DOI: 10.4155/fmc-2016-0212 [Query-Q1: Karishma Bhan(CE) to All(AU)] It is our house style to italicize genes to differentiate them from proteins. Can you please check and confirm that any genes in your article are italicized correctly?

Morales, Ramírez, Morata-Tarifa et al. [Query-Q2: Karishma Bhan(CE) to All(AU)] It is our house style to leave all proteins, genes and growth factors in their abbreviated form only. Please could you ensure none of these have been defined (including the figures and tables of articles)?



Short Communication

# Antitumoral activity of 1,2-diaminocyclohexane derivatives in breast, colon and skin human cancer cells

[Query-Q3: Karishma Bhan(CE) to All(AU)] Please check all author names, in particular your co-author's names, and affiliation details are presented correctly (including in the running header at the top of the page). We will not change these once published in most cases.

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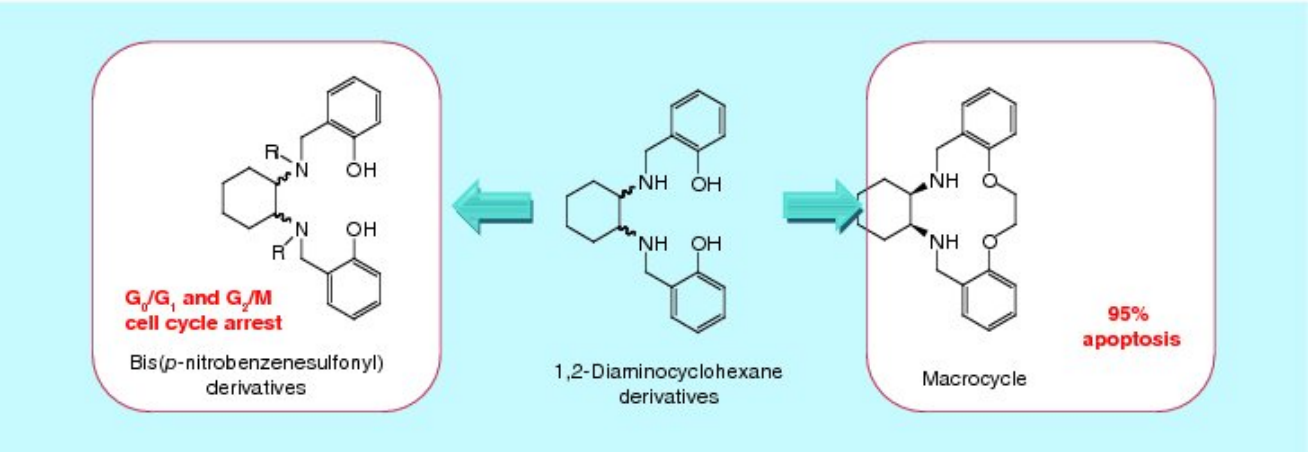
## Abstract

Aim: Cancer is among the leading causes of death worldwide. Medical interest has focused on macrocyclic polyamines because of their properties as antitumor agents. Results/Methodology: We have designed and synthesized a series of 1,2-diaminocyclohexane derivatives with notable in vitro antiproliferative activities against the MCF-7, HCT-116 and A375 cancer cell lines. Cell cycle and apoptosis analyses were also carried out. Our results show that all the compounds are potent cytotoxic agents, especially against the A375 cell line. Conclusion: The selective activity of the macrocyclic derivative against A375, via apoptosis, supposes a great advantage for future therapeutic use. This exemplifies the potential of 1,2-diaminocyclohexane derivatives to qualify as lead structures for future anticancer drug development due to their easy syntheses and noteworthy bioactivity.

## Graphical abstract




Keywords:
1, 2-diaminocyclohexanes • antitumor • apoptosis • benzenesulfonamides • breast cancer • caspases • colon cancer • macrocycles • melanoma

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Cancer is among the leading causes of death worldwide, being responsible for more deaths than all coronary heart disease or stroke, accounting for 8.2 million deaths in 2012. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next two decades. Thus, cancer continues to be a major health problem in developing, as well as undeveloped countries [1,2].

The MCF-7, HCT-116 and A375 cell lines are representative for breast, colon and melanoma tumors, respectively. Breast cancer is the main cancer in women both in the developed and the developing countries [3], with an estimated 1.7 million new cases and 521,900 deaths in 2012, representing 25% of all cancer diagnoses and 15% of all cancer deaths among females. MCF-7 is a luminal epithelial-like cell line that expresses low levels of the enzyme phosphodiesterase type 5 and its phenotype is conserved in their derived tumors [4,5]. Colorectal cancer is a leading cause of cancer death in developed countries, being the third most commonly diagnosed cancer in men and the second in women, with an estimated 1.4 million cases and 693,900 deaths in 2012 [6]. Colon cancer is associated with a high-frequency variation of microsatellite repeats as the HCT-116 cell line shows [7]. A cancer-specific hypermethylation event for the small nucleolar RNAs SNORD123, U70C and ACA59B has recently been found in this cell line [8]. The incidence of both nonmelanoma and melanoma skin cancers has been increasing over the past decades [9], malignant melanoma, being the one that accounts for approximately 75% of all deaths from skin cancer [10]. A375 is a human malignant melanoma cell line derived from skin biopsies that is highly tumorigenic and metastatic in animal models [11,12].

Medical interest has focused on macrocyclic polyamines in the last decades because of their chemical and biological properties as antitumor agents. Their antiproliferative activity is due to their affinity to the DNA [13]. 1,2-diamines possess a wide range of bioactivities, such as the anticancer and antituberculosis ones [14]. The amine groups are useful for modulating the solubility of the drug, as well as for donating or accepting hydrogen bonds to and from a biological receptor [15].

Diamines (-)-trans-, (+)-trans- and meso-1 (Figure 1) showed antiproliferative activity against the MCF-7 cell line [16]. They induce growth inhibition with an $IC_{50}$ of 0.4, 0.6 and 1.8 μM, respectively. The most active compound (-)-trans-1 shows greater cytotoxic activity toward the MCF-7 cells than the normal MCF-10A cells. Real-time RT-PCR analysis

demonstrated that (-)-trans-1 is an extremely efficient regulator of the antiapoptotic genes Bcl-xl, Bcl-2 and cyclin D1. Later studies of the molecule confirmed that it also regulates p53 [16,17].

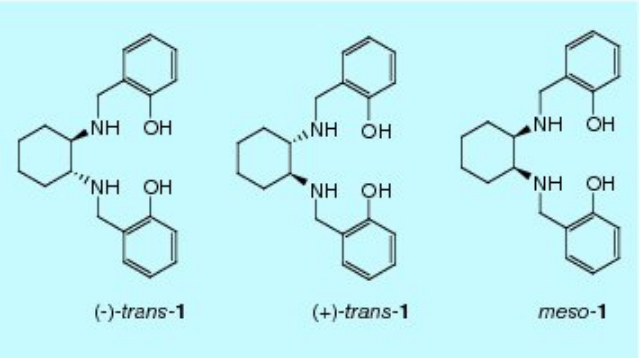


Figure 1. Diamines with antiproliferative activity against the MCF-7 cell line.
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Macrocyclic compounds represent a structural class with an exceptional potential for biological activity and specificity [18]. Nowadays, macrocycles are promising chemotypes, with more than 100 marketed macrocycle drugs that included antitumor drugs [19]. Benzenesulfonamides also show anticancer activity through a variety of mechanisms such as cell-cycle perturbation on the $G_1$ phase, the disruption of microtubule assembly or angiogenesis inhibition [20].

We have previously designed different molecules that target cancer (Figure 2). Compound 2 is one of the most potent human choline kinase inhibitor reported to date, showing activity in the low micromolar range ($IC_{50}$ = 0.3 μM) [21], with similar values to others recently published [22-24]. Macrocycle trans-bis(5-FU O, N-acetal) (3) presents an antiproliferative activity of 5.5 μM against the MCF-7 cell line and provokes a $G_0/G_1$ cell cycle arrest in the MCF-7 breast cancer cells [25]. The benzenesulfonamide-containing compound, named bozepinib (4), is a potent antitumor agent with an $IC_{50}$ against the MCF-7 cell line of 0.355 μM [26] that induces apoptosis in breast and colon cancer cells. The double-stranded RNA-dependent protein kinase PKR is a target of bozepinib (4). Moreover, the combination with IFN-α potentiates the apoptosis induced by bozepinib (4) and also enhances autophagy and senescence, both processes of great importance in tumor cells that show resistance to conventional chemotherapy [27]. Bozepinib (4) also shows in vivo antitumor and antimetastatic efficacy in xenotransplanted nude mice without presenting subacute toxicity and a selective activity against cancer stem cells [28].

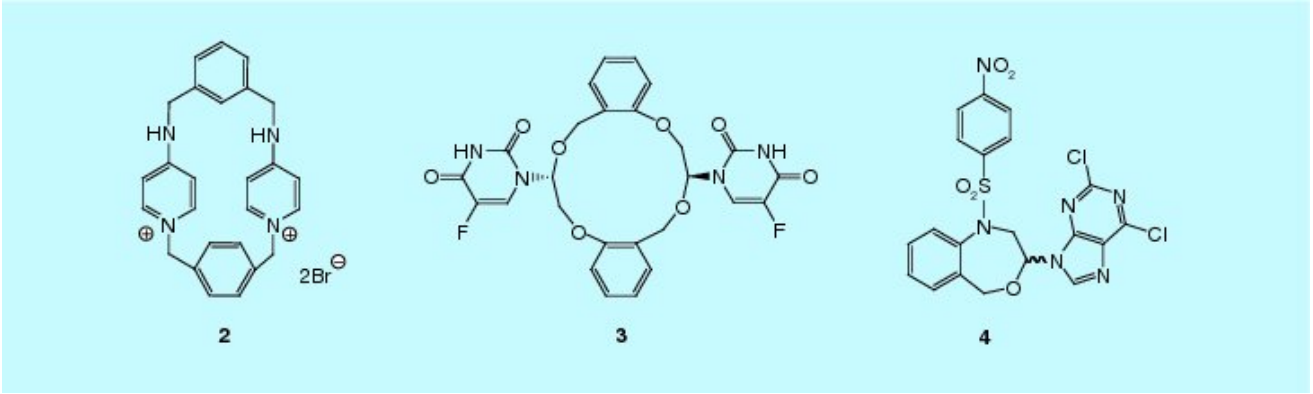


Figure 2. Anticancer agents previously reported by us.

In this paper we present the synthesis and biological evaluation of a series of compounds (Figure 3) designed to study the influence of three structural modifications in the 1,2-diaminocyclohexane derivatives (1): (A) transition of the phenol

group to meta (m-) and para (p-) positions (5 and 6, Figure 3), (B) introduction of two p-nitrobenzenesulfonyl groups (7–9, Figure 3) and (C) its conversion into a 14-membered macrocycle with an ethylene bridge that links the two phenolic groups of 1 (10, Figure 3).[Query-Q7: Leigh Nugent(PE) to All(AU)] What are A, B and C referring to in the preceding sentence?

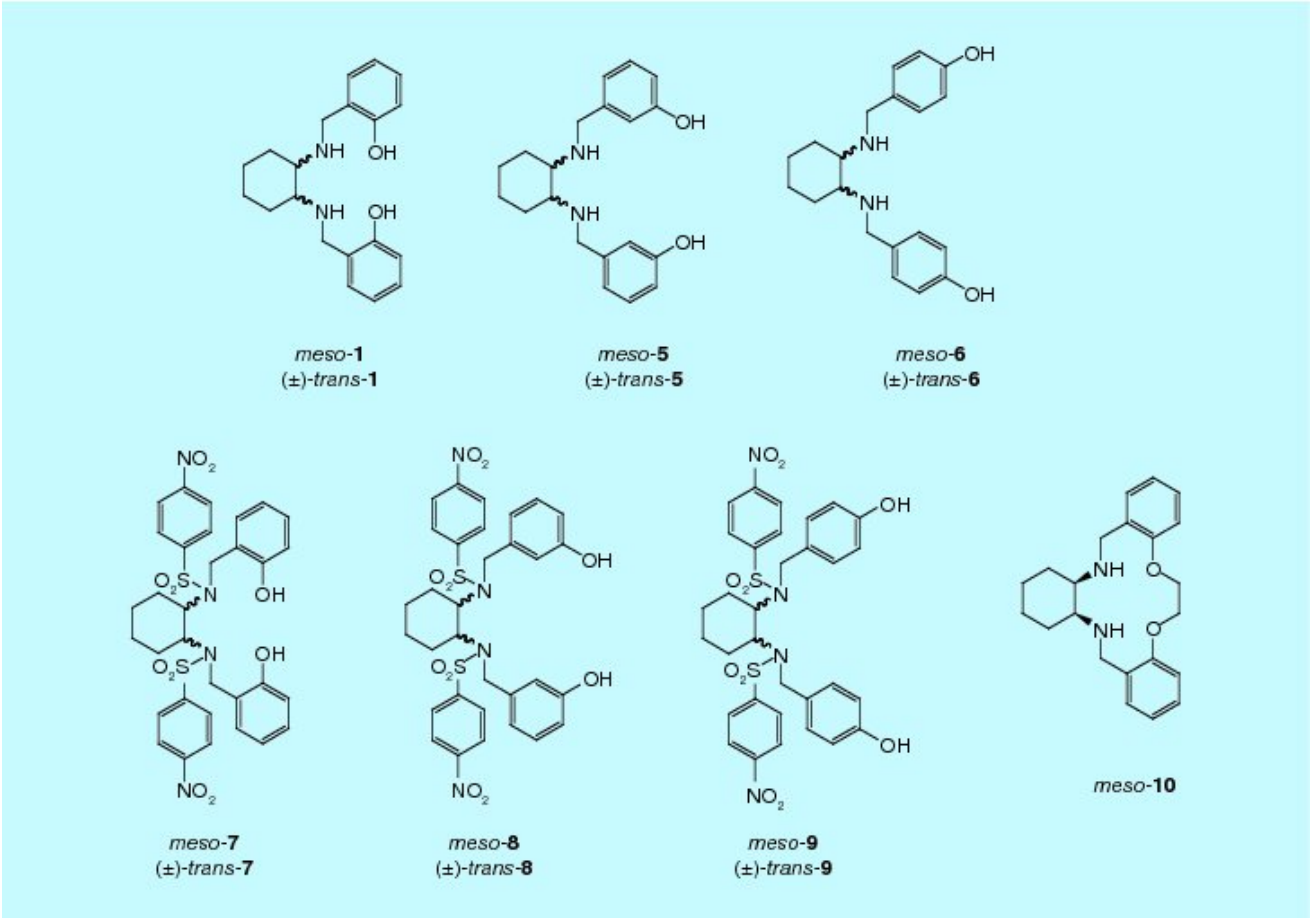


Figure 3. Molecules synthesized and analyzed as antitumor agents.

## Materials & methods

### Chemistry

Melting points were taken in open capillaries on an electrothermal melting point apparatus and are uncorrected. Analytical thin layer chromatography was performed using Merck Kieselgel 60 F254 aluminum sheets, the spots being developed with UV light (λ = 254 nm). All evaporation was carried out in vacuo with a Büchi rotary evaporator and the pressure controlled by a Vacuubrand CVCII apparatus. For flash chromatography, Merck silica gel 60 with a particle size of 0.040–0.063 mm (230–400 mesh ASTM) was used.[Query-Q8: Karishma Bhan(CE) to All(AU)] Please define the following term: 'ASTM.' Purity of all compounds was determined by NMR studies, mass spectroscopy and elemental analysis.[Query-Q9: Karishma Bhan(CE) to All(AU)] Please define the following term: 'NMR.' Elemental analyses were performed on the Thermo Scientific Flash 2000 analyzer and the measured values indicated by the symbols of the elements or functions were within ± 0.4% of the theoretical values. Nuclear magnetic resonance spectra have been carried out at the Centro de Instrumentación Científica (CIC)/Universidad de Granada and recorded on a 300 MHz $^{1}$H and 75 MHz $^{13}$CNMR Varian Inova-TM spectrometers at ambient temperature. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. Signals are designated as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; m, multiplet. High-resolution nano-assisted laser desorption/ionization or electrospray ionization mass spectra were carried out on a Bruker Autoflex or a Waters LCT

Premier Mass Spectrometer, respectively. Anhydrous $CH_2Cl_2$ was purchased from the VWR International Eurolab. Anhydrous conditions were performed under argon. All reagents were purchased from Aldrich. Compounds 1, 5–10 have been named according to IUPAC recommendations for phane structures [29,30].

Intermediate 11 was synthesized as previously described [31]. Target compounds meso-1,5–9, (±)-trans-1,5–9 and meso-10 were synthesized according to the previously reported protocols [16,32,33] with the modifications described in the Supplementary Information.

### Biology

### Cell culture

MCF-7 (ECACC: 86012803) and HCT-116 (ECACC: 91091005) provided by the Cell Bank of the University of Granada and A375 cells, a gift from Bosserhoff (Institute of Pathology, Regensburg University, Germany), were grown at 37°C in an atmosphere containing 5% $CO_2$, with Dulbecco's modified Eagle Medium (Gibco, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 2% L-glutamine, 2.7% sodium bicarbonate, 1% Hepes buffer, 40 mg/l gentamicin and 500 mg/l ampicillin [34-36].

### Drug treatment

Compounds were dissolved in DMSO and stored at -20°C. For each experiment, the stock solutions were further diluted in medium to obtain the desired concentrations. The final solvent concentration in cell culture was ≤0.1% v/v of DMSO, a concentration without any effect on cell replication. Parallel cultures of MCF-7, HCT-116 and A375 cells in medium with DMSO were used as controls.

### Biological assays

The effect of the compounds on cell viability was assessed using the sulforhodamine-B (SRB) colorimetric assay. The assay procedure is explained in the supplementary information file, as well as the cell-cycle distribution analysis, the apoptosis detection by staining with annexin V-FITC and propidium iodide and the caspase assay.

### Statistical analyses

All the quantitative data in the present study are reported as means ± standard derivation from at least three independent experiments. Two-way ANOVA was used for grouped analysis of differences followed by Bonferroni post-tests.

## Results & discussion

### Chemistry

meso- and (±)-trans-1,5,6 were synthesized by reductive amination [16]. The yield of meso-1 was improved to 100% by increasing the reaction time for the Schiff-base condensation from 2 to 48 h. Sulfonylation of diamines meso- and (±)-trans-1,5,6 with p-nitrobenzenesulfonyl chloride gave meso- and (±)-trans-7,8,9 respectively (Figure 4).[Query-Q10: Leigh Nugent(PE) to All(AU)] Please ignore the space below Figure 5; this will be rectified in the final layout. PXE is not representative of the final layout, please only use it to review the content.

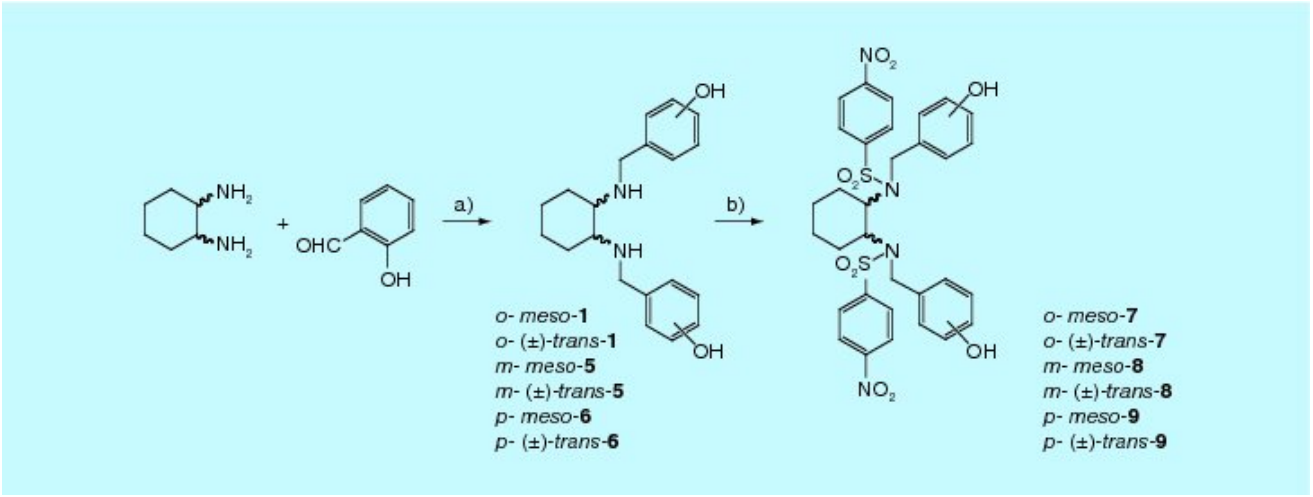


Figure 4. Reagents and conditions. (A) $Et_3N$/EtOH, rt, 48 h; $NaBH_4$, rt, 4 h [100% for meso-1, 41% for (±)-trans-1, 75% for meso-5, 74% for (±)-trans-5, 51% for meso-6, 57% for (±)-trans-6]; (B) p-$NO_2$-Ph-$SO_2Cl$, anhydrous $CH_2Cl_2$, rt, 4.5 h [63% for meso-7, 26% for (±)-trans-7, 24% for meso-8, 24% for (±)-trans-8, 16% for meso-9, 9% for (±)-trans-9].

The first step in the synthesis of meso-10 (Figure 5) was the reaction of 1,2-dibromoethane with salicylaldehyde [31]. The reductive amination of 11 yield meso-10 without further purification [32,33].

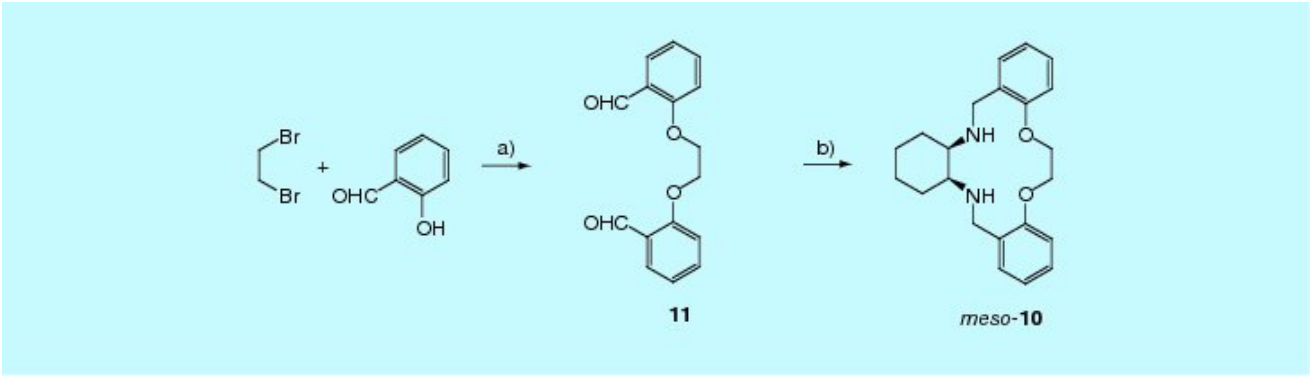


Figure 5. Reagents and conditions. (A) NaOH 2%/EtOH, reflux, 72 h (27%); (B) meso-1,2-diaminocyclohexane, $CH_3OH$, reflux, 5 h; $NaBH_4$, 50°C, 3.5 h (38%).

## Biology

Compounds meso-1, 5–9, (±)-trans-1, 5–9 and meso-10 were assayed for their in vitro antiproliferative activity against the MCF-7, HCT-116 and A375 cell lines. The antitumor drug oxaliplatin, that contains a diamine in its structure, was used as a control in the assays and their $IC_{50}$ values are concurrent with the ones in literature [37]. The results are

summarized in Table 1. All the compounds are potent antiproliferative agents with the highest potency against the A375 melanoma cancer cell line (submicromolar values of $IC_{50}$ for compounds 1, 7–10). meso- and (±)-trans-1 are the best antiproliferative agents in the three cell lines studied (MCF-7, HCT-116 and A375 ($IC_{50}$ = 58 nM for meso-1 vs 2.5 μM for oxaliplatin in MCF-7 cells, $IC_{50}$ = 66 nM for (±)-trans-1 vs 3.8 μM for oxaliplatin in HCT-116 cells). [Query-Q11: Leigh Nugent(PE) to All(AU)] Where do the brackets before MCF-7 and IC50 end in the preceding sentence? We should highlight that the alteration of the phenol group from ortho (o-) to para (p-) position decreases the antiproliferative activity of compounds 5 (m-) and 6 (p-) substantially (from $IC_{50}$ = 0.06–1.2 μM for 1 to $IC_{50}$ = 10.7–54.6 μM for 5 and 6). However, there is not a difference between the molecules with the p-nitrobenzenesulfonyl group (7–9), where the antiproliferative activity is similar between o-, m- and p- derivatives ($IC_{50}$ = 0.94–6.3 μM). The best values for antiproliferative activity of the p-nitrobenzenesulfonyl derivatives are meso-9 against HCT-116 cell line ($IC_{50}$ = 1.1 μM) and (±)-trans-9 against A375 cell line ($IC_{50}$ = 94 nM). meso-10 shows similar values of antiproliferative activity as oxaliplatin against the MCF-7 cell line ($IC_{50}$ = 2.9, 2.5 μM, respectively), being a better agent than oxaliplatin against HCT-116 and A375 cell lines ($IC_{50}$ = 1.5, 1.1 μM against 3.8, 3.4 μM, respectively). As a result, we should mention that the modification of the phenol groups in compounds 5 and 6, the introduction of the p-nitrobenzenesulfonyl group into meso- and (±)-trans-1 to give meso- and (±)-trans-7–9 as well as the macrocyclization to produce meso-10, do not improve the antiproliferative activity in relation to those of meso- and (±)-trans-1.

Table 1. Antiproliferative activity for meso- and (±)-trans-1,5–9, meso-10 and oxaliplatin against the MCF-7, HCT-116 and A375 cell lines.

| Compound | MCF-7 $IC_{50}$ μM | HCT-116 $IC_{50}$ μM | A375 $IC_{50}$ μM |
|---|---|---|---|
| meso-1 | 0.058 ± 0.014 | 0.236 ± 0.060 | 0.099 ± 0.018 |
| (±)-trans-1 | 1.240 ± 0.018 | 0.066 ± 0.017 | 0.205 ± 0.015 |
| meso-5 | 42.30 ± 0.012 | 12.79 ± 1.08 | 40.30 ± 1.01 |
| (±)-trans-5 | 48,40 ± 0.76 | 14.84 ± 0.12 | 35.65 ± 0.92 |
| meso-6 | 54.56 ± 1.16 | 31.30 ± 0.92 | 38.70 ± 1.32 |
| (±)-trans-6 | 40.90 ± 0.78 | 10.65 ± 0.82 | 32.18 ± 0.69 |
| meso-7 | 7.010 ± 0.044 | 5.070 ± 0.032 | 3.413 ± 0.016 |
| (±)-trans-7 | 2.630 ± 0.044 | 3.680 ± 0.045 | 2.398 ± 0.020 |
| meso-8 | 6.320 ± 0.011 | 5.040 ± 0.005 | 2.040 ± 0.001 |
| (±)-trans-8 | 6.100 ± 0.047 | 4.390 ± 0.010 | 2.030 ± 0.001 |
| meso-9 | 4.130 ± 0.014 | 1.090 ± 0.007 | 4.390 ± 0.019 |
| (±)-trans-9 | 4.080 ± 0.024 | 4.330 ± 0.002 | 0.940 ± 0.003 |
| meso-10 | 2.900 ± 0.018 | 1.460 ± 0.001 | 1.106 ± 0.064 |
| Oxaliplatin | 2.500 ± 0.003 | 3.770 ± 0.001 | 3.350 ± 0.002 |

All experiments were conducted in triplicate and gave similar results. Data are the mean ± SD of three independent determinations. μM: Micromolar.

The cell cycle is the process by which cells duplicate themselves, grow and prepare to divide again. It regulates cell division in multicellular organisms and it is essential for the replacement of damaged cells. Regulation of cell proliferation

is one of the strategies for the treatment of cancer. As a consequence, in order to evaluate whether the antiproliferative effect of these molecules involves changes in cell-cycle distribution, A375 cells were treated with the compounds that present better antiproliferative activity: 1, 7–10 and then analyzed by flow cytometry (see Table 2). We found a cell-cycle arrest in the $G_0/G_1$ phase induced by meso- and (±)-trans-7 and 9 and meso-10. meso-1 and meso and (±)-trans-8 did not modify the cell cycle profile and (±)-trans-1 provoked a $G_2$/M and S-phase cell-cycle arrest at the expense of $G_0/G_1$ phase. The antiproliferative properties of several antitumor agents are mediated by the modulation of cell-cycle checkpoint. Palbociclib is an inhibitor of CDK4 and CDK6 that provokes a pronounced $G_0/G_1$ arrest, as molecules 7,9 and 10. It is a highly selective and orally active drug, blocking retinoblastoma phosphorylation in a low nanomolar range [38]. Its antitumor activity is associated with reduced Rb phosphorylation and with a decreased expression of Ki-67 [39], a cell proliferation marker that is associated with ribosomal RNA transcription. It decreased caspase 3/7 activation and CDKN2A and increased levels of RB1 and cyclin D1 [40]. An example of a drug that causes cell-cycle $G_2$/M arrest, as (±)-trans-1, is panobinostat [41-43]. It is demonstrated that it decreases in vivo tumorigenesis of triple-negative breast cancer cells. Panobinostat is a potent inhibitor with activity against Class I, II and IV HDAC enzymes, with hyperacetylation of histones H3 (Lys9) and H4 (Lys8) [41].

Table 2. Cell cycle analysis in the A375 melanoma cell line upon treatment with meso- and (±)-trans-1,7–9, meso-10 compounds and oxaliplatin (at 3 × $IC_{50}$ doses, 48 h).

| Compound | Cell cycle[†‡] | | |
|---|---|---|---|
| | $G_0/G_1$ | S | $G_2$/M |
| Control | 38.97 ± 0.22 | 44.42 ± 0.66 | 16.61 ± 0.45 |
| meso-1 | 39.04 ± 1.63 | 42.24 ± 2.98 | 18.73 ± 1.35 |
| (±)-trans-1 | 19.97 ± 0.13 | 54.78 ± 1.95 | 25.26 ± 1.83 |
| meso-7 | 58.01 ± 0.36 | 30.14 ± 0.24 | 11.85 ± 0.11 |
| (±)-trans-7 | 62.28 ± 0.14 | 28.54 ± 1.33 | 9.19 ± 1.20 |
| meso-8 | 38.95 ± 2.69 | 46.90 ± 2.62 | 14.15 ± 1.70 |
| (±)-trans-8 | 36.68 ± 1.91 | 50.40 ± 0.14 | 12.93 ± 0.92 |
| meso-9 | 55.63 ± 1.98 | 36.56 ± 1.20 | 7.79 ± 0.28 |
| (±)-trans-9 | 42.35 ± 0.99 | 44.16 ± 0.64 | 13.49 ± 0.57 |
| meso-10 | 66.97 ± 3.59 | 15.22 ± 1.05 | 17.82 ± 4.63 |
| Oxaliplatin | 53.15 ± 0.71 | 30.22 ± 0.85 | 16.62 ± 0.42 |

† Determined by flow cytometry [44].
‡ All experiments were conducted in triplicate and gave similar results. Data are the mean ± SD of three independent determinations.

Further studies were performed to determinate if the observed growth inhibition was due to apoptosis. Apoptosis is a genetic orderly process in which cells lead to their own death in response to different stimuli and it is an essential process to maintain homeostasis in multicellular organisms. Therefore, apoptosis activation is a fundamental strategy in the improvement of cancer therapy. Apoptosis was determined using an Annexin V-based assay in A375 cells treated with compounds: 1, 7–10 (see Supplementary Table 1). A light apoptotic response was observed by compound (±)-trans-7 (28.3%) and no significant response by molecules 1-, meso-7 and 9 (6.35–14.91%). However, we can emphasize that

the macrocycle meso-10, which is the compound that induces more $G_0/G_1$ phase accumulation, is also the drug that provokes extremely high levels of apoptosis at 48 h in the A375 cancer cells. This compound increases the percentage of apoptotic cells (early and late apoptosis) from 1.05% in DMSO-treated cells to 95.0%. Highly apoptotic drugs have demonstrated efficient antitumor activities in patients. The potent apoptotic properties of the macrocycle meso-10 in melanoma cells suggest the potential clinical interest of this drug and encourage future deep studies and in vivo experiments.

Finally, studies of apoptosis were performed by the activation of caspases to determine which metabolic pathway activated these drugs. Compound meso-10 (Figure 6) activates the canonical intrinsic caspase-8/caspase-3 apoptotic pathway on the MCF-7 cell line, but also this compound induces slightly caspase-2 activation (Figure 6A). However, this compound does not significantly activate significantly any caspase in HCT-116 and A-375 cell lines (Figures 4B & C). Different cell death modalities exposed to the same molecule in diverse cell lines have been previously described [45]. Indirubin, a CDK inhibitor, induces caspase-independent cell death in human neuroblastoma [46], while other indirubin derivatives induce cell death through the intrinsic caspase-9 pathway and/or activation of caspase-8 in breast cancer cells [47].

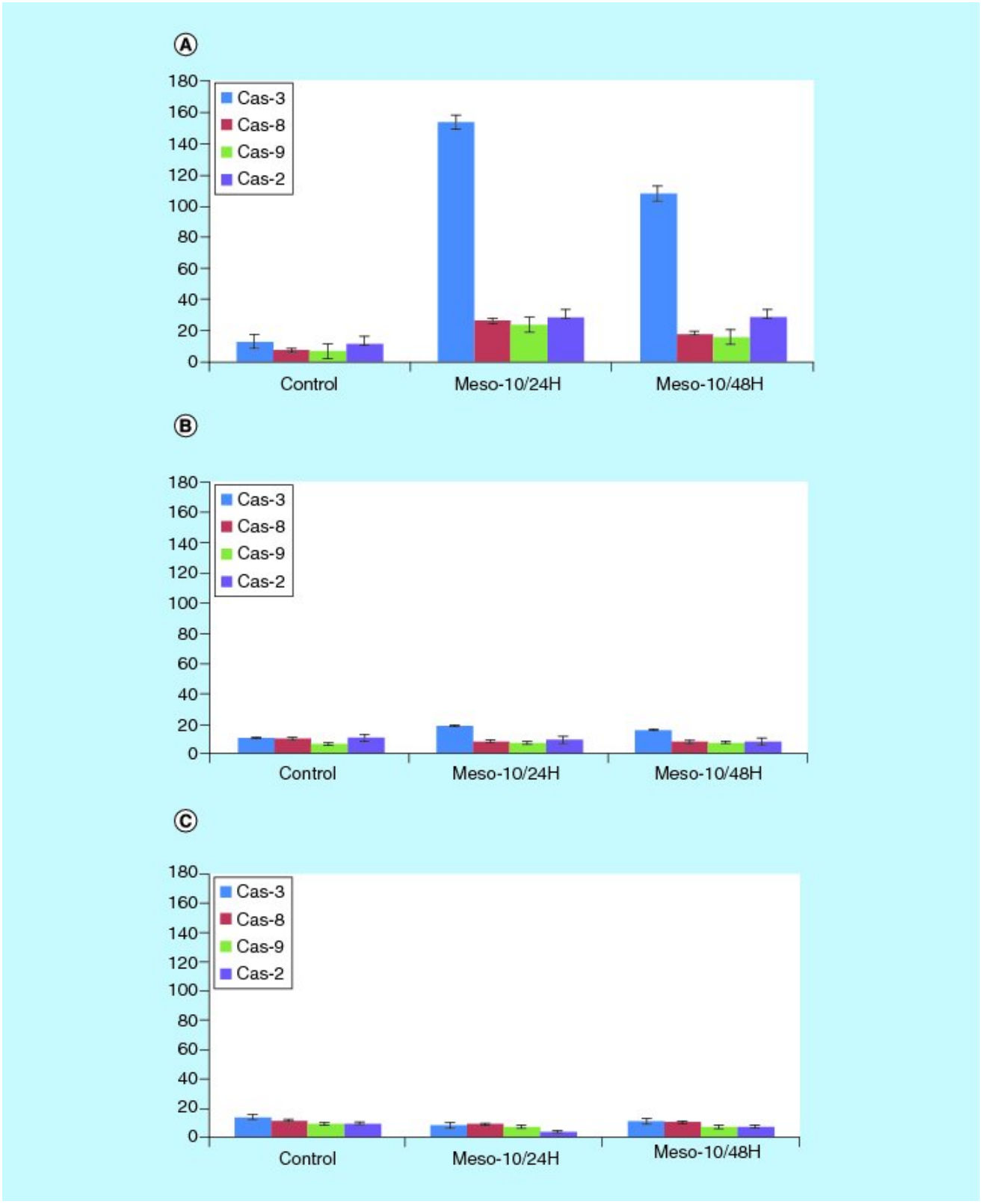


Figure 6. Apoptosis caspase assay in: 4.A: MCF-7, 4.B.: HCT-116 and 4.C.: A-375 cell lines treated with compound meso-10. [Query-Q12: Karishma Bhan(CE) to All(AU)] Please provide a general/main title for Figure 6.

## Conclusion & future perspective

A series of 1,2-diaminocyclohexane derivatives have been designed and synthesized. All the molecules have been assayed against the MCF-7, HCT-116, A375 human cancer cell lines. Apoptosis and cell-cycle studies have also been carried out for the compounds with better antiproliferative activities (1, 7–10) against the A375 cancer cell line. All the molecules are active compounds as antiproliferative agents against all the assayed cell lines. The most active one (meso-1) presents an $IC_{50}$ value of 58 nM against the A375 cell line. Bis(p-nitrobenzenesulfonyl) derivatives (meso- and

(±)-trans-7 and 9) and macrocycle meso-10 provoke a $G_0/G_1$ cell-cycle arrest in the A375 cell lines after 48 h of treatment. The macrocycle meso-10 presents a very significant apoptotic index of 95% after 48 h of treatment in the A375 human melanoma cell line. It is the most apoptotic antiproliferative agent so far reported by our research group.

Macrocycles provide diverse functionality in a conformationally preorganized ring structure. This can result in high affinity and selectivity for protein targets, while preserving sufficient bioavailability to reach intracellular locations. The 1,2-diamine functionality can be found in various compounds displaying a broad spectrum of biological activity. Vicinal diamines can easily be converted into 14-member heterocyclic rings that provide entropic advantage for binding to the biological target.

The results present here indicate that the selective activity of the macrocyclic derivative against the melanoma cell line A375, via apoptosis, supposes a great advantage for a future therapeutic use. This exemplifies the potential of 1,2-diaminocyclohexane derivatives to qualify as lead structures for future anticancer drug development, due to their easy syntheses and noteworthy bioactivity.

Enormous work has been done so far for improving and developing new macrocycles. Medicinal chemists continue to explore new approaches and new molecular modalities in their continued efforts to identify modulators of biological targets. In the future, new macrocycles can be designed applying new synthetic methods in order to increase their selectivity and bioactivity against even unknown enzymes, ion channels and receptors.

**Executive summary**

**Design, synthesis & biological evaluation**

- Cancer is among the leading causes of death worldwide and a major health problem in developing, as well as undeveloped countries.
- Medical interest has focused on polyamines because of their properties as antitumor agents.
- Thus, a series of 1,2-diaminocyclohexane derivatives were designed, synthesized and biologically evaluated as antitumor compounds.

**Antitumor effects**

- All the structures are potent cytotoxic agents, especially against the A375 cell line.
- The most active compound meso-1 presents an $IC_{50}$ value of 58 nM against the A375 cell line.
- The macrocycle meso-10 presents a very significant apoptotic index of 95% after 48 h of treatment in the A375 human melanoma cell line.

**Future anticancer drug development**

- The selective activity of the macrocyclic derivative against A375, via apoptosis, supposes a great advantage for a future therapeutic use.
- These compounds are therefore lead structures for future anticancer drug development due to their easy syntheses and noteworthy bioactivity.

Financial & competing interests disclosure [Query-Q13: Karishma Bhan(CE) to All(AU)] Please check and confirm that the disclosure section is correct.

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PI10/02295) and the ERDF (European Regional Development Fund) for financial supports. The award of a grant from the Ministerio de Educación to Fátima Morales is gratefully acknowledged. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

## References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

1 World Health Organization. Cancer. Fact sheet N. 297. www.who.int/mediacentre/factsheets/fs297/en/

2 Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in globocan 2012. Int. J. Cancer 136, 359–386 (2015).

3 World Health Organization. Breast cancer: prevention and control. www.who.int/cancer/detection/breastcancer/en/

4 Lacroix M, Leclercq G. Relevance of breast cancer cell lines as models for breast tumours: an update. Breat Cancer Res. Treat. 83, 249–289 (2004).

5 Catalano S, Campana A, Giordano C et al. Expression and function of phosphodiesterase type 5 in human breast cancer cell lines and tissues: implications for targeted therapy. Clin. Cancer Res. 22(9), 2271–2282 (2016).

6 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J. Clin. 65(2), 87–108 (2015).

7 Bhattacharyya NP, Skandalis A, Ganesh A, Groden J, Meuth M. Mutator phenotypes in human colorectal carcinoma cell lines. Proc. Natl Acad. Sci. USA 91, 6319–6323 (1994).

8 Ferreira HJ, Heyn H, Moutinho C, Esteller M. CpG island hypermethylation-associated silencing of small nucleolar RNAs in human cancer. RNA Biology 9(6), 881–890 (2012).

9 World Health Organization. Ultraviolet radiation and the INTERSUN Programme. www.who.int/uv/faq/skincancer/en/index1.html

10 Nahar VK, Allison Ford M, Brodell RT et al. Skin cancer prevention practices among malignant melanoma survivors: a systematic review. J. Cancer Res. Clin. Oncol. 142(6), 1273–1283 (2016).

11 Su DM., Zhang Q, Wang X et al. Two types of human malignant melanoma cell lines revealed by expression patterns of mitochondrial and survival-apoptosis genes: implications for malignant melanoma therapy. Mol. Cancer Ther. 8, 1292–1304 (2009).

12 Paulitschke V, Haudek-Prinz V, Griss J et al. Functional classification of cellular proteome profiles support the identification of drug resistance signatures in melanoma cells. J. Proteome Res. 12, 3264–3276 (2013).

13 Dinesh NVS, Bhupathiraju K, Grac M, Vicente H. Synthesis of carborane-containing porphyrin derivatives for the boron neutron capture therapy of tumors. Top. Heterocycl. Chem. 34, 31–52 (2015).

14 Foss MH, Pou S, Davidson PM et al. Diphenylether-modified 1,2-diamines with improved drug properties for development against mycobacterium tuberculosis. ACS Infect. Dis. 2(7), 500–508 (2016).

15 Kim H, So SM, Chin J. Preparation of chiral diamines by the diaza-cope rearrangement (DCR). Aldrichimica ACTA 41, 77–88 (2008).

16 Gao J, Liu YG, Zhou Y. Chiral salicyl diamines. ChemMedChem 2, 1723–1729 (2007).

• An interesting research article regarding diamine open compounds' synthesis.

17 Gao J, Liu YG, Zingaro RA. Cytotoxic activities, celular uptake, gene regulation, and optical imaging of novel platinum (II) complexes. Chem. Res. Toxicol. 22, 1705–1712 (2009).

• An interesting research article regarding diamine open compounds' biological effect.

18 O'Connell KM, Beckmann HS, Laraia L et al. A two-directional strategy for the diversity-oriented synthesis of macrocyclic scaffolds. Org. Biomol. Chem. 10, 7545–7551 (2012).

19 Collins S, Bartlett S, Nie F et al. Diversity-oriented synthesis of macrocycle libraries for drug discovery and chemical biology. Synthesis 48(10), 1457–1473 (2016).

• Interesting review regarding the role of macrocycles in drug discovery.

20 Balderas-Rentería R, González-Barranco P, García A, Banik BK, Rivera G. Anticancer drug design using scaffolds of β-lactams, sulfonamides, quinoline, quinoxaline and natural products. Drug advances in clinical trials. Curr. Med. Chem. 19, 4377–4398 (2012).

21 Conejo-García A, Campos JM, Sánchez-Martín RM, Gallo MA, Espinosa A. Bispyridinium cyclophanes: novel templates for human choline kinase inhibitors. J. Med. Chem. 46, 3754–3757 (2003).

• Published work by us, related to macrocycle research.

22 Trousil S, Kaliszczak M, Schug Z et al. The novel choline kinase inhibitor ICL-CCIC-0019 reprograms cellular metabolism and inhibits cancer cell growth. Oncotarget 7(24), 37103–37120 (2016).

23 Rubio-Ruiz B, Figuerola-Conchas A, Ramos-Torrecillas J et al. Discovery of a New binding site on human choline kinase α 1: design, synthesis, crystallographic studies, and biological evaluation of asymmetrical bispyridinium derivative. J. Med. Chem. 57, 507–515 (2014).

24 Schiaffino-Ortega S, Baglioni E, Mariotto E et al. Design, synthesis, crystallization and biological evaluation of new symmetrical biscationic compounds as selective inhibitors of human Choline Kinase α1 (ChoKα1). Sci. Rep. 6, 23793 (2016).

25 Saniger E, Campos JM, Entrena A et al. Neighbouring-group participation as the key step in the reactivity of acyclic and cyclic salicyl-derived O, O-acetals with 5-fluorouracil. Antiproliferative activity, cell cycle dysregulation and apoptotic induction of new O, N-acetals against breast cancer cells. Tetrahedron 59, 8017–8026 (2003).

26 López-Cara LC, Conejo-García A, Marchal JA et al. New (RS)-benzoxazepinpurines with anti-tumour activity: the chiral switch from (RS)-2,6-dichloro-9-[1-(p-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepin-3-yl]-9H-purine. Eur. J. Med. Chem. 46, 249–258 (2011).

27 Marchal JA, Carrasco E, Ramírez A et al. Bozepinib, a novel small antitumor agent, induces PKR-mediated apoptosis and synergizes with IFNα triggering apoptosis, autophagy and senescence. Drug Des. Devel. Ther. 7, 1301–1313 (2013).

28 Ramírez A, Boulaiz H, Morata-Tarifa C et al. HER2-signaling pathway, JNK and ERKs kinases, and cancer stem-like cells are targets of Bozepinib small compound. Oncotarget 5, 3590–3606 (2014).

29 Powell WH, Weissbach O. Phane nomenclature. Part I: Phane parent names (IUPAC Recommendations 1998). Pure Appl. Chem. 70, 1513–1545 (1998).

30 Favre HA, Hellwinkel D, Powell WH, Smith HA. Phane nomenclature. Part II: modification of the degree of hydrogenation and substitution derivatives of phane parent hydrides (IUPAC recommendations 2002). Pure Appl. Chem. 74, 809–834 (2002).

31 Simion C, Simion A, Mitoma Y et al. Synthesis of new dihydroxy-dioxygenated ortho-[2,x] cyclophanes. Heterocycles 53, 2459–2470 (2000).

•• An interesting research article regarding macrocycle compound's synthesis.

32 Fernández-Fernández MC, Bastida de la Calle R, Macías A, Valencia-Matarranz L, Pérez-Lourido P. Co(II), Ni(II) and Cu(II) complexes of new [1+1] and [2+2] macrocyclic ligands derived from 1,4-bis(2′-formylphenyl)-1,4-dioxabutane and cis-1,2-diaminocyclohexane. Polyhedron 27, 2301–2308 (2008).

33 Correa WH, Scott JL. Synthesis and characterisation of macrocyclic diamino chiral crown ethers. Molecules 9, 513–519 (2004).

34 Soule HD, Vazquez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst. 51(5), 1409–1416 (1973).

35 Brattain MG, Fine WD, Khaled FM, Thompson J, Brattain DE. Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res. 41(5), 1751–1756 (1981).

36 Giard DJ, Aaronson SA, Todaro GT et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. 51, 1417–1423 (1973).

37 Liua F, Zhoua Z, Gou S, Jian Zhaoab J, Chenab F. Synthesis and antiproliferative activity of (1R,2R)-N1-(2-butyl)-1,2-cyclohexanediamine platinum(II) complexes with malonate derivatives. J. Coord. Chem. 67(17), 2858–2866 (2014).

38 Chen P, Lee NV, Hu W et al. Spectrum and degree of CDK drug interactions predicts clinical performance. Mol. Cancer Ther. 15(10), 2273–2281 (2016).

39 Flaherty KT, Lorusso PM, Demichele A et al. Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. Clin. Cancer Res. 18, 568–576 (2012).

40 Roberts PJ, Bisi JE, Strum JC et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. J. Natl Cancer Inst. 104, 476–487 (2012).

41 Kubo M, Kanaya N, Petrossian K et al. Inhibition of the proliferation of acquired aromatase inhibitor-resistant breast cancer cells by

histone deacetylase inhibitor LBH589 (panobinostat). Breast Cancer Res. Treat. 137, 93–107 (2013).

42 Tate CR, Rhodes LV, Segar HC et al. Targeting triple-negative breast cancer cells with the histone deacetylase inhibitor panobinostat. Breast Cancer Res. 14, R79 (2012).

•• Highlights the mechanism of the antitumor effect of panobinostat, a histone deacetylase inhibitor that provokes cell cycle G2/M arrest, as (±)-trans-1.

43 Fortunati N, Catalano MG, Marano F et al. The pan-DAC inhibitor LBH589 is a multi-functional agent in breast cancer cells: cytotoxic drug and inducer of sodium-iodide symporter (NIS). Breast Cancer Res. Treat. 124, 667–675 (2010).

44 Marchal JA, Boulaiz H, Suárez I et al. Growth inhibition, G1-arrest, and apoptosis in MCF-7 human breast cancer cells by novel highly lipophilic 5-fluorouracil derivatives. Invest. New Drug 22, 379–389 (2004).

45 Kamil Rudolf K, Emil Rudolf E. Antiproliferative effects of α-tomatine are associated with different cell death modalities in human colon cancer cells. J. Funct. Foods 27, 491–502 (2016).

46 Kurita M, Hanada S, Ichimaru Y et al. Indirubin 3′-epoxide induces caspase-independent cell death in human neuroblastoma. Biol. Pharm. Bull. 39, 993–999 (2016).

47 Nicolaoua KA, Liapisb V, Evdokioub A et al. Induction of discrete apoptotic pathways by bromo-substituted indirubin derivatives in invasive breast cancer cells. Biochem. Biophys. Res. Commun. 425(1), 76–82 (2012).