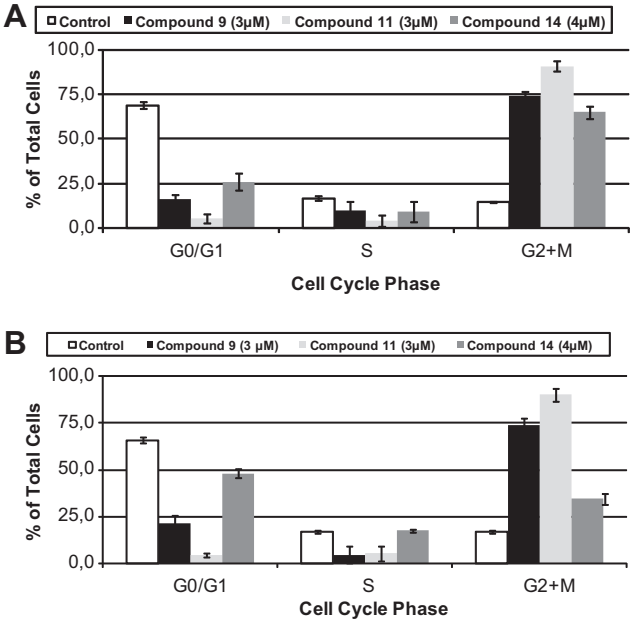


Figure 2. The effect of compounds **9**, **11** and **14** on cell cycle distribution of RT112 cells. RT112 cells were treated with vehicle alone (Control) or with IC_{50} value of each compound for 12 h (**A**) and 24 h (**B**). Cells were then fixed, stained with PI and cell cycle was analyzed by flow cytometry. Each value is the mean ± SD of three independent determinations.

incubation with 4 μM of this molecule, a G2+M accumulation was also observed, but in a lower extent (65%) than with compounds **9** and **11** (Fig. 2A). Furthermore, this accumulation did not persist at 24 h of treatment where only 34% of cells were still arrested at the G2+M phase (Fig. 2B). Therefore, compound **14** seemed to be less effective than molecules **9** and **11** as antimitotic agent.

We next assessed the effect after a longer incubation time. Treatments with the three compounds led to the detection of a

characteristic hypodiploid DNA content peak (sub-G1) when the treatment duration was extended to 48 h (data not shown), indicating the presence of apoptotic cells. The above results indicate that compounds **9**, **11** and **14** induce cell cycle arrest in G2+M phase before cell death occurs.

3. Conclusion

The present study is the first reported investigation dealing with the structure–activity of *N*-methylindolylphenylpropenones as new antitumor agents. It sheds light on the structural elements to be considered for enhancing the antiproliferative activity of *N*-indolyphenylpropenone. The presence of three methoxy groups at 2′,4′,6′-positions of the phenyl ring is essential. The replacement of the latter by an *N*-methylindolyl is allowed, if the linkage to the propenone chain is correctly chosen. Treatment of RT112 bladder cancer cell line with lead compounds led to a G2+M accumulation and showed that they act as antimitotic agents. This activity has been previously described for other chalcones and is often associated with tubulin inhibition. Indeed chalcones have the ability of interfering with microtubule formation, which is essential in cellular processes such as mitosis.[9,10,25,29] Further studies will be conducted in order to determine if our derivatives are able to bind to tubulin. Furthermore, the anticancer potential of the most active chalcones are planned to start shortly on mouse bearing RT112 tumor xenografts in order to determine their bioavailability and their potential toxicity. In conclusion, our lead compounds are promising molecules, as they show a better cytotoxic activity than cisplatin, a conventional chemotherapeutic agent used to treat patients suffering from bladder cancer.

4. Experimental

4.1. Chemistry

4.1.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Brüker AC-400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C). Electrospray ionization ESI mass spectra were acquired by the Analytical Department of Grenoble University on an Esquire 300 Plus Bruker Daltonis instrument with a nanospray inlet. Elemental analyses were performed by the Analytical Department of Grenoble University. Thin-layer chromatography (TLC) used Merck silica gel F-254 plates (thickness 0.25 mm). Flash chromatography used Merck silica gel 60, 200–400 mesh. All solvents were distilled prior to use. Unless otherwise stated, reagents were obtained from commercial sources and were used without further purification.

4.1.2. Synthesis of arylindolylpropenones

To a solution of *N*-methyl-3-acetylindol (1 mmol) and the benzaldehyde derivative (1 mmol) in methanol (10 ml) was added KOH (1 ml from a 50% solution in H_2O). The mixture was heated at 60 °C for 12 h then evaporated to dryness. The crude was dissolved in ethyl acetate (30 ml) then washed with HCl (1N, 10 ml) and H_2O (10 ml), respectively. The organic layer was separated, dried over Na_2SO_4 and evaporated. The product was purified by chromatography column eluted with hexane:ethyl acetate (8:2) to yield the title compound as a yellow powder.

4.1.2.1. 1-(1-Methyl-1H-indol-3-yl)-3-phenyl-propenone (1). Mp: 121–123 °C; ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 7.27–7.42 (m, 7H), 7.64 (d, J = 7.5 Hz, 1H); 7.66 (d, J = 15 Hz, 1H), 7.86 (s, 1H), 8.51 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 42.4, 111.0, 111.4, 119.0, 120.5, 121.9, 122.1, 126.7, 127.1, 128.0, 128.5, 128.8, 135.0, 135.3, 136.6, 144.3, 190.2. MS (ESI) m/z 262 $(M+H)^+$, 284 $(M+Na)^+$. Anal. Calcd for $C_{18}H_{15}NO$: C, 82.73; H, 5.79; N, 5.36. Found: C, 82.70; H, 5.72; N,
5.34.

4.1.2.2. 3-(2-Fluoro-phenyl)-1-(1-methyl-1H-indol-3-yl)-propenone (2). Mp: 151–153 °C; ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H), 7.11–7.45 (m, 6H), 7.50 (d, J = 15 Hz, 1H), 7.63–7.65 (m, 1H), 7.86–7.91 (m, 2H), 8.53 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 42.3, 111.3, 111.6, 115.0 (d, J = 15 Hz), 118.7, 120.2, 121.6, 122.7, 123.0 (d, J = 21 Hz), 124.1, 126.6, 128.3, 129.4, 135.2, 137.1, 145.0, 157.4, (d, J = 249 Hz), 190.3. MS (ESI) m/z 280 $(M+H)^+$, 302 (M+Na)+. Anal. Calcd for $C_{18}H_{14}FNO$: C, 77.40; H, 5.05; N, 5.01. Found: C, 77.26; H, 5.00; N, 4.95.

4.1.2.3. 3-(2,6-Dichloro-phenyl)-1-(1-methyl-1H-indol-3-yl)-propenone (3). Mp: 183–185 °C; ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H), 7.19 (t, J = 7.3 Hz, 1H), 7.27–7.34 (m, 5H), 7.40 (d, J = 15.3 Hz, 1H), 7.80 (s, 1H), 7.84 (d, J = 15.3 Hz, 1H), 8.53 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 41.8, 110.3, 110.9, 119.2, 120.2, 121.4, 122.9, 126.4, 127.4, 130.6, 133.5, 132.2, 134.7, 136.8, 144.1, 189.9. MS (ESI) m/z 330 (M+H)+, 352 $(M+Na)^+$. Anal. Calcd for $C_{18}H_{13}Cl_2NO$: C, 65.47; H, 3.97; N, 4.24. Found: C, 65.39; H, 3.93; N, 4.19.

4.1.2.4. 3-(4-Dimethylamino-phenyl)-1-(1-methyl-1H-indol-3-yl)-propenone (4). Mp: 189–191 °C; ^{1}H NMR ($CDCl_3$): δ 3.04 (s, 6H), 3.86 (s, 3H), 6.70 (d, J = 6 Hz, 2H), 7.20–7.34 (m, 4H), 7.55 (d, J = 6 Hz, 2H), 7.78–7.83 (m, 2H), 8.54 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 39.8, 42.0, 111.0, 111.5, 114.7, 119.3, 120.6, 122.2, 122.4, 124.8, 126.1, 127.4, 134.3, 136.2, 144.4, 149.2, 190.1. MS (ESI) m/z 305 $(M+H)^+$; Anal. Calcd for $C_{20}H_{20}N_2O$: C, 78.92; H, 6.62; N, 9.20. Found: C, 78.88; H, 6.60; N, 9.17.

4.1.2.5. 1-(1-Methyl-1H-indol-3-yl)-3-[4-(4-methyl-piperazin-1-yl)-phenyl]-propenone (5). Mp: 166–168 °C; ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H), 2.59 (br s, 4H), 3.32 (br s, 4H), 3.88 (s, 3H), 6.91 (d, J = 6.2 Hz, 2H), 7.23–7.35 (m, 4H), 7.56 (d, J = 6 Hz, 2H), 7.75–7.84 (m, 2H), 8.55 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 41.5, 43.5, 49.7, 55.3, 110.9, 111.4, 114.7, 119.0, 120.6, 122.0, 122.8, 124.7, 126.0, 127.1, 135.3, 137.2, 145.2, 149.4, 191.2. MS (ESI) m/z 360 $(M+H)^+$; Anal. Calcd for $C_{23}H_{25}N_3O$: C, 76.85; H, 7.01; N, 11.69. Found: C, 76.83; H, 6.99; N, 11.65.

4.1.2.6. 1-(1-Methyl-1H-indol-3-yl)-3-[2-(4-methyl-piperazin-1-yl)-phenyl]-propenone (6). Mp: 152–154 °C; ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H), 2.68 (br s, 4H), 3.05 (br s, 4H), 3.89 (s, 3H), 7.10 (d, J = 6 Hz, 1H), 7.27–7.40 (s, 5H), 7.68 (d, J = 6 Hz, 1H), 7.88 (br s, 1H), 8.19 (d, J = 15.2 Hz, 1H), 8.57 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 42.2, 43.6, 50.9, 55.2, 110.4, 111.6, 115.3, 118.2, 119.3, 120.5, 121.4, 122.7, 123.4, 126.6, 127.8, 129.5, 135.2, 136.9, 146.0, 148.3, 190.9. MS (ESI) m/z 360 $(M+H)^+$. Anal. Calcd for $C_{23}H_{25}N_3O$: C, 76.85; H, 7.01; N, 11.69. Found: C, 76.80; H, 6.96; N, 11.67.

4.1.2.7. 3-(2,4-Dimethoxy-phenyl)-1-(1-methyl-1H-indol-3-yl)-propenone (7). Mp: 181–183 °C; ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H), 3.87 (s, 3H), 3.91 (s, 3H), 6.49 (s, 1H), 6.55 (dd, J_1 = 2 Hz, J_2 = 7 Hz, 1H), 7.34–7.36 (m, 3H), 7.38 (d, J = 15.5 Hz, 1H), 7.58 (d, J = 7 Hz, 1H), 7.83 (s, 1H) (d, J = 15.2 Hz, 1H), 8.06 (d, J = 15.5 Hz, 1H), 8.52 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 41.8, 56.2, 56.5, 100.8, 106.5, 108, 110.2, 111.6, 119.0, 120.3, 121.6, 122.9, 125.7, 128.4, 135.6, 136.2, 145.0, 158.7, 161.1, 191.7; MS (ESI) m/z 322

(M+H)$^+$, 344 (M+Na)$^+$; Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36. Found: C, 74.70; H, 5.93; N, 4.27.

4.1.2.8. 3-(2,6-Dimethoxy-phenyl)-1-(1-methyl-1H-indol-3-yl)-propenone (8). Mp: 161–163 °C; ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H), 3.93 (s, 6H), 6.59 (d, J = 6 Hz, 1H), 7.25–7.34 (m, 5H), 7.80 (s, 1H), 7.85 (d, J = 15.2 Hz, 1H), 8.26 (d, J = 15.2 Hz, 1H), 8.54 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 41.2, 56.0, 106.5, 109.1, 111.2, 111.7, 119.0, 120.8, 122.0, 122.5, 125.9, 130.3, 134.8, 136.9, 145.7, 159.7, 191.1. MS (ESI) m/z 322 (M+H)$^+$, 344 (M+Na)$^+$; Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36. Found: C, 74.72; H, 5.91; N, 4.32.

4.1.2.9. 1-(1-Methyl-1H-indol-3-yl)-3-(2,4,6-trimethoxy-phenyl)-propenone (9). Mp: 183–185 °C; ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H), 3.86 (s, 3H), 3.92 (s, 6H), 6.15 (s, 2H), 7.31–7.37 (m, 3H), 7.80 (s, 1H), 7.74 (s, 1H), 7.78 (d, J = 15.1 Hz, 1H), 8.25 (d, J = 15.1 Hz, 1H), 8.53 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 42.2, 55.9, 56.6, 93.1, 102.4, 110.2, 111.1, 118.1, 120.5, 121.7, 122.4, 126.4, 135.2, 137.2, 160.7, 162.2, 190.8. MS (ESI) m/z 352 (M+H)$^+$, 374 (M+Na)$^+$; Anal. Calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.75; H, 5.99; N, 3.92.

4.1.2.10. 1-(1-Methyl-1H-indol-3-yl)-3-(3,4,5-trimethoxy-phenyl)-propenone (10). Mp: 201–203 °C; ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H), 3.91 (s, 3H), 3.94 (s, 6H), 6.87 (s, 2H), 7.24–7.38 (m, 6H), 7.80 (s, 1H), 7.73 (d, J = 14.9 Hz, 1H), 7.89 (s, 1H), 8.52 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 43.0, 56.0, 56.3, 103.6, 110.8, 111.3, 119.6, 120.5, 122.0, 122.9, 126.1, 130.2, 134.2, 136.6, 139.3, 151.7, 191.8. MS (ESI) m/z 352 (M+H)$^+$, 374 (M+Na)$^+$; Anal. Calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.73; H, 6.00; N, 3.95.

4.1.2.11. 1-(5-Methoxy-1-methyl-1H-indol-3-yl)-3-(2,4,6-trimethoxy-phenyl)-propenone (11). Mp: 206–208 °C; ^{1}H NMR ($CDCl_3$): δ 3.39 (s, 3H), 3.86 (s, 3H), 3.41 (s, 3H), 3.46 (s, 9H), 5.70 (s, 2H), 6.50 (d, J = 7 Hz, 1H), 6.78–6.85 (m, 2H), 7.26 (d, J = 15 Hz, 1H), 7.30 (s, 1H), 7.65 (s, 1H), 7.79 (d, J = 15.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 41.4, 55.9, 56.2, 56.6, 93.0, 101.6, 102.3,, 109.2, 110.2, 112.4, 121.4, 127.6, 129.3, 135.4, 145.0, 155.1, 159.2, 162.3, 190.1. MS (ESI) m/z 382 (M+H)$^+$, 404 (M+Na)$^+$; Anal. Calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.24; H, 6.04; N, 3.62.

4.1.2.12. 1-Methyl-3-[3-(2,4,6-trimethoxy-phenyl)-acryloyl]-1H-indole-5-carboxylic acid (12). Mp: 205–207 °C; ^{1}H NMR (CD_3OD): δ 3.91 (s, 3H), 3.94 (s, 3H), 4.01 (s, 6H), 3.46 (s, 9H), 6.29 (s, 2H), 7.45 (d, J = 6.5 Hz, 1H), 7.93 (d, J = 15.6 Hz, 1H), 8.03 (d, J = 6.5 Hz, 1H), 8.14 (s, 1H), 8.23 (d, J = 15.5 Hz, 1H), 9.10 (s, 1H); ^{13}C NMR (CD_3OD): δ 43.3, 55.9, 56.7, 93.2, 103.2, 111.6, 112.4, 115.0, 122.0, 122.9, 126.9, 135.5, 143.6, 146.7, 160.1, 162.2, 169.1, 192.1. MS (ESI) m/z 396 (M+H)$^+$; Anal. Calcd for $C_{22}H_{21}NO_6$: C, 66.83; H, 5.35; N, 3.54. Found: C, 66.79; H, 5.28; N, 3.52.

4.1.2.13. 1,3-Bis-(1-methyl-1H-indol-3-yl)-propenone (13). Mp: 238–240 °C; ^{1}H NMR (CD_3OD): δ 3.86 (s, 6H), 7.25–7.50 (m, 8H), 7.68–7.71 (m, 2H), 8.31–8.38 (m, 2H); ^{13}C NMR (CD_3OD): δ 42.0, 42.5, 110.4, 111.0, 111.2, 111.9, 112.4, 118.1, 119.2, 120.1, 120.5, 122.3, 123.5, 126.7, 126.9, 134.4, 135.5, 136.8, 137.3, 145.0, 192.2. MS (ESI) m/z 315 (M+H)$^+$; Anal. Calcd for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.20; H, 5.79; N, 8.89.

4.1.2.14. 3-(1-Methyl-1H-indol-5-yl)-1-(1-methyl-1H-indol-3-yl)-propenone (14). Mp: 202–204 °C; ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3H), 3.85 (s, 3H), 6.54 (d, J = 3 Hz), 7.07(d, J = 3 Hz), 7.28–7.39 (m, 7H), 7.57 (dd, J_1 = 3 Hz, J_2 = 6.5 Hz, 1H), 7.85–7.91 (m, 3H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 42.3, 42.8, 110.0, 110.1, 111.4, 111.7, 112.0, 118.0, 119.2, 120.9, 121.5, 123.2, 127.2, 128.0, 128.9, 135.1, 135.6, 136.9, 138.2, 138.8, 191.9. MS (ESI) m/z 315 (M+H)$^+$; Anal. Calcd for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.17; H, 5.72; N, 8.90.

4.1.2.15. 3-(1-Methyl-1H-indol-5-yl)-1-(2,4,6-trimethoxy-phenyl)-propenone (15). Mp: 159–161 °C; ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 6H), 3.80 (s, 3H), 3.95 (s, 3H), 6.18 (s, 2H), 6.30 (br s, 1H), 6.97 (d, J = 15.7 Hz, 1H), 7.07 (br s, 1H), 7.29 (d, J = 15.6 Hz, 7.48 (d, J = 15.7 Hz, 1H), 7.76 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 42.4, 55.7, 56.1, 93.7, 100.6, 103.7, 110.4, 111.2, 118.9, 122.4, 128.0, 129.3, 136.1, 138.2, 145.5, 163.0, 168.2, 189.2. MS (ESI) m/z 352 (M+H)+, 374 (M+Na)$^+$; Anal. Calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.72; H, 5.97; N, 3.95.

4.1.3. Preparation of arylindolylpropenones solutions

Compounds were dissolved in DMSO to a final concentration of 10^{-2} M. These solutions were then directly diluted into culture medium. In order to test its innocuousness, DMSO alone was used at the same dilution than the other compounds.

4.2. Cell culture

Human bladder cancer cell line RT112 was obtained from Cell Lines Service (Eppelheim, Germany). RT112 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (FCS) and 2 mM glutamine (Invitrogen Life Technologies, Paisley, UK). Cells were maintained at 37 °C in a 5% CO_2-humidified atmosphere and tested to ensure freedom from mycoplasma contamination.

4.3. Cell proliferation assay

Inhibition of cell proliferation by arylindolylpropenones derivatives was measured by a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. RT112 cells were seeded into 96-well plates (3×10^3 cells/well) in 100 µl of culture medium. After 24 h, cells were treated with cisplatin (Sigma-Aldrich, Lyon France) or arylindolylpropenones compounds at various concentrations. In parallel, a control with DMS0 (vehicle alone) at the same dilutions was done. Following incubation for 48 h, 10 µl of a MTT (Euromedex, Mundolsheim, France) stock solution in PBS at 5 mg/ml was added in each well and the plates were incubated at 37 °C for 3 h. Plates were then centrifugated 5 min at 1500 rpm before the medium was discarded and replaced with DMSO (100 µl/well) to solubilize water-insoluble purple formazan crystals. After 15 min under shaking, absorbance was measured on an ELISA reader (Tecan, Männedorf, Switzerland) at a test wavelength of 570 nm and a reference wavelength of 650 nm. Absorbance obtained by cells treated with the same dilution of the vehicle alone (DMSO) was rated as 100% of cell survival. Each data point is the average triplicates of three independent experiments.

4.4. Cell cycle analysis

RT112 cells were cultured in 6-wells plates (2×10^5 cells/well) for 24 h before treatment with DMSO or compounds **9**, 11 and **14** for 24 or 48 h. Trypsinized and floating cells were then pooled, fixed with 70% ethanol, washed with PBS and stained with 20 µg/ml propidium iodide (PI) in the presence of 0.5 mg/ml of RNAse (Sigma-Aldrich, Lyon France). Data acquisitions were performed with a FACScan (Becton Dickinson France, Le Pont-De-Claix, France) equipped with a 488 nm laser Argon and PI fluorescence

was collected with a 585 ± 44 nm filter. Parameters from 2×10^4 cells were acquired using the Cell Quest Pro software (Becton Dickinson). The percentage of cell cycle distribution in the G1, S and G2+M phases was determined using FCS Express 3 software (De Novo Sofware, Los Angeles, CA).

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