RESEARCH COMMUNICATION

Evaluation of Apoptosis-Induction by Newly Synthesized Phthalazine Derivatives in Breast Cancer Cell Lines

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Abstract

Newly synthesized phthalazine derivatives including copper and platinum complexes were evaluated for cytotoxicity in human breast cancer cell lines. The cells were incubated with the compounds (100 μM) for 72 h and cytotoxicity, apoptosis and DNA content were measured by flow cytometery. Our results suggest that the parent (H1-2), copper (C1-2)- and platinum (P1-2)-derivatized compounds were relatively more active in inducing apoptosis and cell killing in both human breast cancer cell lines, MDA-MB-231 cells being the more sensitive. Other compounds showed weak or no response towards these parameters except H-5 causing 40% apoptosis in MDA-MB-231 cells. Addition of copper or platinum in the structures generally reduced the apoptotic potential. Possible roles for structure activity relationships are discussed.

Key Words: Phthalazine - platinum compounds - human breast carcinoma cells - MCF-7 - MDA-MB-231

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Introduction

Cisplatin is currently used as a first line chemotherapeutic agent for the treatment of testicular, bladder and some other cancers (Ho et al., 2003; Wang and Lippard, 2005; Kostova, 2005) but significant side effects and intrinsic resistance limit the clinical success (Kostova, 2005).To overcome evolving resistance pathways of cisplatin, the strategies are being developed to design new anticancer agents with the incorporation of different carrier groups that can target specific tumor cells. Numerous copper and platinum complexes have been found to possess anticancer activity against cell lines (Singh et al., 1997; Miller III et al., 1998; Wheate and Collins, 2005).

Several phthalazine derivatives as well as phthalazine-1,4-diones are known to be cytotoxic agents (Easmon et al., 1997; Savini et al., 1997; Wood et al., 2000; Lazo et al., 2001). The discovery that naturally occurring pyrazole C-nucleoside, pyrazofurin (4-hydroxy-3-β-D-ribofuranosyl-1H-pyrazole-5-carboxamide), possesses broad spectrum of antimicrobial and antiviral activities in addition of being active against several tumor cell lines (Petrie et al., 1986; Chen et al., 1993) has pointed the way for many pyrazole derivatives to be screened for comparable activities (Daidone et al., 1998; Kücükgüzel et al., 2000; Genin et al., 2000a,b; Gamage et al., 2002). Therefore, it is of interest to synthesize molecules containing both phthalazine and pyrazole functionalities in the same structure aiming to obtain improved activity.

For our ongoing search of new anticancer compounds (Fahmy et al., 2002; Habib et al., 2003; Bekhit et al., 2004), we synthesized some new platinum and copper analogues mounted on 4-hydrazonophthalazinones as carrier ligands. In the present study, we report anticancer potential in human breast cancer cell lines.

Materials and Methods

Chemicals and cell lines

A novel series of phthalazine derivatives namely, 4-(1,3-diaryl-1H-pyrazol-4-yl)methylidenehydrazino-1(2H)-phthalazinones (H1-8) and their copper (C1-8) and platinum (P1-8) complexes were synthesized (see the Table). The summarized scheme for their synthesis will be published in detail elsewhere.

Human breast carcinoma cell lines (MCF-7, MDA-MB-231) were purchased from American Type Culture Collection (Rockville, MD, USA). All the cell culture reagents and media were obtained commercially from Sigma Chemical Company (St. Louis, MO, USA). A Vybrant apoptosis assay kit #2 was purchased from Molecular Probes, Inc. (Eugene, OR, USA).

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Table. List of 4-(1,3-diaryl-1H-pyrazol-4-yl) Methyl-idenehydrazino-1(2H)-phthalazinones (H1-8) and their Copper (C1-8) and Platinum Complexes (P1-8)

Compounds	R^1	R^2
H1-C1-P1	H	H
H2-C2-P2	H	CH_3
H3-C3-P3	H	Cl
H4-C4-P4	H	Br
H5-C5-P5	CH_3	H
H6-C6-P6	CH_3	CH_3
H7-C7-P7	CH_3	Cl
H8-C8-P8	CH_3	Br

Treatment of cell lines

MCF-7 and MDA-MB-231 cells were cultured in RPMI1640 medium using 10% serum to confluence, then subcultured using 60 mm dishes and treated with 100 µM of the test compounds dissolved in DMSO (final conc. ~1%) for 72 h at 37°C in an incubator under 5% carbon dioxide. The cells were harvested by trypsinization for analysis of apoptosis as described below.

Induction of apoptosis

Cells were washed in cold phosphate-buffered saline and centrifuged followed by staining with annexin V and propidium iodide in annexin-binding buffer. After 15 min incubation at room temperature, the fluorescence was measured using a flow cytometer (FACScan, Becton Dickenson, USA). The results were analyzed using CellQuest Pro software and represented as percentage of normal (viable) and apoptotic cells at various stages. Simultaneously, treated cells were stained for nuclear DNA and the sub-G1 apoptotic population was analyzed (Krishan, 1975).

Results and Discussion

Our results showed cell-specific differential response with these phthalazine derivatives. The parent, copper- and platinum-derivatized compounds 1-2 were relatively more active in inducing the apoptosis and cell killing in both the human breast cancer cell lines, MCF-7 and MDA-MB-231 (Figures 1-5). Other compounds had shown weak or no response towards these parameters except H-5, which showed 40% apoptosis in the MDA-MB-231 cells (Figure 5 B). In general, MDA-MB-231 cell lines showed greater sensitivity to these compounds regardless of the metal derivatives (Figures 2 and 4). Further, compounds H-1, C-1 and P-1 were the most active compounds in both the cell lines followed by compounds H-2 = H-5 = P-2 > C-2 only in the MDA-MB-231 cell lines, causing up to 60% apoptosis (Figure 5 B,D,F). MCF-7 cells showed maximum 30% apoptosis with H-1 and the remaining compounds were ineffective.

Further the differential response for these phthalazine derivatives in the MDA-MB-231 cells could well be dependent on their structures. The compounds with both R^1 and R^2 positions containing hydrogen were the most active in inducing the apoptosis. However, the R^1 position with hydrogen and R^2 (methyl) also shown significant induction of apoptosis. Compound H-5 where R^1 and R^2 positions contained methyl and hydrogen groups respectively was quite active. Addition of copper or platinum metal in the structures significantly affected their apoptotic abilities. Addition of platinum inhibited the apoptotic ability of the

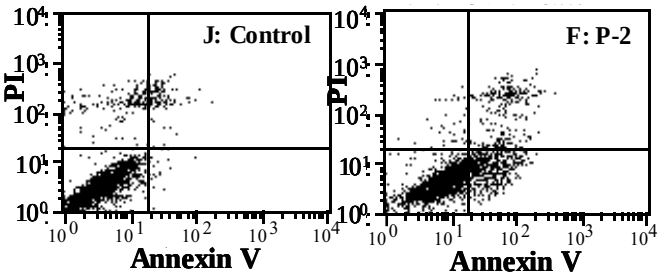

Figure 1. Representative Flow Cytometric Analysis Data for MCF-7 Cells

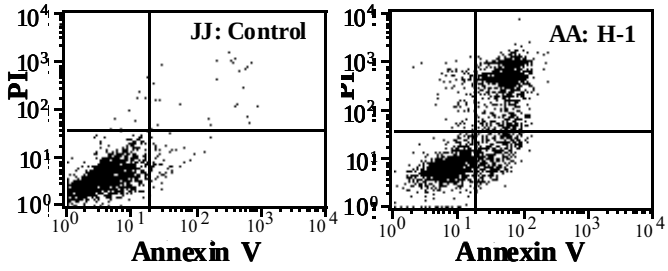

Figure 2. Representative Flow Cytometric Analysis Data for MDA-MB-231 Cells

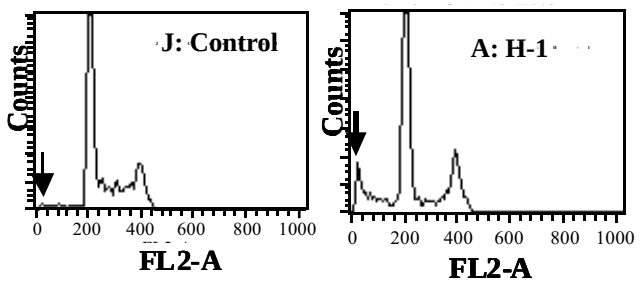

Figure 3. DNA Content Analysis of MCF-7 Cells. Arrows represent the sub-G1 apoptotic population

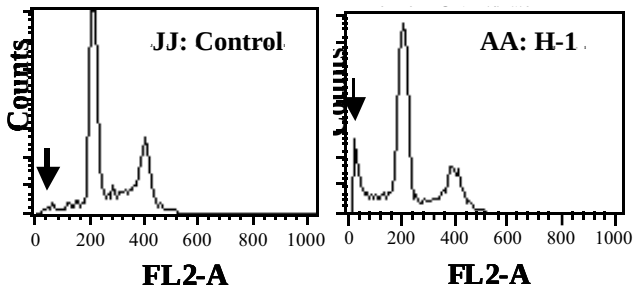

Figure 4. DNA Content Analysis of MDA-MB-231 Cells. Arrows represent the sub-G1 apoptotic population

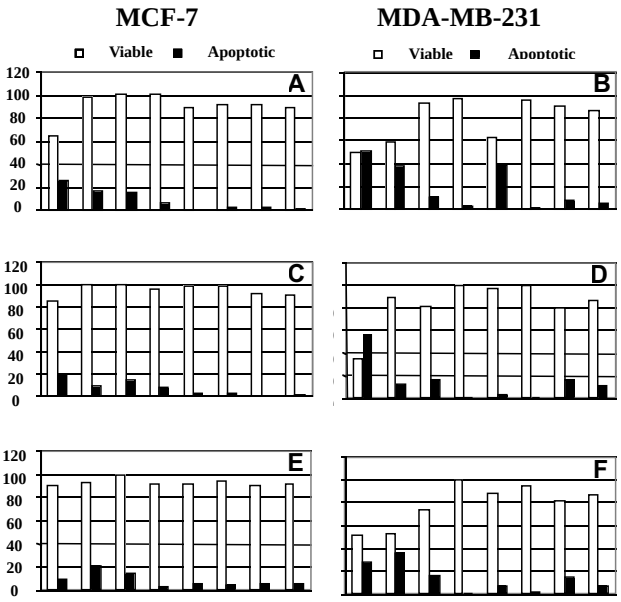

Figure 5. Percentages of Viable and Apoptotic MCF-7 (panels A, C, E) and MDA-MB-231 (panels B, D, F) Cells Following Treatment with the Test Compounds 1-8 (100 μM). Panels A/B, C/D and E/F show the parent (H1-8), copper (C1-8) or platinum (P1-8) substituted compounds. The data was normalized against the control (100% for viability or 0% for apoptosis).

compounds (P-1 and P-5) by 50-90% when compared to their counterparts H-1 and H-5 (Figure 5 B, F). Further, addition of copper did not affect the ability of H-1 while the apoptotic potential was significantly reduced in case of H-2 (Figure 5 B, D). These observations tend to suggest that the structure activity relationship plays an important role in the apoptosis caused by these compounds.

Though the mechanism(s) of action of apoptosis by these compounds was beyond the scope of this manuscript, however, it seems that estrogen receptor (ER)-alpha which is absent in the MDA-MB-231 cell lines, may play important role in inducing higher degree of apoptosis compared to the MCF-7 cell lines, which are ER-alpha positive (Lacroix et al., 2004). In addition, bcl2, an apoptosis inhibitory protein, which is not expressed in the MDA-MB-231 cell lines, may also be responsible for an increased apoptosis in this cell line (Srivastava et al., 1998). Moreover, it has been shown that caspases play significant role in execution of apoptosis. Caspase-3 plays a critical role in a proteolytic cascade within the apoptosis signal pathway and is activated by numerous death signals. Our results showing higher apoptosis in MDA-MB-231 cell lines compared to the MCF-7 cell lines, which are deficient of caspase-3, further strengthens the role of caspase-3 in the apoptosis by these compounds (Yang et al., 2005). Presence of mutant p53 in the MDA-MB-231 cells compared to the wild type p53 in the MCF-7 cells may also play a role in the increased susceptibility of the MDA-MB-231 cells towards these phthalazine derivatives.

In conclusion, some of these compounds seems to have potential anticancer ability specifically against the MDA-MB-231 cells, however, detailed studies are required using the normal breast epithelial cell lines to pinpoint the mechanism(s) of action of these compounds with the least associated genotoxic activity.

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