Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

# Antiproliferative activity of Juglone derivatives on rat glioma

Valeria Pavan, Giovanni Ribaudo, Maira Zorzan, Marco Redaelli, Raffaele Pezzani, Carla Mucignat-Caretta & Giuseppe Zagotto

**To cite this article:** Valeria Pavan, Giovanni Ribaudo, Maira Zorzan, Marco Redaelli, Raffaele Pezzani, Carla Mucignat-Caretta & Giuseppe Zagotto (2016): Antiproliferative activity of Juglone derivatives on rat glioma, Natural Product Research, DOI: 10.1080/14786419.2016.1214830

**To link to this article:** http://dx.doi.org/10.1080/14786419.2016.1214830

NATURAL PRODUCT RESEARCH, 2016
http://dx.doi.org/10.1080/14786419.2016.1214830

Taylor & Francis
Taylor & Francis Group

# Antiproliferative activity of Juglone derivatives on rat glioma

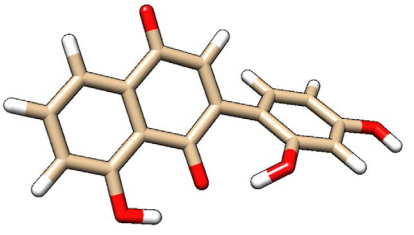
Valeria Pavan[a], Giovanni Ribaudo[a], Maira Zorzan[b], Marco Redaelli[b,d], Raffaele Pezzani[c,d], Carla Mucignat-Caretta[b] and Giuseppe Zagotto[a]

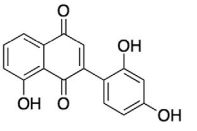
[a]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy; [b]Department of Molecular Medicine, University of Padova, Padova, Italy; [c]Department of Medical and Surgical Sciences-Operative Unit of Endocrinology, University of Padova, Padova, Italy; [d]AIROB, Italian Association for Basic Oncological Research, Padova, Italy



**ABSTRACT**

Malignant gliomas are aggressive and life-threatening tumours that still show a poor prognosis: the current therapeutic approach based on surgical resection and chemotherapy combined with radiotherapy does not provide a satisfactory chance of long-term survival to patients. Natural bioactive compounds represent a precious source of molecules with antiproliferative activity, potentially effective also against glioma cells. Among these, Juglone is a known allelopathic compound extracted from the eastern black walnut (*Juglans nigra*) whose antimitotic effect has been extensively described in mammalian cells. We investigated the antiproliferative effect of a synthetic derivative of this natural compound, 2-(2,4-dihydroxyphenyl)-8-hydroxy-1,4-naphthoquinone (DiNAF), in rat glioma cells. We compared this molecule and its effect with the natural reference compound and with newly synthesised derivatives to build a preliminar structure–activity relationship. Biological assays and NMR-based redox experiments confirmed that DiNAF is a promising lead and supported the hypothesis of a redox mechanism underlying its cytotoxic activity.






DiNAF, 1

**ARTICLE HISTORY**
Received 16 February 2016
Accepted 27 June 2016

**KEYWORDS**
Glioma; Juglone; TMZ; naphthoquinones

## 1. Introduction

Malignant gliomas are characterised by a high tendency to infiltrate through the brain parenchyma and a high resistance to the standard anticancer therapies (Capper & Reifenberger 2015).

**CONTACT** Giuseppe Zagotto giuseppe.zagotto@unipd.it

Supplemental data for this article can be accessed here at http://dx.doi.org/10.1080/14786419.2016.1214830.

The current FDA-approved therapeutic approach consists in neurosurgical resection, followed by chemotherapy with temozolomide (TMZ) in combination with radiotherapy (Stupp et al. 2005), though only the 26% of treated patients reach 2 years of survival (Preusser et al. 2011). Juglone is a natural compound acting as growth-stunting agent (Kiran Aithal et al. 2009). It can be extracted from *Juglans nigra* (Juglandaceae family) and it has been also associated with the induction of oxidative stress in both healthy and cancerous mammalian cells (Öllinger & Brunmark 1991; Xu et al. 2012). Thus, it is known that naphthoquinones may induce cytotoxicity through two main mechanisms: redox cycling and reactivity towards nucleophiles. Concerning redox activity, we recently studied, in accordance with the work of other research groups, the influence of the redox properties of some Juglone derivatives (such as DiNAF, compound 1) and other compounds of the same class on their cytotoxic activity, also towards glioma cells (Zagotto et al. 2011; Chen et al. 2012; Redaelli et al. 2015; Yang et al. 2014). On the other hand, for what concerns electrophilic reactivity, it has been observed that naphthoquinones can react with thiols and amines to give covalent adducts inside the cell, depending on the electrophilicity of C-3. Moreover, it cannot be ruled out that both the mechanisms contribute to developing the cytotoxic effect (Klotz 2014). To confirm our observations and to push our search for novel therapeutic leads against glioma to a further level, we prepared a small set of 1,4-naphthoquinone and DiNAF derivatives, with focused modifications on the scaffold to build a preliminary structure–activity relationship. We also extended the investigation of the antiproliferative effects to the F98 rat glioma cell line, a widely accepted cellular model of glioma (Barth & Kaur 2009), comparing the performance of the synthesised compounds with the effects of Juglone, TMZ and paclitaxel (PTX).

Although it is generally accepted that naphthoquinones can promote cell death inducing oxidative stress (Öllinger & Brunmark 1991; Chen et al. 2012; Xu et al. 2012; Yang et al. 2014), we recently highlighted the relevance of the kinetic aspect of this phenomenon. In fact, we preliminary demonstrated that in solution, even in the presence of an excess of $Na_2S_2O_4$, 3-(2,4-dihydroxyphenyl)naphthalene-1,4,5-triol, the non-toxic, reduced form of compound 1, has a high tendency to quickly reconvert to its oxidised, cytotoxic form (Figure 1), while juglone promptly undergoes a non-reversible reduction to the non-toxic hydroxy naphthalene under the same conditions (Redaelli et al. 2015). To better describe this observation, we designed an NMR-based experiment to study the reduction kinetic of this and similar naphthoquinones in the presence of 1 equivalent of $Na_2S_2O_4$.

## 2. Results and discussion

The new approaches to target glioma cells exploit their action through different mechanisms, such as the activation/inhibition of specific receptors, by interfering with cellular pathways

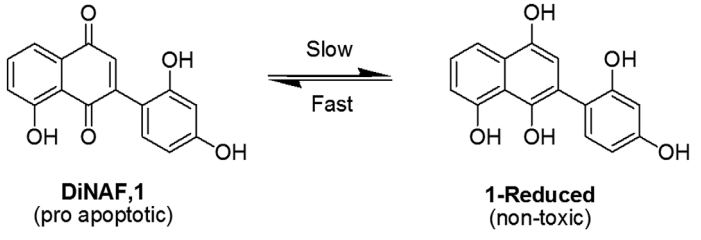



Figure 1. Redox and toxicity properties of compound 1.

or gene expression, or by influencing redox activities (Jung et al. 2012; Phillips et al. 2012; Yang et al. 2014). With this work, we provide further evidence that the redox reactivity parallels with the cytotoxic effect, at least in some of the cases. We prepared a small, focused set of compounds sharing the 1,4-naphthoquinone scaffold and showing light modifications in the substituents (Figure 2). The number and the positioning of the hydroxyl groups on both the naphthoquinone scaffold and on the phenyl ring were modified, and the effect of the introduction of an electron-withdrawing nitro group was also evaluated.

We investigated the redox properties of some of the compounds by measuring their degree of reactivity towards $Na_2S_2O_4$, a reducing agent. We previously demonstrated the high tendency of the reduced, non-toxic, form of compound 1, 3-(2,4-dihydroxyphenyl)naphthalene-1,4,5-triol, to be promptly reoxidised to its parent compound in solution and we show here that the opposite reaction, i.e. the reduction from the naphthoquinone to the hydroxynaphtalene form, is the slowest when compared to the behaviour of the other tested compounds (for a detailed review on the electrochemical properties of quinones see Guin 2011).

We designed this NMR-based experiment to highlight the role that the number and position of electron donor groups (hydroxyls) have in redox cycling, in contrast with the effect of the deactivating nitro group that we inserted in compound 6. Curiously, most of the compounds, including Juglone and compound 6, were promptly converted to the reduced form just after the addition of the $Na_2S_2O_4$ solution. Compounds 1 and 5 showed otherwise a lower tendency to be converted to the hydroxy naphthalene form, with 1 demonstrating a $t_{1/2}$ three times longer than the one of 5. Compound 5 anyway showed a curious degree of conversion to the reduced form just after the addition of $Na_2S_2O_4$ (see Supplementary Material for representative spectra and reduction profiles). Even if a more evidently different trend for the behaviour of the compounds with an electron-rich scaffold and compound 6 may have been expected, this observation anyway suggests that, among the screened compounds, compound 1 performs peculiarly and shows the highest tendency to remain in solution in its oxidised, toxic form.

MTT-based cell viability assay on F98 rat glioma cells reported a significant reduction in cell viability induced by 5 and 0.5 μM DiNAF (1), compared to the same concentrations of Juglone, TMZ and PTX (Figure 3(A)).

Morphological analysis of F98 cells treated with DiNAF (1) showed a statistically significant increase in apoptosis (Figure 3(B)), which was noteworthy compared to the results obtained for Juglone, TMZ and PTX. The difference was found to be significant at all tested concentrations. Comparison between DiNAF (1), Juglone, TMZ and PTX also showed that the necrosis

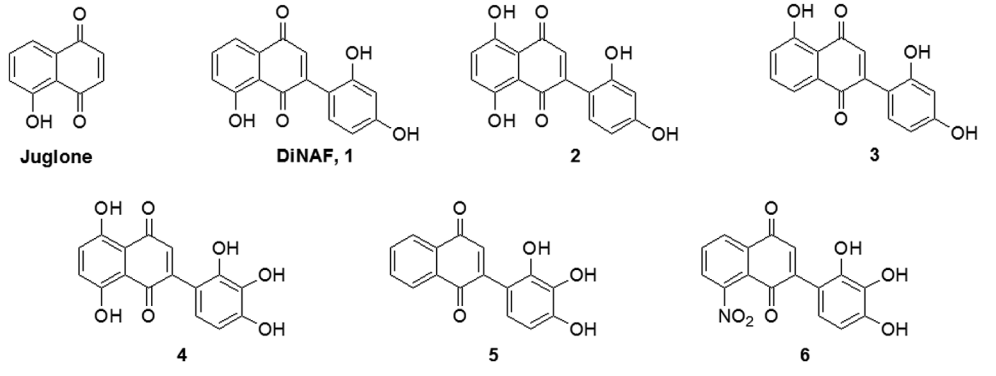



**Figure 2.** Structures of the studied compounds.

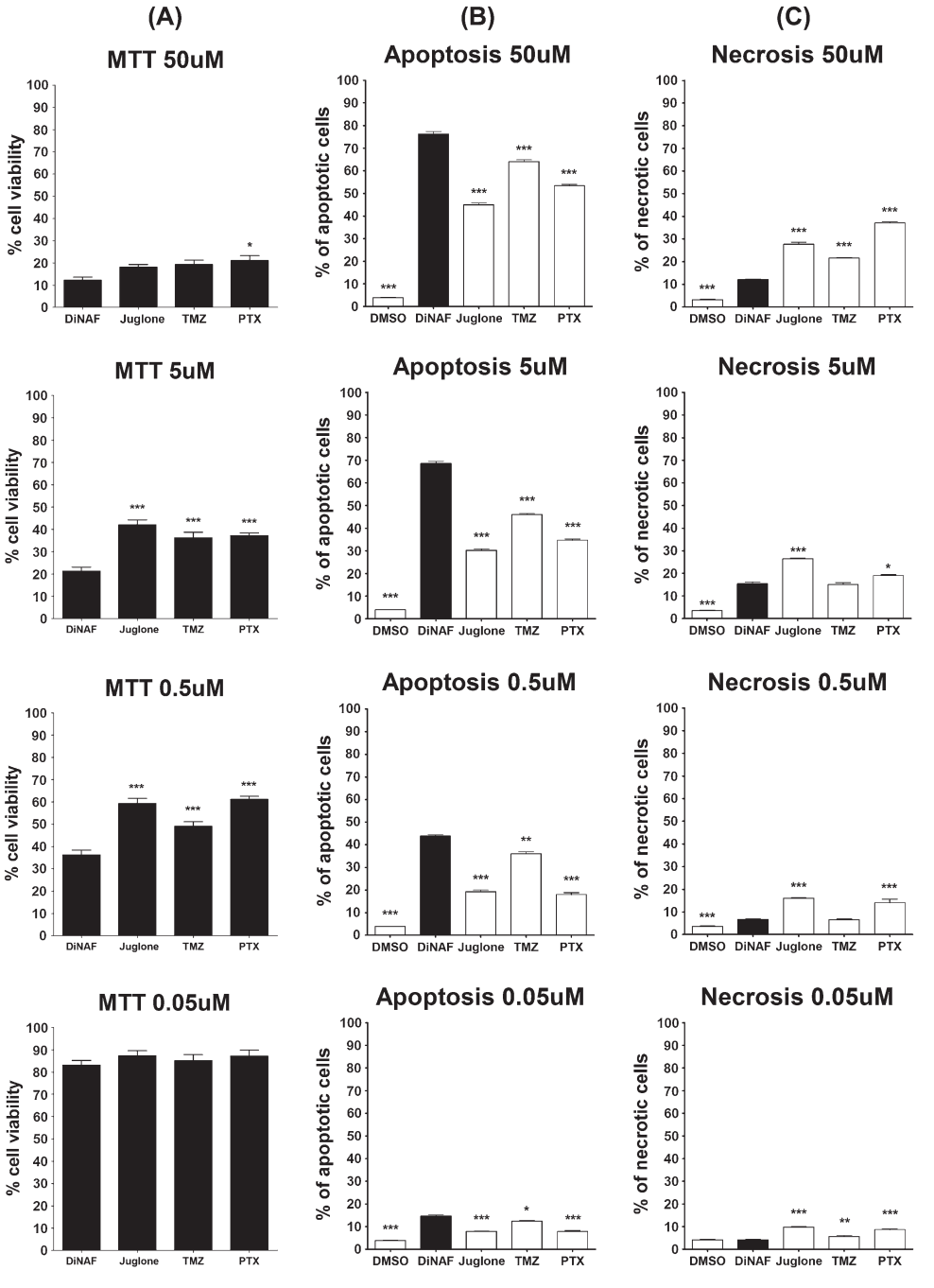



**Figure 3.** A MTT assay results for F98 cells treated with DiNAF (1), Juglone, TMZ and PTX. *t*-Test compares DiNAF (1) with other treatments *$p < 0.05$, ***$p < 0.001$. Morphological analysis of F98 cells treated with DiNAF (1), Juglone, TMZ and PTX: percentages of apoptotic (B) and necrotic (C) cells (Mean ± SEM). *t*-Test compares DiNAF (1) with other treatments *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

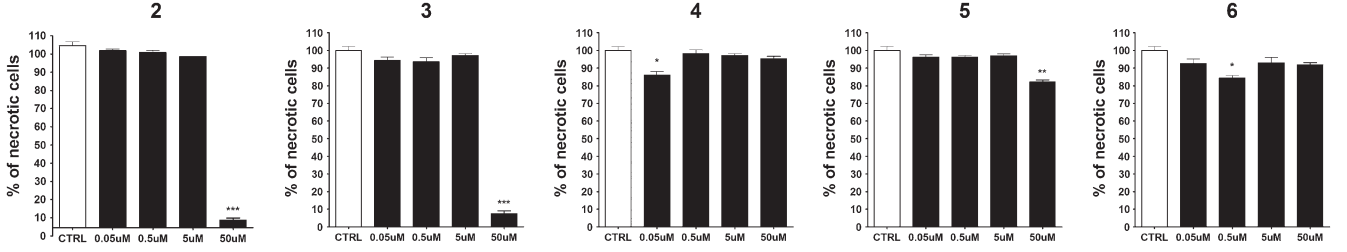



**Figure 4.** MTT assay results for F98 cells treated with compounds 2–6. Histograms represent the percentages of cell viability (Mean ± SEM). *t*-Test compares treatment concentration levels with shame control $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

(Figure 3(C)) induced by DiNAF (1) is significantly reduced at the highest and the lowest concentrations (50 and 0.05 μM) in comparison with TMZ. In addition, necrosis induced by DiNAF (1) resulted to be significantly lower for all the tested concentrations in comparison with PTX and Juglone.

The other synthetic Juglone derivatives sharing the same naphthoquinone scaffold (compounds 2–6) were tested for their cytotoxic effect on F98 cells. A significant reduction in cell viability compared to control cells was observed only at the highest concentration (50 μM) of compounds 2, 3 and 5 (Figure 4).

Preliminary *in vivo* experiment were carried out on three adult Wistar rats, after 3, 7 and 10 day animals did not show any neurological alteration related to DiNAF brain injection.

The biological results, combined with those observed from the NMR experiments, suggest that a lower tendency of DiNAF (1) to undergo reduction is accompanied by a higher reduction in glioma cell viability and a more efficient pro-apoptotic effect compared to Juglone and the other derivatives.

## 3. Conclusion

We prepared a set of naphthoquinones with the aim of describing their ability of reducing glioma cells viability. 2-(2,4dihydroxyphenyl)-8-hydroxy-1,4-naphthoquinone (DiNAF, 1) represents the most promising compound of the series, confirming our preliminary observation in other cellular models. The biological results supported those observed from NMR experiments, strongly suggesting the influence of redox kinetics in exploiting the toxic effect. Moreover, DiNAF (1) showed a significantly increased apoptosis induction *in vitro* and cell viability reduction on rat glioma cells when compared with Juglone, TMZ and PTX. Interestingly, small focused modifications in DiNAF (1) resulted in a decreased cytotoxicity, confirming the peculiar features of the lead derivative. The results reported in this study therefore prompted further investigation including other cell lines and using animal models in order to evaluate the tolerability and the viability of treatment based on DiNAF (1) or on novel Juglone derivatives, whose testing is currently being carried out.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

The research project was financially supported by University of Padova.

## References

Barth RF, Kaur B. 2009. Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. J Neurooncol. 94:299–312.

Capper D, Reifenberger G. 2015. Classification of gliomas: current progress and perspectives. Nervenarzt. 86:672–683.

Chen C, Lin M, Ong P, Yang J. 2012. Novel multiple apoptotic mechanism of shikonin in human glioma cells. Ann Surg Oncol. 19:3097–3106.
Guin PS, Das S, Mandal PC. 2011. Electrochemical reduction of quinones in different media: a review. Int. J. Electrochem. Article ID 816202. Available from: http://dx.doi.org/10.4061/2011/816202
Jung J, Jung K, Kim D, Kim H. 2012. Selective inhibition of MMP-9 gene expression by mangiferin in PMA-stimulated human astroglioma cells: involvement of PI3K/Akt and MAPK signaling pathways. Pharmacol Res. 66:95–103.
Kiran Aithal B, Sunil Kumar MR, Nageshwar Rao B, Udupa N, Satish Rao BS. 2009. Cytotoxic, genotoxic and oxidative stress induced by 1,4-naphthoquinone in B16F1 melanoma tumor cells. Cell Biol Int. 33:1039–1049.
Klotz LO, Hou X, Jacob C. 2014. 1,4-Naphthoquinones: from oxidative damage to cellular and inter-cellular signaling. Molecules. 19:14902–14918.
Öllinger K, Brunmark A. 1991. Effect of hydroxy substituent position on 1,4-naphthoquinone toxicity to rat hepatocytes. J Biol Chem. 266:21496–21503.
Phillips JJ, Huillard E, Robinson AE, Ward A, Lum DH, Polley M, Rosen SD, Rowitch DH, Werb Z. 2012. Heparan sulfate sulfatase SULF2 regulates PDGFRα signaling and growth in human and mouse malignant glioma. J Clin Invest. 122:911–922.
Preusser M, de Ribaupierre S, Wöhrer A, Erridge SC, Hegi M, Weller M, Stupp R. 2011. Current concepts and management of glioblastoma. Ann Neurol. 70:9–21.
Redaelli M, Mucignat-Caretta C, Isse AA, Gennaro A, Pezzani R, Pasquale R, Pavan V, Crisma M, Ribaudo G, Zagotto G. 2015. New naphthoquinone derivatives against glioma cells. Eur J Med Chem. 96:458–466.
Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al. 2005. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 352:987–996.
Xu HL, Yu XF, Qu SC, Qu XR, Jiang YF, Sui DY. 2012. Synthesis and photoluminescence properties of low-T(g), photorefractive polymers of double function. Food Chem Toxicol. 50:590–596.
Yang J, Li Z, Wu J, Lu F, Chen C. 2014. An oxidative stress mechanism of shikonin in human glioma cells. PLoS ONE. 9. Available from: http://dx.doi.org/10.1371/journal.pone.0094180
Zagotto G, Redaelli M, Pasquale R, D'Avella D, Cozza G, Denaro L, Pizzato F, Mucignat-Caretta C. 2011. 8-Hydroxynaphthalene-1,4-dione derivative as novel compound for glioma treatment. Bioorg. Med. Chem. Lett. 21:2079–2082.