Mol Cell Toxicol (2016) 12:237-242
DOI 10.1007/s13273-016-0028-8

ORIGINAL PAPER

Inhibition of cancer cell growth and migration by dihydroxynaphthyl aryl ketones

Julio Benites[1,2], Jaime A. Valderrama[1,2], David Ríos[1,3], Rosalba Lagos[5], Octavio Monasterio[5] & Pedro Buc Calderon[1,3,4]

Received: 15 January 2016 / Accepted: 26 April 2016

Abstract Dihydroxynaphthyl aryl ketones **1-5** exhibit activity as tubulin polymerization inhibitors by targeting the colchicine binding site of microtubules making them potential anticancer drugs. Therefore, analogues **1-5** have been evaluated for their cytotoxic activity against the cancer cell lines DU-145 (prostate), T24 (bladder) and MCF-7 (breast). Notable differences in biological activity were observed for compounds **1-5**, most likely related to the nature of the aryl substituent bonded to the carbonyl group. Among the tested compounds, only compound **5** showed selectivity for cancer cells over healthy, non-transformed cells. T24 cancer cells treated with compound **5** presented a concentration-dependent decrease in cell proliferation and a loss of migration ability. The cytotoxicity of compounds **1-5** on the selected cell-based assays is discussed in terms of it lipophilicity and polarizability parameters.

Keywords Cytotoxicity, Dihydroxynaphthyl aryl ketones, Cell migration, Cell proliferation, Lipophilicity

[1]Facultad de Ciencias de la Salud, Universidad Arturo Prat, Iquique, Chile
[2]Instituto de Ciencias Exactas y Naturales (ICEN), Universidad Arturo Prat, Iquique, Chile
[3]Instituto de EtnoFarmacologia (IDE), Universidad Arturo Prat, Iquique, Chile
[4]Toxicology and Cancer Biology Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium
[5]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
Correspondence and requests for materials should be addressed to P. B. Calderon (✉pedro.buccalderon@uclouvain.be)

The critical role play by microtubules in cell division makes them a desirable target for the development of chemotherapeutic agents directed against rapidly dividing cancer cells[1]. Numerous drugs that are currently used in clinical as antimitotic agents, including vincristine, vinblastine[2] and epothilones[3,4], exert their action by interrupting the dynamic instability of microtubules[5-7]. Nevertheless, due to the appearance of severe side effects[8] their applicability is limited for the treatment of cancer.

We have recently reported a flexible and eco-friendly procedure to synthesize diaryl ketones by means of solar photo-Friedel-Crafts acylation of 1, 4-naphthoquinone with aldehydes[9]. This procedure was successfully extended to the synthesis of a range of antiproliferative dihydroxynaphthyl aryl ketones[10]. Taking advantage of the simple access to ketones **1-5** and their structural analogy to the tubulin polymerization inhibitors nocodazole[11,12] and naphthylphenstatins[13,14], compounds **1-5** (Figure 1) were tested as potential tubulin polymerization inhibitors leading to disruption of microtubule assembly *in vitro*. The study revealed that compounds **1-4** exhibited higher affinity than **5** for tubulin. This fact was attributed to the polar heterocyclic fragments of the former compounds, which favoured molecular interactions with the protein[15].

Based on these precedents and due to the importance of microtubules in cancer cell growth, the aim of this work was to evaluate compounds **1-5** as cytotoxic agents against cancer cell proliferation and migration. To this end, the biological activity was assessed using the reduction of MTT to formazan blue in a panel of three cancer cell lines, namely T24 (a human bladder carcinoma cell line), MCF-7 (a human breast adenocarcinoma cell line) and DU-145 (a human prostate carcinoma cell line). The activity was expressed as

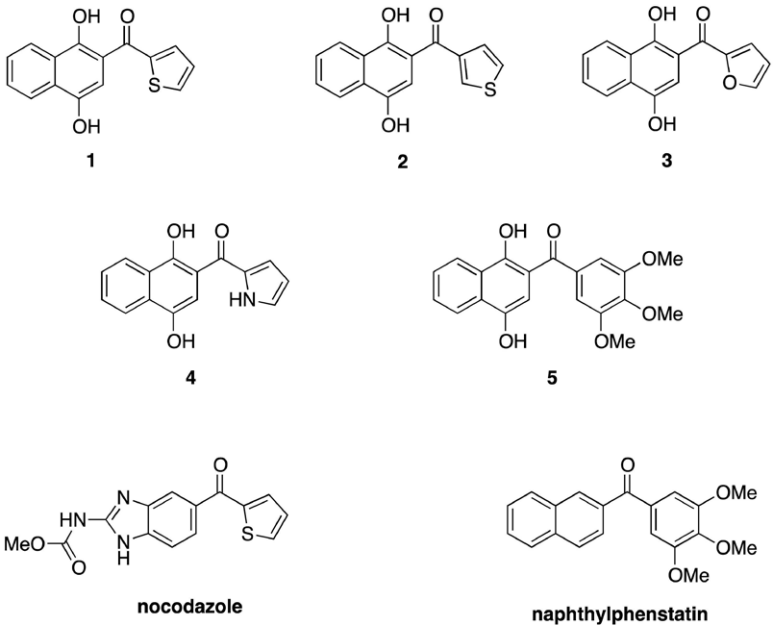

Figure 1. Structures of compounds **1-5**, nocodazole and naphthylphenstatin. Structural analogy between nocodazole and quinones **1** to **4** showing the substituent group of 5-atoms. Naphtylphenstatins is close related to quinone **5**.

Table 1. IC_{50}, ClogP and CMR values for compounds **1-5**.

	$IC_{50} \pm SEM$ (μM)[a]						
N°	T24[b]	DU-145[c]	MCF-7[d]	NIH 3T3[e]	SI[f]	ClogP[g]	CMR[g]
1	32.29 ± 3.1	32.75 ± 2.9	37.87 ± 4.2	33.36 ± 3.6	0.98	3.89	7.50
2	35.00 ± 4.1	33.42 ± 4.3	38.08 ± 3.2	36.93 ± 4.6	1.05	3.89	7.50
3	33.67 ± 2.5	32.14 ± 3.4	31.23 ± 3.5	27.23 ± 2.4	0.84	3.36	6.90
4	20.37 ± 1.2	30.38 ± 2.9	29.04 ± 2.3	22.58 ± 1.6	0.88	2.83	7.12
5	17.49 ± 1.3	8.67 ± 1.1	17.70 ± 0.9	28.28 ± 1.8	2.16	3.36	9.54

[a]*In vitro* inhibitory effect of compounds **1-5** expressed as IC_{50} values of the human-derived tumour cell lines, [b]T24 (bladder), [c]DU-145 (prostate) and [d]MCF-7 (breast), and [e]non-tumour fibroblasts (NIH 3T3). Data represent mean average values ± SEM for three separate experiments. [f]Selective Index = IC_{50} values for non-tumour cells/IC_{50} values for tumour cells. [g]ClogP = calculated lipophilicity and CMR = calculated molar refractivity index were determined by using the Chemdraw Ultra software.

IC_{50} values and was compared then to that obtained against Balb/3T3 cells (normal fibroblast). In addition, a clonogenic assay and a cell migration test were also performed to confirm the *in vitro* anti-tumour activity of the dihydroxynaphthyl aryl ketones **1-5**.

Cytotoxic effects of compounds 1-5 on cancer and non-cancer cells

Table 1 shows the effects of compounds **1-5** on cell survival using a panel of 3 different cancer cells types (T24, DU-145 and MCF-7) and non-transformed Balb/3T3 cells. Cytotoxicity is expressed as IC_{50} values at concentrations ranging from 0.001 to 100 μM and these values were calculated by measuring the reduction of MTT to blue formazan. In addition, by calculating the ratio between the IC_{50} of normal fibroblasts and the IC_{50} of tumour cells, a specific antitumor effect, termed selectivity index was determined.

Compound **5** showed more potent cytotoxicity than compounds **1-4**, with a selectivity index (IC_{50} non-tumour cells/IC_{50} tumour cells) of 2.16. Indeed, compound **5** was cytotoxic toward the three cancer cell lines, but it was less active in the non-transformed cells. No particular difference was observed for isomers **1** and **2**, indicating that additional pathways other than tubulin polymerization are involved in the loss of cellular viability. Compound **4**, the best tubulin polymerization inhibitor[15], retained its activity against the 3 cancer cell lines but also against normal fibroblasts

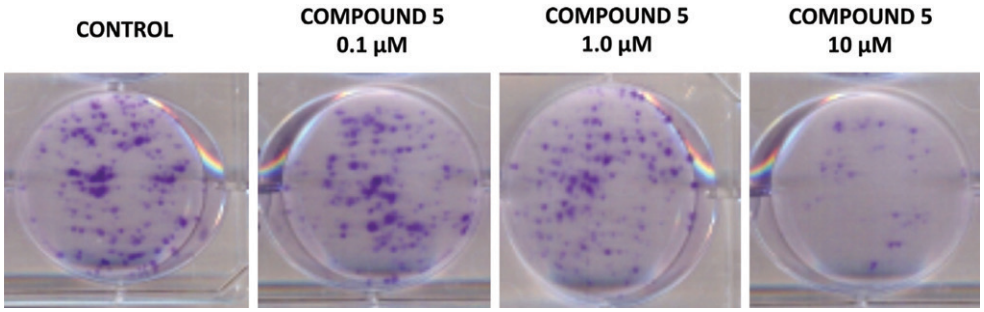

Figure 2. Effect of compound **5** on cell proliferation. T24 cells were incubated for 24 h with different concentrations of compound **5**, as indicated in the figure. After 10 days, the colonies were stained and counted. The figures are representative of three independent experiments.

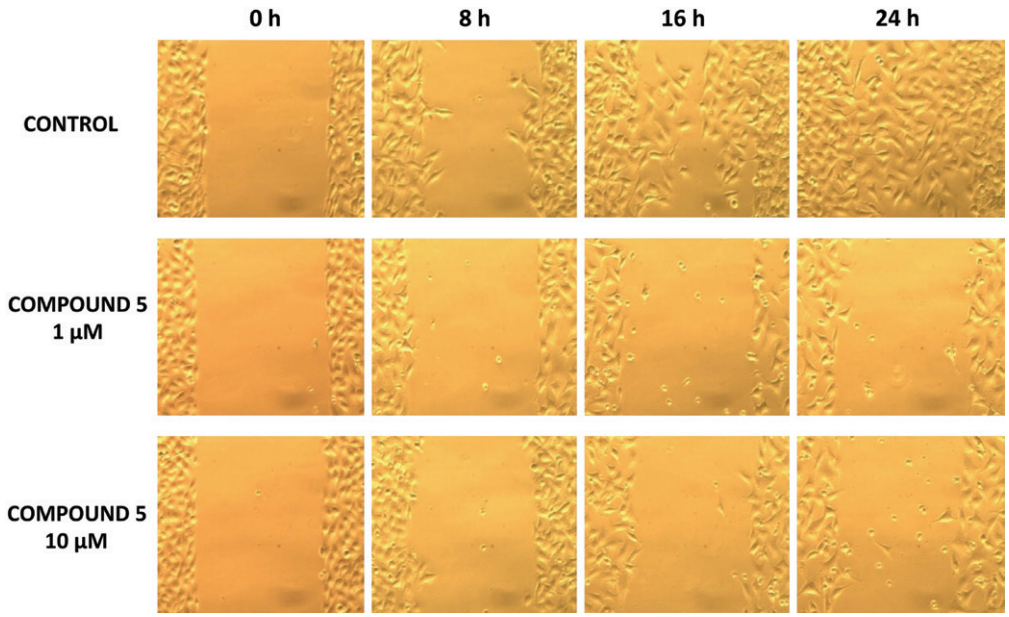

Figure 3. Effect of compound **5** on cell migration. The motility of the T24 cells was assessed by incubating the cells for different times in a culture insert plate in the absence (control) or in the presence of 1 and 10 µM compound **5**, as described in Materials and Methods.

(Balb/3T3 cells), indicating a lack of selectivity. In addition, none of the compounds **1-5** was able to induce caspase-3 activation (data not shown) ruling out an apoptosis-like cell death.

Effect of compound 5 on cancer cell proliferation and cell migration

In T24 cells treated with compound **5** (from 0.1 to 10 µM), a concentration-dependent decrease in cell proliferation was observed (Figure 2). At the highest concentration used (10 µM), which is below its IC_{50} value, compound **5** impaired the capacity of T24 cells to proliferate, and it induced clonogenic death. Notably, increased cell proliferation is a key characteristic of malignancy.

Figure 3 shows that migration of the control cells (untreated T24 cells) was detectable after 12 hours of incubation, and from this point forward, migration was consistent, resulting in layer closure within 24 hours. T24 cells notably lost their motility when treated with compound **5** at either 1 or 10 µM.

Compound **5** induced a concentration-dependent inhibition of proliferation in T24 cells, resulting in clonogenic death (i.e., a strong loss of proliferation). Moreover, in T24 cells treated with compound **5**, cell motility was markedly reduced compared to that observed in the untreated control cells.

Discussion

We have recently shown that compounds **1-5** are competitive inhibitors of colchicine binding to tubulin[15], therefore the question was raised about their potential inhibitory activity on eukaryotic cell growth. To this end, experiments of cell proliferation using normal and cancer cell lines were performed. In addition, the effect of compound **5** was also examined with regard to cancer cell motility. Indeed, the inhibition of T24 cancer cell migration by compound **5** (Figure 3) is important because the transit of cancer cells towards the tissue surrounding the tumour and the vasculature are the first steps in the spread of metastatic cancers[16]. The IC_{50} values (Table 1) for the inhibition of cell proliferation show that compound **5** is the most active member of the group.

Molecular descriptors such as atomic masses, van der Waal volume, electronegativity, dipole moments and polarizabilities are associated with pharmacokinetic profiles, which affect the activities of compounds. These chemical properties together with lipophilicity and steric bulk are important factors facilitating cell entry, a crucial step that allows compounds to reach the intracellular target sites and to interact with them. The lipophilicity of a compound, logP[17], is often correlated with activity as well as molar refractivity (MR) that serve as convenient estimate of molecular polarizability and steric bulk[17-21]. Comparison of the ClogP and CMR parameters of compounds **1-5** (Table 1) indicated that such compounds do not exhibit significant differences on their lipophilicities. Nevertheless, it can be observed that among the ketones **1-5**, compound **5** have the greatest CMR value. Hence, it may be assumed that the polarizability or steric bulk properties in this class of ketones could be associated with the cytotoxic activity.

In conclusion, the results reported here indicate that compounds **1-5** have good activities in biochemical assays and good translation to cellular assessment of cytotoxicity and motility. The limitations of the ketones **1-5** include their selectivity and likely metabolic liabilities in the hydroquinone moiety. Indeed, hydroquinones are generally less reactive than semiquinone radicals and are conjugated with glucuronic acid or sulphate, and eliminated from the cell[22]. However, a potential metabolic effect can be discarded because the hydroquinone residue, which is common in compounds **1-5**, is stabilized in part by intramolecular hydrogen bond through the *ortho* carbonyl group and also because compound **5** shows a higher cytotoxic effect compared to their analogues **1-4**. Accordingly, prior to conclude about a specific mechanism of action and to move on with sophisticated *in vivo* experiments, more active molecules are needed. Studies are now in progress taken compound **5** as a template to develop a new generation of more potent ketone compounds.

Materials & Methods

Dihydroxynaphthyl aryl ketones **1-5**: Compounds **1-4** were prepared from 1,4-naphthoquinone and the corresponding aldehydes: thiophene-2-carbaldehyde; thiophene-3-carbaldehyde; furan-2-carbaldehyde and 1*H*-pyrrole-2-carbaldehyde[9]. The structure of compounds **1-4** were confirmed by comparing the ^{1}H-, ^{13}C-NMR spectral data to the data reported in the literature[9]. Compound **5** was prepared from 1,4-naphthoquinone and 3,4,5-trimethoxybenzaldehyde, and the spectral data were in agreement with those reported in the literature[10,23].

Chemicals and cells culture conditions

MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). All other chemicals were ACS reagent grade.

Human cancer cell lines (T24, DU-145, MCF-7) were cultured in high-glucose Dulbecco's modified Eagle medium (Gibco, Grand Island, NY, USA) supplemented with 10% foetal calf serum, penicillin (100 U/mL), and streptomycin (100 µg/mL). Balb/3T3 cells (normal mouse fibroblasts) were cultured in the same medium, except that the foetal calf serum was replaced by 10% new-born calf serum. All cultures were kept at 37°C in 95% air/5% CO_2 at 100% humidity. Phosphate-buffered saline (PBS) was purchased from Gibco. Cells were incubated at the indicated times at 37°C with or without dihydroxynaphthyl aryl ketones **1-5** at various concentrations.

Cell viability assay

The cytotoxicity of compounds **1-5** was evaluated for their *in vitro* antiproliferative activity against a panel of four cell lines, including non-tumour Balb/3T3 fibroblasts and three human-derived tumour cell lines, namely DU-145 (prostate), T24 (bladder) and MCF-7 (breast), using conventional microculture MTT tetrazolium reduction assays[24]. Briefly, 10^4 cells/well were plated in 96-well plates, and after confluence, they were exposed to different dihydroxynaphthyl aryl ketones at concentrations ranging from 0.001 to 100 µM for 48 h. The cells were then washed twice with PBS and incubated for 2 h with MTT (0.5 mg/mL). DMSO was added to dissolve the formazan crystals (100 µL/

well), and the resulting absorption of the coloured solutions was read at 550 nm. The IC_{50} values were calculated using the GraphPad Prism software (San Diego, CA, USA).

Clonogenic survival assay

The potential to induce clonogenic death was evaluated according to Franken *et al*.[25] using the T24 cell line. These cells were selected due to their facility to enumerate when they form colonies. Indeed, the other cell lines proliferate by forming cellular aggregates making difficult the colony counting. Using 6-well plates, trypsinized cells (500) were seeded at a single cell density and were allowed to adhere overnight. Cells were treated with vehicle alone as a negative control or with compound **5** (0-10 μM). After 24 h, a double wash with warm PBS was performed, and fresh medium was added. Colony formation was allowed to proceed for 10 days. Finally, the colonies were fixed and stained with crystal violet to measure the clonogenic survival.

Cellular migration assay

Cell culture plates with inserts (Culture insert - Ibidi^{TM}) were used to evaluate cell migration, according to the manufacturer's protocol. In each plate-set, the inserts formed two small chambers separated by a septum. Each chamber was filled with 70 μL of a T24 cell suspension at 7×10^5 cells/mL. We preferred the use of these cells because their rapidity to migrate. Cells were allowed to set overnight. At time zero, the inserts were removed using sterile tweezers, resulting in two confluent cell populations separated by a 500-μm gap in each plate. Then, fresh serum-free medium containing vehicle alone as a negative control or compound **5** (0-10 μM) was added. Thereafter, images were captured at regular intervals under a microscope coupled to a camera.

Acknowledgements Supported by Grants n° 1100376 (JVG) and n° 1130711 (OM) from the Fondo Nacional de Ciencia y Tecnología, Chile; and FSR-2013-UCL, Belgium (PBC).

Conflict of Interest The authors declare no conflict of interest.

Authors contribution Julio Benites and Jaime Valderrama contributed to this work by performing the chemical synthesis of molecules, the discussion of results specially those about molecular descriptors; David Rios actively participated in the discussion of the whole results; Rosalba Lagos and Octavio Monasterio contributed by discussing the design of biological assays and during the discussion of both chemical and biological results; and Pedro Buc Calderon performed the biological assays and he was actively involved in the discussion of results.

References

1. Zhou, J. & Giannakakou, P. Targeting microtubules for cancer chemotherapy. *Curr Med Chem Anti-Cancer Agents* **5**:65-71 (2005).
2. Lobert, S., Vulevic, B. & Correia, J. J. Interaction of vinca alkaloids with tubulin: a comparison of vinblastine, vincristine, and vinorelbine. *Biochemistry* **35**: 6806-6814 (1996).
3. Bollag, D. M. *et al*. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res* **55**:2325-2333 (1995).
4. Altmann, K. H., Wartmann, M. & O'Reilly, T. Epothilones and related structures - a new class of microtubule inhibitors with potent in vivo antitumor activity. *Biochim Biophys Acta* **1470**:79-91 (2000).
5. Kamath, K. & Jordan, M. A. Suppression of microtubule dynamics by epothilone B is associated with mitotic arrest. *Cancer Res* **63**:6026-6031 (2003).
6. Nettles, J. H. *et al*. The binding mode of epothilone A on α,β-tubulin by electron crystallography. *Science* **305**:866-869 (2004).
7. Hastie, S. B. Interactions of colchicine with tubulin. *Pharmacol Ther* **51**:377-401 (1991).
8. Lange, U. Current aspects of colchicine therapy: classical indications and new therapeutic uses. In Columbus FH, editors. Arthritis research: treatment and management, Nova Sci. Publishers Inc; Chap III. 2005.
9. Benites, J., Ríos, D., Díaz, P. & Valderrama, J. A. The solar-chemical Photo-Friedel-Crafts heteroacylation of 1,4-quinones. *Tetrahedron Lett* **52**:609-611 (2011).
10. Arenas, P. *et al*. Eco-friendly synthesis and antiproliferative evaluation of oxygen substituted diaryl ketones. *Molecules* **18**:9818-9832 (2013).
11. Hoebeke, J., Van Nijen, G. & DeBrabander, M. Interactions of nocodazole (R 17934), a new anti-tumoral drug, with rat brain tubulin. *Biochem Biophys Res Commun* **69**:319-324 (1976).
12. DeBrabander, M. J. *et al*. The effects of methyl [5-(2-thienylcarbonyl)-lH-benzimidazol-2-ylI carbamate (R 17,943: NSC 238159), a new synthetic antitumoral drug interfering with microtubules, on mammalian cells cultured in vitro. *Cancer Res* **36**:905-916 (1976).
13. Álvarez, C. *et al*. Synthesis and biological activity of naphthalene analogues of phenstatins: Naphthylphenstatins. *Bioorg Med Chem Lett* **17**:3417-3420 (2007).
14. Álvarez, C. *et al*. Naphthylphenstatins as tubulin ligands: Synthesis and biological evaluation. *Bioorg Med Chem* **16**:8999-9008 (2008).
15. Gutierrez, E. *et al*. Binding of dihydroxynaphthyl aryl ketones to tubulin colchicine site inhibits microtubule assembly. *Biochem Biophys Res Commun* **466**:418-425

(2015).
16. Sahai, E. Mechanisms of cancer invasion. *Curr Opin Genet Dev* **15**:87-96 (2005).
17. Leo, A. J. Calculating log Poct from structures. *Chem Rev* **93**:1281-1308 (1993).
18. Hansch, C. *et al*. On the role of Polarizability in Chemical-Biological Interactions. *J Chem Inf Comput Sci* **43**: 120-125 (2003).
19. Hansch, C. & Kurup, A. QSAR of Chemical Polarizability and Nerve Toxicity. *J Chem Inf Comput Sci* **43**: 1647-1651 (2003).
20. Verma, R. P., Kurup, A. & Hansch, C. On the role of polarizability in QSAR. *Bioorg Med Chem* **13**:237-255 (2005).
21. Verma, R. P. & Hansch, C. A comparison between two polarizability parameters in chemical-biological interactions. *Bioorg Med Chem* **13**:2355-2372 (2005).
22. Powis, G. Metabolism and reactions of quinoid anticancer agents. *Pharmac Ther* **35**:57-162 (1987).
23. Iribarra, J. *et al*. Synthesis and antitumor evaluation of 6-arylsubstituted benzo[*j*]phenanthridine- and benzo[*g*] pyrimido[4,5-c] isoquinolinequinones. *Molecules* **17**: 11616-11629 (2012).
24. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**:55-63 (1983).
25. Franken, N. A. P., Rodermond, H. M., Stap, J., Haveman, J. & Van Bree, C. Clonogenic assay of cells *in vitro*. *Nat Protoc* **1**:2315-2319 (2006).