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8-Hydroxynaphthalene-1,4-dione derivative as novel compound for glioma treatment

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ABSTRACT

Malignant gliomas continue to demand the search for improved chemotherapeutic solutions. In this work the results of a preliminary in vitro screening performed on a small library of compounds are disclosed. As a result 2-(2,4-dihydroxyphenyl)-8-hydroxy-1,4-naphthoquinone emerged as a promising therapeutic lead.

Malignant gliomas affect the Central Nervous System (CNS) and are the most frequent subtype of primary brain tumours, being considered one of the most aggressive human malignant diseases.[1] These tumours are characterized by diffuse infiltration through the brain parenchyma and have high resistance to the standard cancer therapies. At present, the Food and Drug Administration (FDA) approved approach consists of neurosurgical resection, followed by chemotherapy with an alkylating agent, such as temozolomide (TMZ) (Fig. 1), in combination with radiotherapy.[2] It is widely accepted that the anticancer activity of TMZ is due to methylation of the oxygen-O^6 of the guanine DNA base, thus inducing apoptosis and therefore tumor regression.[3]

In previous years, oncogenesis has been increasingly associated with an altered protein kinase expression.[4,5b] In particular it was noticed that the interference with the protein kinase A pathway induced modulations in the proliferation and cell death of glioma cells.[5a,6] Recently we described the distribution of protein kinase A in rodent glioma models and in human medulloblastoma.[5] On this basis, a possible biological effect of compounds able to interact with the pathways involving kinases appeared reasonable.[7]

This study investigated novel potential species for malignant glioma treatment.

Preliminary screening of a small, *in house,* library seemed a good approach to find a new lead in the search of drugs for the treatment of gliomas. So a panel of 30 chemical entities, many of which

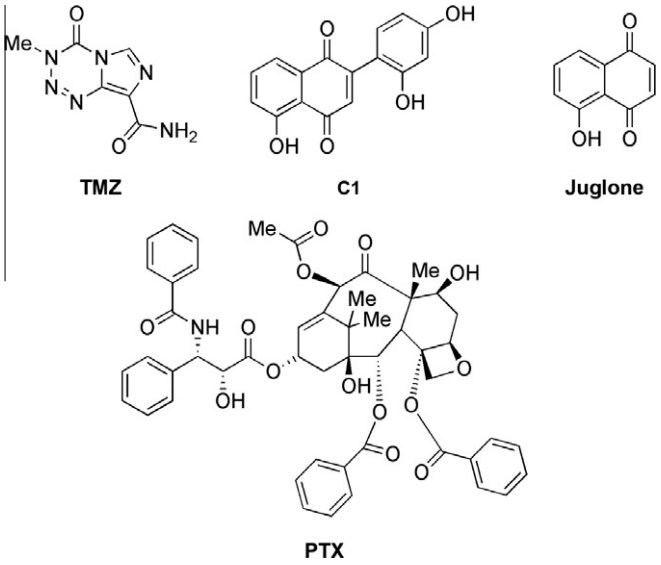

Figure 1. Chemical structures of the cited compounds.

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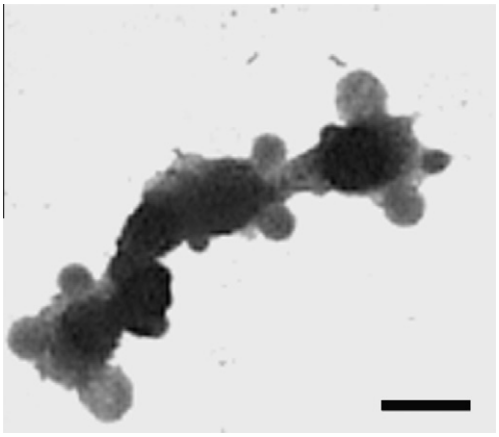

Figure 2. GLI36 cell treated with **C1** 50 μM. Apoptotic cells stained with Wright stain; direct light 40×; scale bar 30 μm.

showed a kinase inhibitory activity, were selected on the basis of their chemical diversity and investigated in search of compounds having a good cytotoxicity on glioma cells and, as a result, 2-(2,4-dihydroxyphenyl)-8-hydroxy-1,4-naphthoquinone (**C1**) (Fig. 1) emerged as a promising therapeutic lead.[7]

Adaptation and improvement of the procedure reported by Zonta et al.[8] gave a very straightforward process to synthesize the compound **C1**. Resorcinol and phosphoric acid were added to a suspension of juglone in acetic acid giving the desired compound in satisfactory yield (62%), upon stirring the mixture for less than 2 h at room temperature.[7]

The cytotoxic effect of **C1** was evaluated in the immortalized human glioma cell line GLI36 and in a primary human glioblastoma cell culture, and compared with the effect induced by TMZ. In addition, a comparison with paclitaxel (PTX) (Fig. 1), a mitotic spindle poison clinically used, which stabilizes microtubules and inhibits their depolymerization to tubulin,[9] was also carried out (Fig. 2).[10]

Remarkably, GLI36 cells treated with the juglone derivative **C1** showed a statistically significant increase in apoptosis (Fig. 3) which was noteworthy compared to the results obtained for the other reference substances TMZ and PTX. In addition, such a difference was found to be significant for all the tested concentrations (Fig. 3A–D). Comparison between **C1**, TMZ and PTX also showed that the necrosis induced by **C1** was found to be significantly lower at higher concentration (50 μM, 5 μM—Fig. 4A and B). Interestingly, necrosis increased in the presence of lower concentration of **C1** (0.5 μM, 0.05 μM—Fig. 4C and D).

The primary human glioblastoma cell culture treated with the juglone derivative **C1** showed a statistically significant increase in apoptosis compared to the cells treated with other substances (Fig. 5 A and B). As expected, the necrosis induced by **C1** was significantly lower than the other substances (Fig. 5 C and D).

In conclusion, the synthesis of 2-(2,4-dihydroxyphenyl)-8-hydroxynaphthalene-1,4-dione **C1** was successfully achieved as described by Zonta et al.[8] and improved. Characterisation of **C1** by NMR spectroscopy was rather difficult, although it provided evidence to prove its molecular structure. Pharmacological evaluation

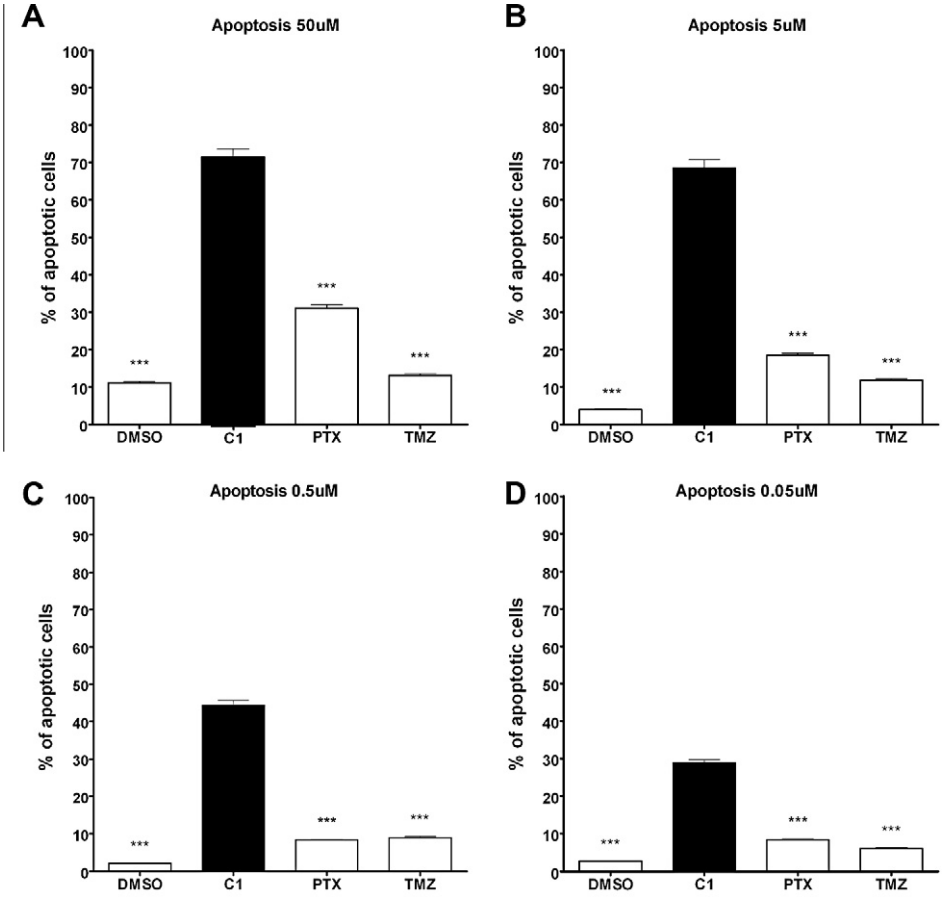

Figure 3. The percentage of apoptotic GLI36 cells induced by treatment with DMSO, **C1**, PTX and TMZ at different concentration of the tested substances (A = 50 μM, B = 5 μM, C = 0.5 μM and D = 0.05 μM). *T-test* $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001, different form **C1**.

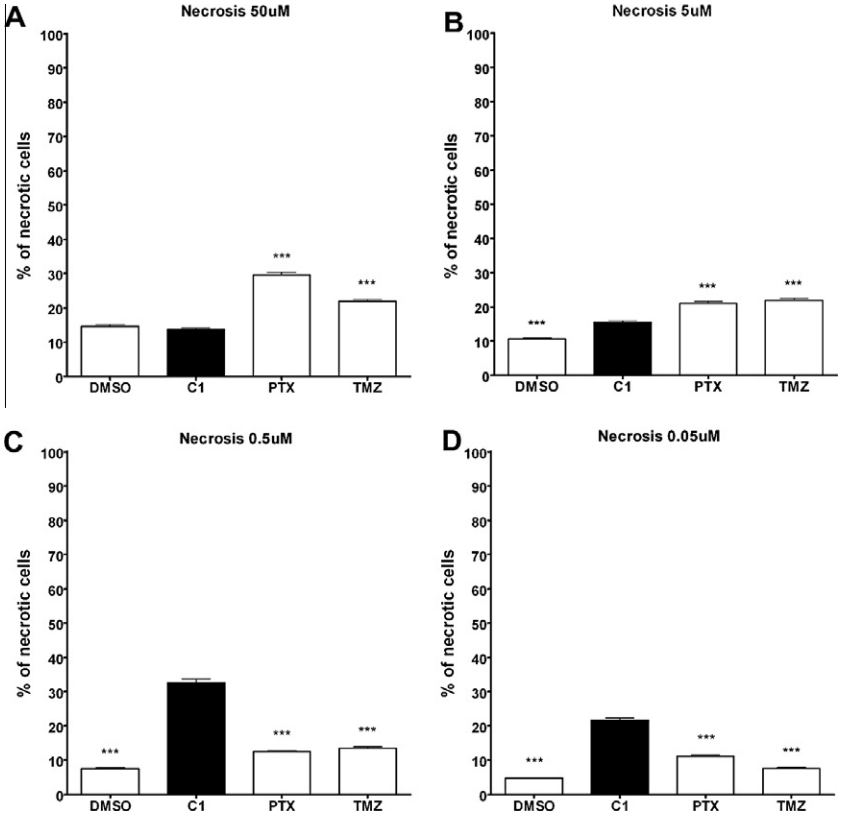

Figure 4. The percentage of necrotic GLI36 cells induced by treatment with DMSO, **C1**, PTX and TMZ at different concentration of the tested substances (A = 50 μM, B = 5 μM, C = 0.5 μM and D = 0.05 μM). *T-test* $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001, different form **C1**.

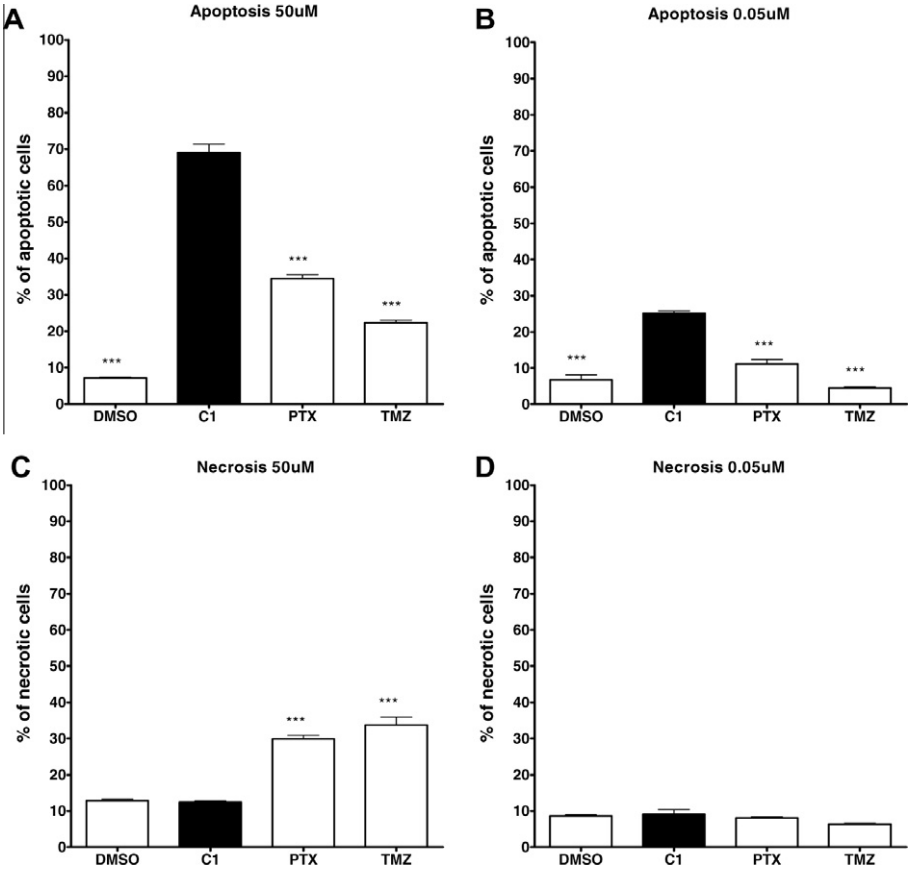

Figure 5. The percentage of apoptotic (A and C) and necrotic (B and D) cells from a primary human glioblastoma induced by treatment with different concentration of DMSO, **C1**, PTX and TMZ (A and C = 50 μM, B and D = 0.05 μM). *T-test* $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001, different form **C1**.

showed a remarkable increase of the in vitro apoptotic induction on human glioma cells when compared with TMZ and PTX. Aiming for the discovery of novel therapeutic compounds which could significantly improve contemporary glioma chemotherapy, these preliminary results prompted the preparation of novel derivatives, whose testing is currently being carried out under the same conditions. In addition, future cytotoxicity assays on different cell lines will be considered, thus giving significant and comprehensive data to better understand the cytotoxic effect of such compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.001.

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10. Cells were maintained in a monolayer using complete growth medium (CGM) in combination with 90% Dulbecco Modified Eagle's Medium (DMEM), 10% FBS, 100 I.U./ml penicillin, 10 μg/ml streptomycin, 10 μg/ml tetracycline, 25 μg/ml Plasmocin (*InVivogen*, Milan, Italy). Cells were incubated at 37 °C in a humidified environment with 95% air and 5% CO_2, up to 80–90% confluence (4–6 days). For cytotoxicity assay, cells were plated on 24-wells plates and grown on 9 mm sterile cover glass. After 48 h, addition of the following species was carried out whilst maintaining the CGM medium: **C1** (50 μM, 5 μM, 0.5 μM or 0.05 μM), PTX (50 μM, 5 μM, 0.5 μM or 0.05 μM) and TMZ (50 μM, 5 μM, 0.5 μM or 0.05 μM). Cells were incubated for 24 h, and then fixed with methanol and stained with Wright's stain; a total of 2400 cells were counted from each slide. The percentage of apoptotic and necrotic cells was then calculated, following an established procedure;[5b] *t-test* was used to estimate the amount of apoptotic and necrotic cells (in percentage) between TMZ and treated cells. The primary cell culture was prepared from glioblastoma biopsy (patient: male 70 years). The biopsy (3 mm^3) was shaken for 5 min in 3 ml of 0.25% Trypsin, 0.02% EDTA solution. The suspension was inactivated with CGM and centrifuged at 37 °C for 10 min at 1350 rpm. The supernatant was discharged, the pellet was re-suspended carefully in 10 ml of CGM and plated in a cell culture flask. The culture was monitored for three weeks changing the medium every 3 days.[5c] Cells were maintained as described above and the cytotoxicity assay has been carried out as described for the GLI36 cell lines using two different concentrations of **C1**, PTX and TMZ (50 μM or 0.05 μM).