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SHORT COMMUNICATION

Antiproliferative activity of some 1,4-dimethylcarbazoles on cells that express estrogen receptors: part I

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Abstract
Several 9*H*-carbazole derivatives are used for various pharmacological applications. Many of these compounds demonstrated cytotoxic and anticancer activities. In this work, we have investigated the cytotoxic activity of some substituted carbazoles against cancer cell lines (MCF-7, and ISK). The derivative **2a** showed the highest inhibitory activity against both cell lines.

Keywords: Carbazoles, anticancer, MCF-7 cell line, Ishikawa cell line

Introduction

The indole nucleus is often found embedded in compounds with a wide range of biological activities[1]. Carbazoles, whether synthetic or naturally occurring, are important members of indole-containing heterocycles[1,2]. Several 9*H*-carbazoles possess various pharmacological applications and also they are used in dyes-, insecticide-, and plastic industries. In addition, several derivatives of 1,2,3,4-tetrahydro-9*H*-carbazole and 9-methyl-9*H*-carbazole have been known by their medicinal use as antitubercular, antifungal, antibacterial, β_3-adrenoceptor agonists and anticancer activities[2–4].

The precise mechanisms of their anti-neoplastic activity have not yet been explained. Many of these carbazoles are cytostatic and their activity results from alternative cytotoxic effects. It was suggested that the prevalent mechanisms of the antitumor, mutagenic and cytotoxic activities are their intercalation into DNA and inhibition of DNA-topoisomerase II activity[5,6].

Based on this, the aim of this work is to investigate the cytotoxic activity of the prepared carbazoles (Figure 1) against a cancer cell line. We have used MCF-7 cell line derived from a pleural effusion of a postmenopausal 69-year-old patient with metastatic breast cancer in 1970[7]. This cell line was grown in Dulbecco's modified Eagle's medium (DMEM), without phenol red, containing 10% foetal calf serum (Invitrogen, Milan, Italy) and 1 mg/mL penicillin-streptomycin. The MCF-7 cell line is the most widely used and best characterized of all the human breast cancer cell lines[8]. MCF-7 cells respond to estrogens and anti-estrogens, have differential expression of estrogen receptors (ER), progesterone receptor, and have high proliferation rates[9]. MCF-7 cells are also a perfect model to study the pathway of malignant progression as they can be subjected to appropriate endocrinologic and physiologic selective pressures for the derivation of variants with more progressed phenotypes[8].

Further in this work, some tests were performed on Ishikawa cell line (ISK) derived from an endometrial adenocarcinoma of a 39-year-old Asian woman[10]. This cell line was maintained in Dulbecco's modified Eagle's

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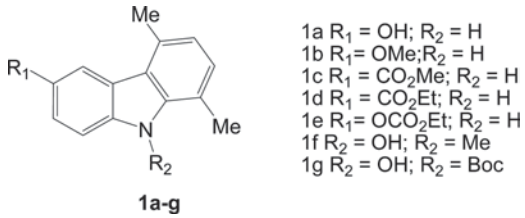

Figure 1. Carbazole derivatives (**1a–g**).

medium-F12 (DMEM/F12), without phenol red, supplemented with 10% foetal bovine serum (Invitrogen, Milan, Italy) and 1 mg/mL penicillin-streptomycin.

Ishikawa cells express estrogen[11], progesterone[12], androgen[13] and aryl hydrocarbons receptors[14]. There is a big evidence for the hormonal responsiveness of Ishikawa cells. This evidence is based mainly on hormonal modification of proliferation, cellular functions or gene expression[15]. In particular, these cells are

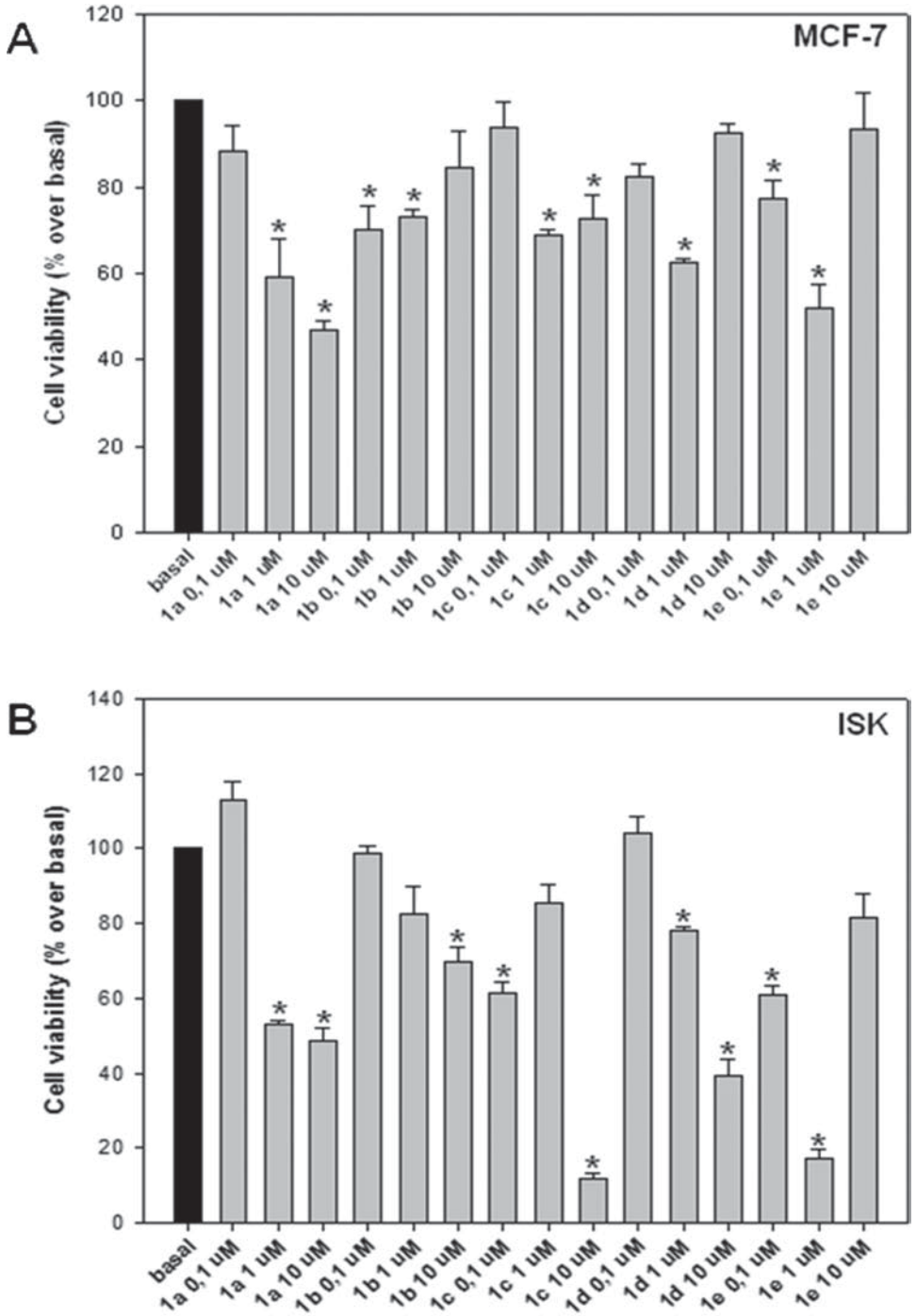

Figure 2. Effect of carbazole derivatives (**1a–e**) against MCF-7 and ISK cell proliferation. MCF-7 (**A**) and ISK (**B**) cells were treated for 96 or 24 h, respectively, after 24-h starvation, with the indicated concentrations of substances **1a**, **1b**, **1c**, **1d** and **1e**. Cells proliferation was evaluated by MTT assay. Statistically significant differences are indicated. Histograms; mean of three independent experiments each performed with triplicate samples expressed as percent of basal; bars, SE (*$P < 0.01$ compared with basal).

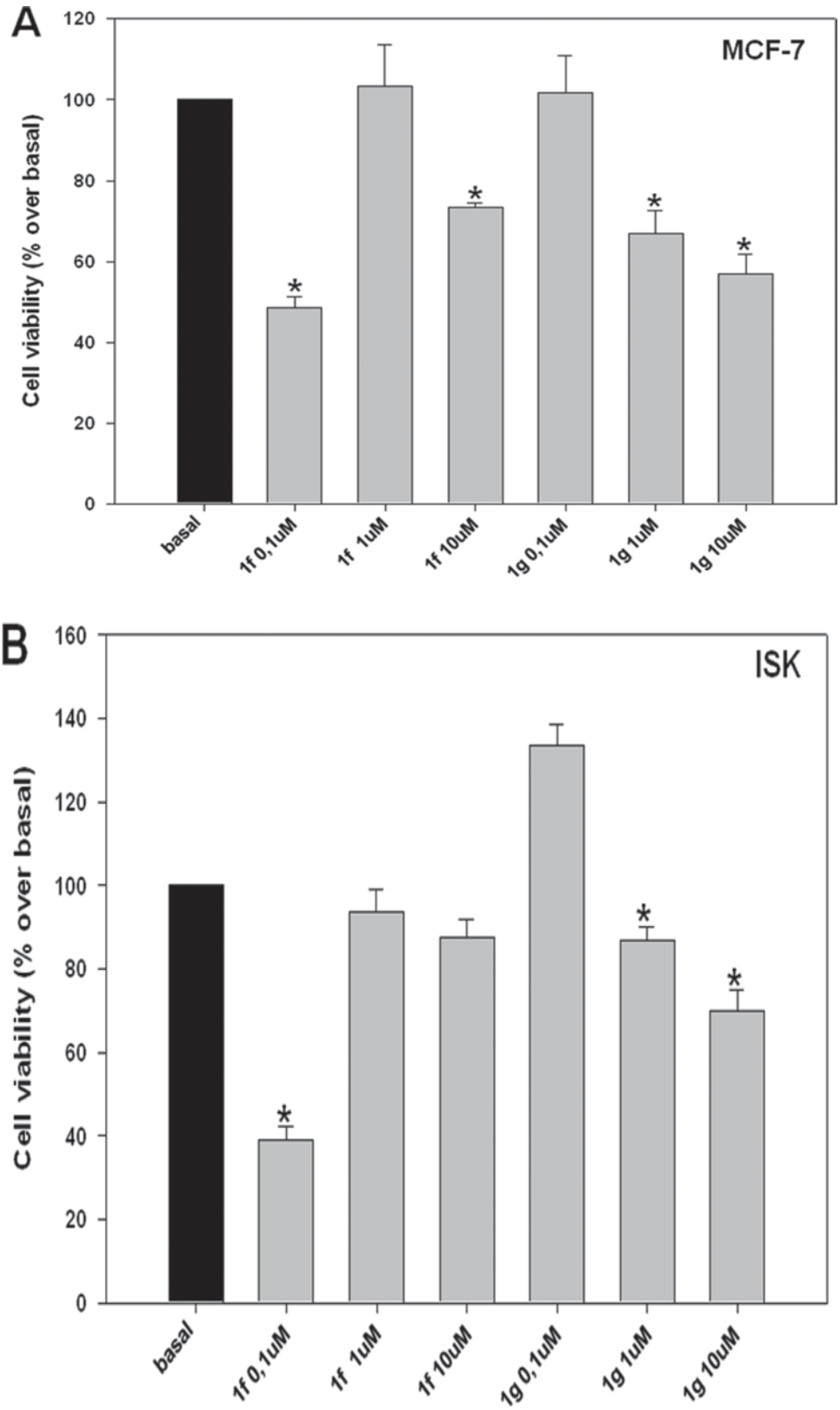

Figure 3. Effect of carbazole derivatives (**1f–g**) against MCF-7 and ISK cell proliferation. MCF-7 (**A**) and ISK (**B**) cells were treated for 96 or 24 h, respectively, after 24-h starvation, with the indicated concentrations of substances **1f** and **1g**. Cells proliferation was evaluated by MTT assay. Statistically significant differences are indicated. Histograms; mean of three independent experiments each performed with triplicate samples expressed as percent of basal; bars, SE (*$P < 0.01$ compared with basal).

used *in vitro* for the elucidation of molecular mechanisms of hormone action (e.g., in drug and discovery processes), testing of potential agonistic functions of anti-estrogens or selective estrogen receptor modulators in an endometrial-derived model[16], in studies of ligand independent activation of the estrogen receptor and anchorage independent tumour growth[17]. In addition, they are used for studies on factors controlling hormonal receptivity[18] and on environmental toxicology on the function of phytoestrogens[19] and endocrine-disrupting chemicals[20] in an endometrial model; paracrine cell/cell-interaction[21], signalling cross-talk[14] and others[22]. Because of their hormonal responsiveness *in vitro*, Ishikawa endometrial adenocarcinoma cells have also been developed as an estrogen sensitive *in vivo* tumour model, which is particularly suitable for the study of hormonal growth control[23].

Results and discussion

Carbazoles **1a–g** were synthesized[24–27] and characterized[28–34] using earlier reported methods including ours.

In particular, the 9*H*-carbazoles **1a–d** were prepared by the reaction of appropriate indoles with hexane-2,4-dione in the presence of *p*-toluenesulphonic acid. The 9*H*-carbazole **1e** was instead prepared by reaction of **1a** with ethyl chloroformate under standard conditions[35]. Furthermore, we have used **1a** as starting compound for the preparation of **1f–g**. In fact, **1a** was *N*-methylated by iodomethane and in the presence of sodium hydride to give compound **1f**. The carbazole NH could be *N*-protected by $(Boc)_2O$ to give **1g** (Figure 1).

In order to evaluate the cytotoxic activity of prepared compounds (**1a–g**) against MCF-7 and ISK cell lines, an MTT assay was performed[36] using increasing concentrations (0.1, 1, 10 μM) of the compounds under testing. Compound **1a** showed a significant inhibition at 1 and 10 μM, against both MCF-7 and ISK cell lines (Figure 2A, and B). Concerning the MCF-7 cells, a 30% inhibition was observed by the lower concentrations of 0.1 and 1 μM of **1b** and **1c**, respectively (Figure 2A). However, with ISK cells, compound **1b** displayed a significant inhibitory effect on cell proliferation only at 10 μM concentration, while the inhibitory effect of **1c** was observed at a concentration of 0.1 and 10 μM (Figure 2B). On the other hand, **1d** showed an inhibitory effect against MCF-7 cells at 1 μM concentration (Figure 2A), and the same compound, however, displayed more inhibition against ISK cells at a concentration of 1 and 10 μM (Figure 2B). Compound **1e** significantly reduced cell proliferation of both MCF-7 and ISK cells at a concentration of 0.1 μM and 1 μM (Figure 2A, and B).

Based on the above results indicating the ability of compound **1a** to inhibit proliferation of both cell lines (Figure 2A, and B), structural modifications of **1a** were made in order to find out whether the introduction of a substituent such as a methyl- (**1f**) or a Boc substituent (**1g**), at the 9 position of the carbazole nucleus could affect the antiproliferative activity.

Figure 3 showed that the introduction of a methyl group (an electron-donating small substituent), improved the inhibitory activity, whereas the introduction of Boc group did not show any improvement of activity. The derivative **1f** showed the highest inhibitory activity against both cell lines (at low concentration of 0.1 μM) (Figure 3A, and B), the matter that encourages its use as a possible antitumor agent. However, the observation that higher concentrations (1, 10 μM) of **1f** do not induce dose-response inhibitory effect on both MCF-7 and ISK cell viability suggests a possible role of **1f** in blocking a survival pathway binding to receptor desensitized at high concentration. This hypothesis is currently under investigation in our laboratories.

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Declaration of interest

The authors report no conflicts of interest.

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28. (1a) White solid, mp 174°C. IR (KBr) (cm-1): 3517, 3415, 1461, 1165, 847, 809, 543. 1HNMR (DMSO-d6): δ 2.50 (s, 3H, CH3), 2.61 (s, 3H, CH3); 6.83 (d, J = 7.36 Hz, 1H, Ar); 6.95 (d, J = 8.56 Hz, 1H, Ar); 7.08 (d, J = 7.36 Hz, 1H, Ar); 7.40 (d, J = 8.56 Hz, 1H, Ar); 7.53 (s, 1H, Ar); 9.12 (br, 1H, OH); 10.88 (s, 1H, NH). MS (ESI+): 212 (M+ +1).
29. (1b) White solid, mp 150°C. IR (KBr) (cm-1): 3406, 2959, 1481, 1210, 1045, 812, 545. 1H NMR (DMSO-d6): δ 2.62 (s, 3H, CH3), 2.69 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 6.85 (d, J = 7.08 Hz, 1H, Ar); 7.09 (d, J = 8.28 Hz, 2H, Ar), 7.48 (d, J = 8.56 Hz, 1H, Ar), 7.64 (s, 1H, Ar), 11.01 (br, 1H, NH). MS (ESI+): 226 (M+ +1), 224 (M+ -1).
30. (1c) White solid, mp 266°C. IR (KBr) (cm-1): 3337, 1702, 1615, 1430, 1290, 1122, 989, 803, 764, 735, 557. 1H NMR (DMSO-d6): δ 2.52 (s, 3H, CH3), 2.71 (s, 3H, CH3), 3.84 (s, 3H, OCH3), 6.89 (d, J = 7.84 Hz, 1H, Ar); 7.11 (d, J = 7.32 Hz, 1H, Ar), 7.55 (d, J = 8.08 Hz, 1H, Ar), 7.98 (dd, J1 = 1.48 Hz, J2 = 8.56 Hz, 1H, Ar), 8.67 (s, 1H, Ar), 11.62 (s, 1H, NH). MS (ESI+): 254 (M+ +1).
31. (1d) White solid, mp 253°C. IR (KBr) (cm-1): 3339, 1705, 1618, 1430, 1295, 1129, 990, 806, 765, 738, 560. 1H NMR (DMSO-d6): δ 1.67 (s, 3H, CH3), 2.53 (s, 3H, CH3), 2.80 (s, 3H, CH2CH3), 4.30-4.60 (m, 2H, OCH2), 6.95 (d, J = 7.50 Hz, 1H, Ar); 7.16 (d, J = 7.20 Hz, 1H, Ar), 7.55 (d, J = 8.70 Hz, 1H, Ar), 8.04 (d, J = 8.70 Hz, 1H, Ar), 8.72 (s, 1H, Ar), 11.59 (br, 1H, NH). MS (ESI+): 268 (M+ +1).
32. (1e) White solid, mp 130°C. IR (KBr) (cm-1): 3396, 1744, 1462, 1253, 1181, 1002, 811, 780, 552. 1H NMR (DMSO-d6): δ 1.32-1.40 (t, 3H, CH2CH3), 2.57 (s, 3H, CH3), 2.78 (s, 3H, CH3), 4.28–4.35 (q, 2H, CH2CH3), 6.91 (d, 1H, J = 7.08 Hz, Ar), 7.16 (d, 1H, J = 7.32 Hz, Ar), 7.29 (dd, 1H, J1 = 1.72 Hz, J2 = 8.56 Hz, Ar), 7.58 (d, 1H, J = 8.56 Hz, Ar), 7.94 (s, 1H, Ar), 11.36 (s, 1H, NH). MS (ESI+): 284 (M+ +1).
33. (1f) White solid, mp 202°C. IR (KBr) (cm-1): 3415, 1587, 1460, 1165, 809, 543. 1H NMR (CDCl3): δ 8.23 (d, J = 1.96 Hz, 1H, Ar); 7.52 (dd, J = 8.80 Hz, 1H, Ar); 7.23 (d, J = 8.80 Hz, 1H, Ar); 7.07 (d, J = 7.32 Hz, 1H, Ar); 6.88 (d, J = 7.32 Hz, 1H, Ar); 4.07 (s, 3H, NCH3); 2.80 (s, 3H, CH3); 2.78 (s, 3H, CH3). MS (ESI+): 226 (M+ +1).
34. (1g) White solid, mp 205°C. IR (KBr) (cm-1): 3410, 1701, 1449, 1329, 1157, 807. 1HNMR (CDCl3): δ 1.64 (s, 9H, CH3); 2.43 (s, 3H, CH3); 2.74 (s, 3H, CH3); 6.91 (d, J = 8.56 Hz, 1H, Ar); 6.98 (d, J = 7.32 Hz, 1H Ar,); 7.09 (d, J = 7.32 Hz, 1H, Ar); 7.46 (s, 1H, OH); 7.63 (d, J = 8.56 Hz, 1H, Ar); 7.85 (s, 1H, Ar). MS (EI) m/z (%): 311 (M+, 15), 255 (48) (M+, -tBu), 211 (100) (M+, -CO2tBu).
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