Investigation of a new 1,3-diarylpropenone as a potential antimitotic agent targeting bladder carcinoma

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1-(2,4-dimethoxyphenyl)-3-(1-methylindolyl) propenone, namely IPP51, was identified by screening a library of 3-indolyl-1-phenylpropenones. IPP51 was investigated for its ability to inhibit proliferation and/or to induce apoptosis of human bladder cancer cell lines and to assess its potential use in bladder carcinoma treatment. After treating the cells with IPP51 for 24 h, the title compound induced a predominant and reversible G2 + M accumulation at the prometaphase stage of mitosis. However, when used for a longer period, it leads to cell apoptosis. These results suggest that the compound has potential anticancer activities, which could be useful in bladder cancer treatment. ***Anti-Cancer Drugs*** **20:469–476 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Bladder cancer is estimated to be the seventh most common malignancy worldwide, accounting for approximately 3.2% of the international cancer burden. Bladder tumors can be divided into two groups, depending on the pathologic stage, as superficial and invasive [1]. More than 80% of superficial carcinoma remain confined to the submucosa and tend to have a benign clinical course. However, up to 25% will progress to invasive form which has a high propensity to invade the muscle wall, to metastasize, and has a poor prognosis with less than 50% survival at 5 years [2].

Treatment modalities for bladder cancer depend on the form of the tumor and consist of a combination of surgical resection, intravesical immunotherapy, and chemotherapy [3]. For chemotherapy, research has been focused on finding new drugs and combinations of several molecules. After cisplatin-based regimens in the first-line setting, there is no standard treatment and it is necessary to find new treatments to improve patient's outcome. Single agents (vinflunine, pemetrexed), and multidrug combinations with cytotoxic and targeted agents, including trastuzumab and bevacizumab, were tested to improve the second-line treatment [4]. Vinflunine is a semisynthetic drug derived from the vinca alkaloid vinorelbine with antitubulin, antineoplastic, and antiangiogenic activities. Vinflunine inhibits tubulin assembly without any stabilization of assembled microtubules at concentrations comparable with those of other vinca alkaloids such as vincristine, vinblastine, and vinorelbine. The effect on microtubule dynamics results in cell cycle arrest in mitosis and apoptosis. In contrast, pemetrexed is a tight-binding inhibitor of folate-dependent enzymes, which inhibits enzymes involved in folate metabolism and purine–pyrimidine synthesis. Trastuzumab is a recombinant humanized monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2). After binding to HER2 on the tumor cell surface, trastuzumab induces an antibody-dependent cell-mediated cytotoxicity against tumor cells that overexpress HER2. Bevacizumab is also a recombinant humanized monoclonal antibody, which is directed against the vascular endothelial growth factor (VEGF), a proangiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance of tumor blood vessels [5].

In a recent study, we investigated 2-arylidenedihydroindole-3-ones as potent antiproliferative agents targeting bladder cancer cells [6]. In pursuing our efforts to identify more potent and safe compounds for the treatment of bladder cancer, we investigated a series of 3-indolyl-1-phenylpropenones (IPPs) (Fig. 1). The latter scaffold was investigated on the basis of two recent studies reported from our group which assess the potential of 2-arylidenedihydroindol-3-ones [6] and methoxylated chalcones (compounds b) as potent antimitotic agents [7]. The analysis of compounds a and b structures (Fig. 1) allowed us to consider the importance of at least three

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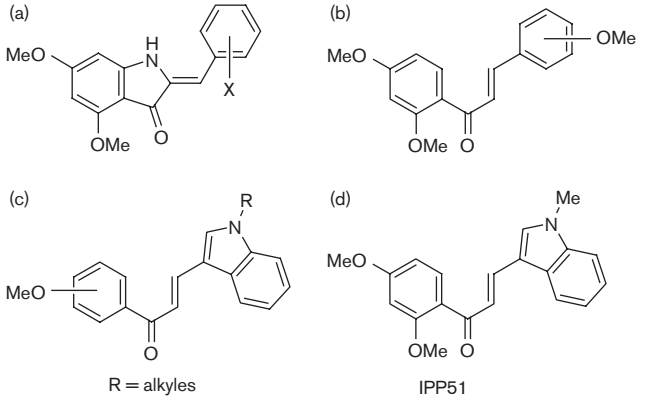
Fig. 1



(a–c) Structures of screened molecule (Refs [6,7]) and (d) structure of the lead compound (IPP51).

structural elements: a dimethoxyphenyl, a propenone chain, and an indol moeity. Taken together, the latter three elements allowed us to emphasize a series of 1-dimethoxyphenyl-3-indolylpropenones (compounds c in Fig. 1). The screening of a large series of derivatives led us to identify 1-(2,4-dimethoxyphenyl)-3-(1-methylindolyl) propenone, namely IPP51 (compound d in Fig. 1), as the most potent derivative, which was selected for global investigation on different bladder cancer cell lines.

Methods

Chemistry

Compound IPP51 was prepared according to Fig. 2. KOH (1 ml from a 50% solution in H_2O) was added to a solution of 2′,4′-dimethoxyacetophenone (1 mmol) and 1-methylindolyl-3-carboxaldehyde (1 mmol) in methanol (10 ml). The mixture was heated at 60°C for 12 h then evaporated to dryness. The crude pruduct was dissolved in ethyl acetate (30 ml) then washed with HCl (1 N, 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 pure IPP51 as a yellow powder.

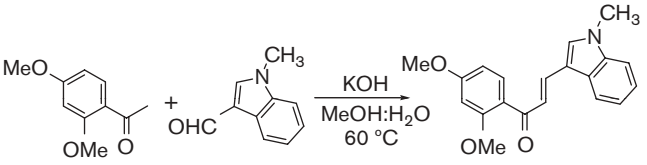
Cell culture

Bladder cancer cell lines were cultured in RPMI 1640 medium (Invitrogen Life Technologies, Cergy Pontoise, France) supplemented with 10% (v/v) fetal calf serum, 2 mmol/l glutamine, 2.5 U/ml penicillin, and 2.5 µg/ml streptomycin (Invitrogen, Life Technologies). Each cell type was cultured at 37°C in a 5% CO_2-humidified atmosphere and tested to ensure freedom from mycoplasma contamination.

Preparation of IPP51 solution

IPP51 was dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10^{-2} mol/l. This solution was

Fig. 2



Chemical synthesis of IPP51.

then directly diluted into culture medium. For control, DMSO was used at the same concentration to test its innocuousness.

Cell proliferation assay

Bladder cancer cell lines were seeded into a 96-well plate (3×10^3 cells/well). After 24 h of culture, cells were treated with DMSO or IPP51. After incubation for 24 or 48 h, 10 µl of a MTT (Euromedex, Mundolsheim, France) stock solution in PBS (5 mg/ml) was added into each well and the plate was then incubated at 37°C for 3 h. A solution of 10% SDS, 0.01 N HCl was then added to each well (100 µl/well) to solubilize water-insoluble purple formazan crystals. After 90 min at 37°C, absorbance was measured at 570 nm using an ELISA reader (Sunrise, Tecan France, Lyon).

Flow cytometry

Cell cycle analysis

Cancer cell lines were cultured in 6-well plates (1.2×10^4 cells/well) for 24 h. Cells were then treated with DMSO or IPP51 for 24 or 48 h. Trypsinized and floating cells were pooled, fixed with 70% ethanol, and stained with 20 µg/ml propidium iodide (PI) in the presence of 0.5 mg/ml of RNAse (Sigma-Aldrich, Saint Quentin-Fallavier, France). PI fluorescence was collected with a 585 ± 44 nm filter.

Reversibility of IPP51 effect

After 24 h of treatment with IPP51, the medium was eliminated and replaced with fresh medium without drug. Incubation was then prolonged for 24 or 48 h, after which, analysis was performed as previously mentioned.

Apoptosis detection

Fluorescein isothiocyanate-coupled annexin V was obtained from BD Bioscience and labeling of cells was performed following the manufacturer's instructions.

Analysis of mitotic fraction

After treatment for 24 h with IPP51, the culture medium and cells were harvested. After fixation with 70% ethanol (30 min at 4°C) and two PBS washes, cell pellet was incubated with an antibody specific for Ser-10 phosphorylated H3 histone (Ozyme, Saint-Quentin-

en-Yvelines, France) diluted at 1/50 in PBS supplemented with 10% of normal goat serum. After 2 h at room temperature, cells were washed twice with PBS and an Alexa 488-goat anti-mouse antibody at 1/4000 was then added and incubated for 1 h at room temperature. After PBS washes, RNase and propidium iodide were added as described in the Cell cycle analysis section.

Data acquisitions were performed with a FACScan (BD Bioscience, Le Pont de Claix, France) equipped with a 488 nm argon laser. Parameters from 2×10^4 cells were acquired using the Cell Quest Pro software (BD Biosciences). Data were analyzed with FCS Express 3 software (De Novo Software, Los Angeles, California, USA).

Quantification of mitotic cells

After incubation for 24 h with 5×10^{-6} mol/l of IPP51, culture medium and cells were washed with PBS. Cells were resuspended in PBS and cytospinned (800 rpm for 3 min) on glass slides. Cytospots were then air-dried for 24 h after which they were stained by Feulgen coloration. For each cytospot, 500 cells were counted and three independent experiments were conducted.

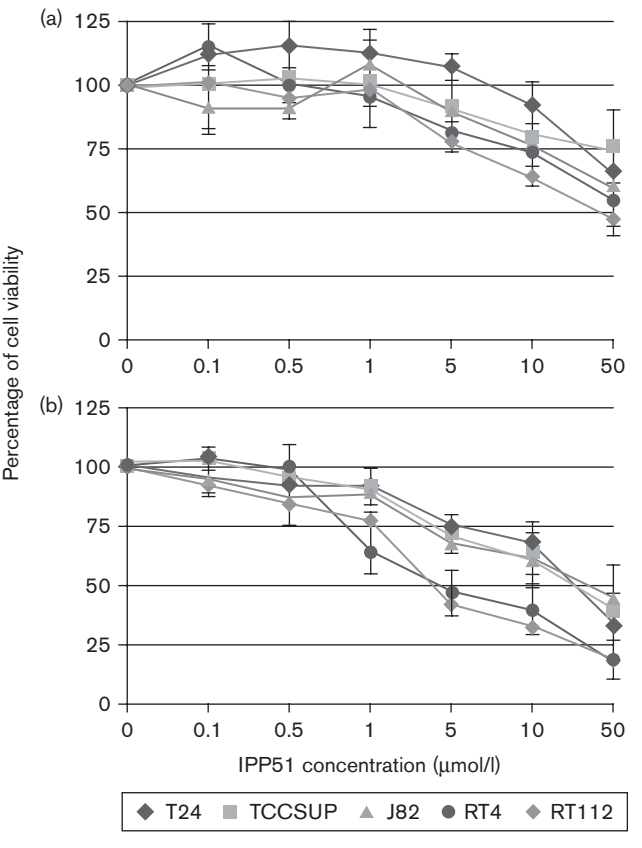
Western blotting

Adherent cells and cells in suspension were harvested, washed twice with PBS, and finally lysed in a PBS buffer containing 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.2 µg/ml leupeptin, 5 µg/ml pepstatin, and 1 mmol/l phenylmethylsulfonyl fluoride. After placing on ice for 45 min, lysates were centrifuged at 20 000*g* for 15 min and soluble proteins were quantitated by the bichinconic acid method (Pierce, Rockford, Illinois, USA). The lysates (75 µg of the total protein) were resolved on 10% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked for 1 h with 5% nonfat dry milk dissolved in TBS solution (10 mmol/l Tris-HCl, pH 7.5, 150 mmol/l NaCl). Membranes were then successively incubated overnight at 4°C with the primary antibodies: cleaved caspase 3 antibody (Cell Signaling Technology, Danvers, Massachusetts, USA) and actin antibody (Sigma-Aldrich) in TBST (TBS with 0.1% Tween 20) with 5% nonfat dry milk. After washing in TBST solution, the secondary antibody (goat anti-rabbit or anti-mouse IgG horseradish peroxidase-conjugated antibodies) diluted in TBST/5% nonfat dry milk was added for 45 min at room temperature. The membranes were rinsed with TBST before being developed by an enhanced chemiluminescence detection system (ECL, GE Healthcare, UK) according to the manufacturer's instructions.

Results

In this study, we used five bladder cancer cell lines, isolated from bladder tumors of different grades and stages, which mimic the bladder tumor progression process. RT4 (pTaG1) and RT112 (pTaG2) derived from transitional cell papilloma were well differentiated and moderately differentiated, respectively. Other cell lines T24 (pT2G3) and J82 (pT3G3) were obtained from invasive transitional cell carcinoma and TCCSUP (pT4G4) was derived from a metastatic form of a transitional cell carcinoma. The viability of the cell lines treated with various concentrations of IPP51 was determined by an MTT assay. Our results indicated that massive cell death occurred after 48 h of treatment (Fig. 3). Therefore, the IC_{50} of IPP51 (which corresponds to the concentration resulting in 50% loss of cell viability relative to untreated cells) was determined after 48 h of incubation (Fig. 3b). This was evaluated at 5×10^{-6} mol/l for cell lines derived from low-grade tumors (RT4 and RT112) and about 5×10^{-5} mol/l for cell lines derived from high-grade tumors (T24, TCCSUP, and J82) suggesting that IPP51 could be used as an inducer of

Fig. 3



Effect of IPP51 on the viability of bladder cancer cell lines. RT4, RT112, T24, TCCSUP, and J82 cell lines were treated with increasing concentrations of IPP51 for 24 h (a) or 48 h (b). Cell density was then determined by an MTT assay. The effect of IPP51 is expressed as percentage of cell viability relative to control without IPP51 in the medium. Each data point is the average duplicates of three independent experiments and standard deviations are shown by error bars.

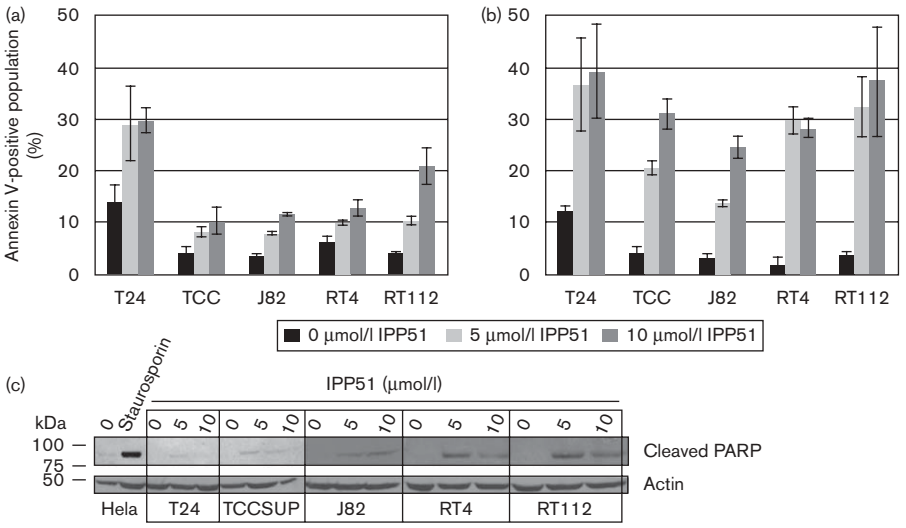
bladder cancer cell lines mortality and this was time and concentration dependent.

Microscopic observation of cells after 48 h of treatment at concentrations higher than 10^{-6} mol/l showed evident cell death (cell shrinkage, nuclear fragmentation). To determine whether cells entered apoptosis after treatment with IPP51, cells were labeled with annexin V to detect translocation of phosphatidylserine from the inner leaflet of the plasma membrane to the outer (cell surface). The results obtained confirmed that after treatment for 48 h with 5×10^{-6} mol/l of IPP51, 14–37% of cells were apoptotic (depending on the cell line studied). These values reached 25–40% at 10^{-5} mol/l concentration (Fig. 4b). No correlation was found between the efficacy and the grade of the tumor, as the most sensitive cell lines were T24 (derived from a high-grade tumor) and RT112 (derived from a low-grade tumor). As it can be expected, the extent of apoptosis was lower at 24 h of treatment (Fig. 4a).

Furthermore, it was important to confirm that the observed cell death was because of apoptosis and not because of necrosis. Indeed, apoptosis, or programmed cell death, is a tightly regulated process under normal conditions. This process is controlled by a hierarchical set of cell death molecules identified originally in *Caenorhabditis elegans* and later in mammalian cells. One prominent event during apoptosis is the selective cleavage of poly(ADP-ribose) polymerase (PARP) by several caspases, especially by caspase-3. After induction of apoptosis, caspase-3 cleaves the 113-kDa PARP at the DEVD site between Asp214 and Gly215, to generate 89-kDa and 24-kDa polypeptides. PARP cleavage is a universal phenomenon observed during programmed cell death induced by a variety of apoptotic stimuli. This prompted us to study the potential PARP cleavage in the extracts of bladder cancer cell lines being treated with different concentrations of IPP51. The cells were incubated with IPP51 for 48 h at concentrations (5×10^{-6} or 10^{-5} mol/l), which induced massive cell death (Fig. 4b). The PARP proteolysis was monitored by western blotting with an antibody specific for the cleaved 89 kDa large fragment of PARP. As shown in Fig. 4c, PARP cleavage is detectable in almost all cell lines, except in T24 cells, which could appear inconsistently with the massive apoptosis observed with annexin V labeling (Fig. 4a). It is worth noting that these techniques did not reveal the same apoptosis stage. The first few steps of apoptosis were detected by the annexin V labeling, whereas later events were revealed by PARP cleavage. The apparent discrepancy in the case of T24 cells could be explained by a different kinetic in the execution of apoptosis in comparison with other cell lines.

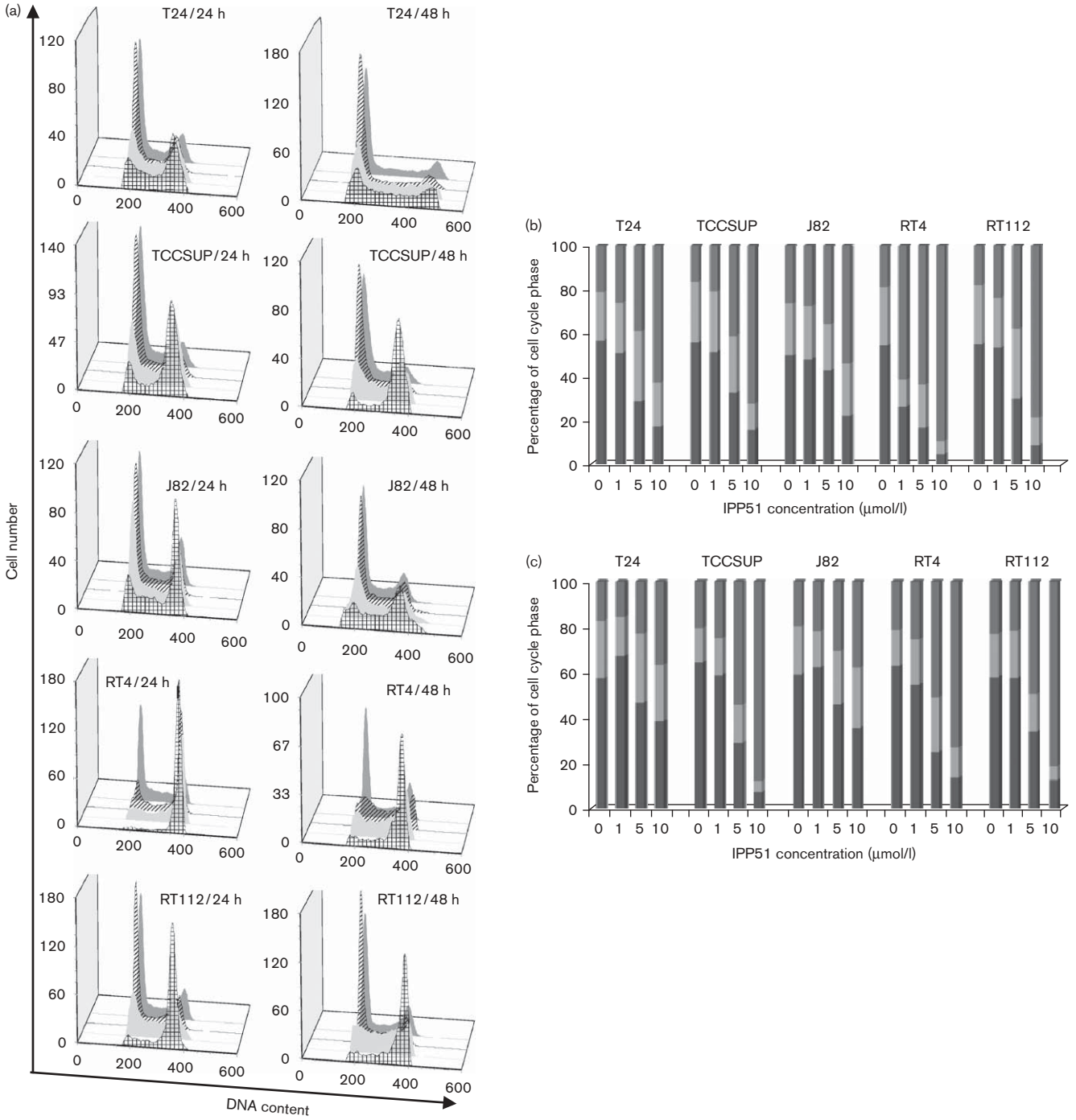
Another interesting feature of cells treated with IPP51 for 24 h is the significant amount of rounded cells, which could correspond to cells under mitosis process. Furthermore, the cell cycle distribution of treated cells was studied by flow cytometry after 24 or 48 h of incubation with different concentrations of IPP51. These analyses

Fig. 4



IPP51-induced apoptosis of bladder cancer cell lines. Cell lines were incubated with various concentrations of IPP51 for 24 h (a) or 48 h (b). Annexin V labeling of cells was then quantified by flow cytometry. Values noted on the graphs correspond to the percentage of annexin V positive cells relative to the total number of cells. Each point is the mean of two independent experiments where 20 000 cells were analyzed and standard deviations are shown by error bars. (c) Cell lysates from untreated (0) or IPP51 treated (5 and 10 μmol/l) bladder cancer cell lines were resolved by 10% SDS-polyacrylamide gel electrophoresis and immunoblotted successively with antibodies directed against cleaved poly(ADP-ribose) polymerase (PARP) and actin, which served as a loading control. Western blot analysis of Hela cells untreated (0) or treated with 1 μmol/l of staurosporin for 6 h was used as positive control from PARP cleavage.

Fig. 5



Arrest of cell cycle progression induced by IPP51. (a) Untreated (dark gray) or treated with varying concentrations (hatched, 1 μmol/l; gray, 5 μmol/l; grid, 10 μmol/l) of IPP51 cancer bladder cell lines were harvested, fixed, and subjected to fluorescence-activated cell sorter analysis after propidium iodide labeling. Presented data are from three independent experiments with similar results. (b) Relative percentages of cells in G1, S, and G2 + M stages after 24 h (b) or 48 h (c) of IPP51 treatment. Data were derived from cytometry analysis in (a).

showed that IPP51 induced an increase in the proportion of cells in G2 + M phase after 24 h of treatment (Fig. 5a). This effect was observed on all cell lines but was most important on RT4 and RT112 cell lines, derived from low-grade tumors and for TCCSUP cells derived from a metastatic carcinoma. This cell cycle accumulation was dependent on IPP51 concentration. For example, treatment with 10^{-5} mol/l for 24 h led to the accumula-

tion of 90, 79, and 72% of RT4, RT112, and TCCSUP cells, respectively (Fig. 5b). This G2 + M accumulation remained statistically significant after 48 h treatment, but to a lesser degree than that observed at 24 h, except for the TCCSUP cell line (Fig. 5c). This could be explained by the massive cell death observed after 48 h of treatment (Fig. 4b), which probably affects the cells arrested in cell cycle first.

Taken together, our experiments showed that the marked effect of IPP51 corresponds to an accumulation of bladder cancer cell lines in G2 + M after 24 h of treatment. Furthermore, high concentrations or a longer period of treatment induced apoptosis. To characterize the effect of IPP51 on the cell cycle arrest, we decided to study the cell behavior after 24 h of treatment at 5×10^{-6} mol/l.

To distinguish between cells arrested in the G2 phase from those in mitosis, we analyzed the expression of a mitosis marker, the phosphorylated histone H3. Indeed, mitotic chromosome condensation is associated with phosphorylation of histone H3 at Ser-10, making it a frequently used marker of mitosis [8]. Treatment with 5×10^{-6} mol/l of IPP51 for 24 h increased significantly the number of cells labeled by the antibody specific for Ser-10 phosphorylated H3 histone relative to control, which is in agreement with a cell cycle arrest at the mitosis phase (Fig. 6a). The proportion of mitotic cells appeared lower than the results shown in Fig. 5b. In fact, in contrast to the cell cycle analysis after PI labeling, which does not distinguish between cells in G2 phase and those in mitosis phases, Ser-10 phosphorylated H3 histone staining discriminated between these two populations.

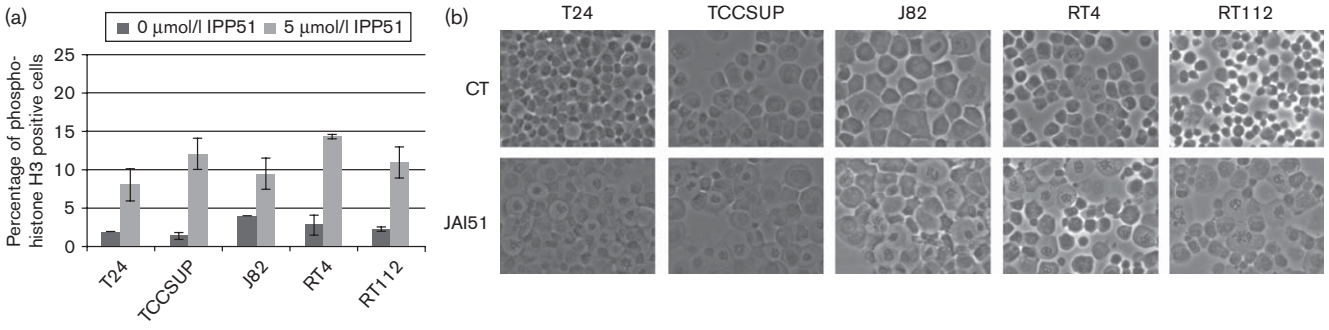
On the basis of the mitotic arrest, we determined the stages where bladder cancer cell lines were accumulated by analyzing their chromosome distribution after staining by the Feulgen coloration (Fig. 6b). As observed by flow cytometry analysis after labeling with antibody directed against Ser-10 of H3 histone, it appears clearly that the title compound induced an increase in mitotic cells. The mitotic index, corresponding to the ratio between the number of mitosis cells and the total number of cells, was enhanced from 2.3 to 6 fold relative to control cells, depending on the cell line studied. Table 1 shows the repartition of mitotic cells in the different phases of mitosis, and led us to conclude that cells accumulate preferentially in the prometaphase stage of mitosis. The later results suggested that IPP51 interferes with cell cycle progression, arresting cell division specifically at the transition prometaphase to metaphase stage.

Finally, we sought to determine whether IPP51 was a cytostatic or a cytotoxic molecule by evaluating its reversible effect. Indeed, cytostatic drugs inhibit cellular growth and multiplication. Generally, their use in chemotherapy involves a combination with another drug and a long-term treatment. In contrast, cytotoxic molecules induced apoptosis and could be used alone. The different bladder cancer cell lines were incubated with IPP51 at 5×10^{-6} or 10^{-5} mol/l for 24 h, in the same conditions

Table 1 Relative percentage of mitotic cells in the different stages of mitosis

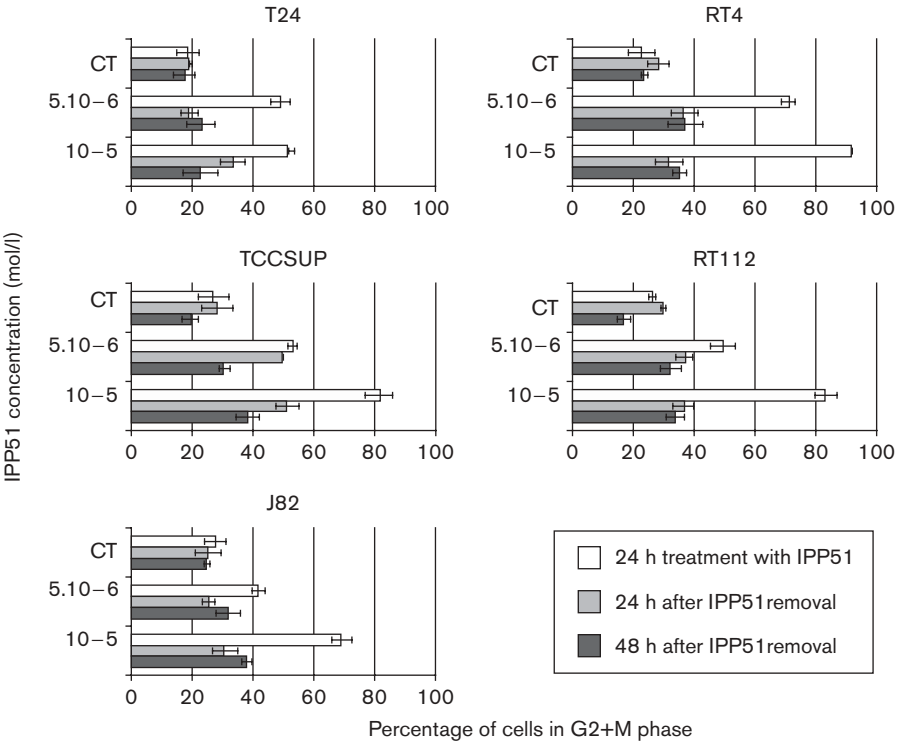
		Prophase (%)	Prometaphase (%)	Metaphase (%)	Anaphase (%)
T24	CT	29.1	9.1	58.2	3.6
	JAI51	24.3	62.1	13.1	0.5
TCC-SUP	CT	19.0	31.0	50.0	0.0
	JAI51	9.4	74.5	15.4	0.7
J82	CT	26.25	47.50	23.75	2.50
	JAI51	6.56	55.74	37.16	0.55
RT4	CT	27.66	23.40	42.55	6.38
	JAI51	15.56	77.78	6.67	0.00
RT112	CT	46.27	34.33	17.91	1.49
	JAI51	16.95	69.49	13.56	0.00

Fig. 6



Quantification of mitotic cells in IPP51-treated bladder cancer cell lines. (a) After incubation with 5×10^{-6} mol/l of IPP51 for 24 h, cells were harvested and their double labeling with an antibody specific for Ser-10 phosphorylated H3 histone and propidium iodide was analyzed by flow cytometry. The data are means ± SD of three independent experiments. (b) Observation of chromosome structures after incubation for 24 h with 5×10^{-6} mol/l of IPP51 for 24 h and cell coloration by Feulgen technique. Photographs are from three independent analyses with similar results.

Fig. 7



Reversibility of the effect of IPP51. Cell lines were untreated (CT) or incubated for 24 h with 5 or 10 μmol/l of IPP51. After 24 h, the drug was removed and replaced by fresh medium without IPP51. Cell cycle was analyzed 24 or 48 h later by cytometry after labeling of cells with propidium iodide. Only the percentage of cells in G2 + M phase relative to the total number of cells is presented on these histograms. The data are the mean of three independent experiments where 10 000 cells were analyzed at each time. Standard deviations are shown by error bars.

where the G2 + M phase is arrested without massive cell death. Then, the drug was removed, fresh medium was added, and the cycle of treated cells was studied 24 and 48 h after withdrawal of the compound. The results showed that the proportion of cells in G2 + M phase after 48 h in medium without IPP51 was in the same order as control cells, except for the TCCSUP cell line, where proportion of cells in G2 + M stage remained high (Fig. 7). Cells resumed normal proliferation comparable with untreated cells (data not shown), concluding that IPP51 displayed a cytostatic behavior on most bladder cancer cell lines when used for periods that did not exceed 24 h.

Discussion

In the past few years, therapeutic strategies for killing cancer cells by induction of apoptosis have been investigated extensively. In general, the initiation of cellular apoptosis progressed through two distinct pathways: an extrinsic pathway involving the death of receptor signaling, and an intrinsic pathway involving the mitochondrial cascade. Activation of either of the pathways by cleavage of the procaspase-8 or procaspase-9 will in turn result in downstream activation of caspase-3, caspase-activated DNase, and finally lead to DNA fragmentation. Activation of caspase-3 is often considered as the point-of-no-return in the apoptotic signaling cascade.

The 1-(2,4-dimethoxyphenyl)-3-(1-methylindolyl) propenone described in this study was initially identified after screening a library of 3-indolyl-1-phenylpropenones for their capacity to inhibit the proliferation of bladder cancer cell lines. The hit compound, IPP51, was able to decrease the percentage of viable bladder cancer cell lines in a dose-dependent and time-dependent manner when used at micromolar concentrations. Indeed, the induction of apoptosis by IPP51 was confirmed by labeling the cells with annexin V and observing the cleavage of PARP, which are hallmarks of apoptosis. Preliminary experiments to explore the apoptotic pathway induced by IPP51 indicated caspase-3 activation but this needs to be further confirmed, particularly for T24 and RT112 cell lines (data not shown).

The other interesting finding of this study is that the first effect of IPP51 was to arrest cells in G2 and early mitosis phase (prometaphase stage); it was not lethal if the compound was removed after 24 h of incubation. Taken together, the later results indicate that the effect of IPP51 was reversible, which is of interest for using it in

chemotherapy. It should be highlighted that it is not true for the TCCSUP cell line, which remained accumulated in the G2 + M phase after 24 h of treatment even if IPP51 was removed. TCCSUP cells were derived from a high-grade transitional cell bladder carcinoma from a patient with metastases to the bone.

Previous studies have shown that these cells respond differently than other bladder cancer cell lines. For example, they display a higher resistance to apoptosis induced by drugs such as adriamycin or cisplatin [9,10]. TCCSUP cells are p53 +/+ and it was postulated that the nature of p53 mutations might affect the cellular response to anticancer drugs. Therefore, it will be of interest to study the potential relationship between the p53 status of the different bladder cancer cell lines used and their sensitivity to IPP51.

Numerous drugs induce mitotic blockade by interfering with the normal organization of the microtubule cytoskeleton. The close structure of the title compound to those of chalcones (Fig. 1) allows us to assume that it can either hamper the polymerization or disassembly of microtubules [11]. It was shown that chalcones have the capacity to inhibit tubulin polymerization [12–14]. Our molecule was tested in a tubulin polymerization assay, but we observed no notable effect at the concentrations tested. However, it is necessary to observe the effect of the molecule on microtubule organization directly into cells. Immunofluorescence studies with antibodies directed against mitotic apparatus proteins are in progress to investigate the occurrence of abnormalities in spindle organization. It should be noted that spindle damage cells frequently undergo apoptosis [15], which could explain the obtained results when cells were treated for 48 h. In particular, cell death triggered in response to spindle damage is characterized by the activation of caspases and/or mitochondrial membrane permeabilization with the release of cell death effectors such as apoptosis-inducing factor and the cytochromec [16].

Eukaryotic cell cycle progression involves sequential activation of Cdks, whose activation is dependent upon their association with cyclins. Cell cycle progression is also regulated by the relative balance between the cellular concentrations of cyclin-dependent kinase inhibitors and inhibitor of cyclin-dependent kinases family. The level of these different proteins should also be studied in cells treated with IPP51 to understand the mechanisms implicated in cell cycle blockade induced by our compound.

In conclusion, we demonstrated the ability of IPP51, which belongs to a new class of compounds, to inhibit bladder cancer cell proliferation through cell cycle arrest at G2 and M phases. This accumulation was followed by the induction of apoptosis if cells were incubated with the compound for 48 h. Delineation of the precise biological effects of IPP51 on mitotic arrest and its chemotherapeutic potential for human bladder cancer treatment need further investigation.

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References

1 Patard JJ, Rodriguez A, Leray E, Rioux-Leclercq N, Guille F, Lobel B. Intravesical Bacillus Calmette-Guerin treatment improves patient survival in T1G3 bladder tumours. *Eur Urol* 2002; **41**:635–641.
2 Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Palou J, Vicente-Rodriguez J. Multivariate analysis of the prognostic factors of primary superficial bladder cancer. *J Urol* 2000; **163**:73–78.
3 Ghoneim MA, Abol-Enein H. Management of muscle-invasive bladder cancer: an update. *Nat Clin Pract Urol* 2008; **5**:501–508.
4 Laffaioli RV, Milano A, Caponigro F. Therapy of metastatic bladder carcinoma. *Ann Oncol* 2007; **18**:153–156.
5 Gallagher DJ, Milowsky MI, Bajorin DF. Advanced bladder cancer: status of first-line chemotherapy and the search for active agents in the second-line setting. *Cancer* 2008; **113**:1284–1293.
6 Gerby B, Boumendjel A, Blanc M, Bringuier PP, Champelovier P, Fortuné A, *et al.* 2-Arylidenedihydroindole-3-ones: design, synthesis and biological activity on bladder carcinoma cell lines. *Bioorg Med Chem Lett* 2007; **17**:208–213.
7 Boumendjel A, Boccard J, Carrupt PA, Nicolle E, Blanc M, Geze A, *et al.* Antimitotic and antiproliferative activities of chalcones: forward structure-activity relationship. *J Med Chem* 2008; **51**:2307–2310.
8 Hendzel MJ, Wei Y, Mancini MA, Van Hooser A, Ranalli T, Brinkley BR, *et al.* Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma* 1997; **106**:348–360.
9 Chang FL, Lai MD. The relationship between p53 status and anticancer drugs-induced apoptosis in nine human bladder cancer cell lines. *Anticancer Res* 2000; **20**:351–355.
10 Chang FL, Lai MD. Various forms of mutant p53 confer sensitivity to cisplatin and doxorubicin in bladder cancer cells. *J Urol* 2001; **166**:304–310.
11 Chen JG, Horwitz SB. Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs. *Cancer Res* 2002; **62**:1935–1938.
12 Lawrence NJ, McGown AT. The chemistry and biology of antimitotic chalcones andrelated enone systems. *Curr Pharm Des* 2005; **11**:1679–1693.
13 Kim do Y, Kim KH, Kim ND, Lee KY, Han CK, Yoon JH, *et al.* Design and biological evaluation of novel tubulin inhibitors as antimitotic agents using a pharmacophore binding model with tubulin. *J Med Chem* 2006; **49**:5664–5670.
14 Romagnoli R, Baraldi PG, Carrion MD, Cara CL, Cruz-Lopez O, Preti D, *et al.* Design, synthesis, and biological evaluation of thiophene analogues of chalcones. *Bioorg Med Chem* 2008; **16**:5367–5376.
15 Mollinedo F, Gajate C. Microtubules, microtubule-interfering agents and apoptosis. *Apoptosis* 2003; **8**:413–450.
16 Castedo M, Perfettini JL, Roumier T, Andreau K, Medema R, Kroemer G. Cell death bymitotic catastrophe: a molecular definition. *Oncogene* 2004; **23**:2825–2837.