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N,N-Bis-(8-hydroxyquinoline-5-yl methyl)-benzyl substituted amines (HQNBA): Peroxisome proliferator-activated receptor (PPAR-γ) agonists with neuroprotective properties

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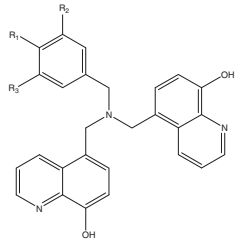
ABSTRACT

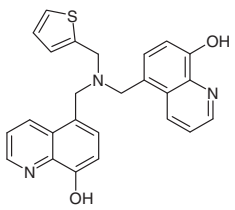
We report on the neuroprotective effects of *N*,*N*-bis-(8-hydroxyquinoline-5-yl methyl)-benzyl substituted amines (HQNBA) in a model of oxidative stress-induced nerve cell death using mouse hippocampal-derived HT22 cells. The four derivatives (JLK1472, JLK1486, JLK1522 and JLK1535) protected the HT22 cells from death at concentrations ranging from 0.1 to 1 μM. Their action is partially dependent on their ability to act as PPARγ agonists. These analogues also maintain GSH levels suggesting that they have indirect anti-oxidant effects.

8-Hydroxyquinolines represent a privileged substructure which is found in bioactive natural products.[1–3] Chemically speaking, the coupling of this 8-hydroxyquinoline substructure at the four position with benzylamine moieties or aromatic heterocycles results in chemically reactive molecules prone to fragmentation, thereby creating reactive species (quinone methide) which can undergo nucleophilic attacks from biological nucleophiles such as cysteine thiol groups, the side chain amino groups of Lys and His or amino groups in nucleosides. Moreover such structures can also strongly chelate bioinorganic cations such as Cu^{2+} and Zn^{2+}. Keeping in mind that chemical reactivity often correlates with biological activity, we have developed a chemical library focused on the *N*,*N*-bis-(8-hydroxy quinolineylmethyl)-substituted benzyl amine scaffold (HQNBA). Some analogues of this series were found to be particularly active as cytostatic anticancer agents (nanomolar range), especially on glioblastoma cells lines.[4] We have also shown that depending on the substituent R on the benzyl ring (JLK 1472, JLK1486, JLK1522) or on the substituent directly bound to the nitrogen atom (JLK1535), the compounds (Fig. 1) exert potent anti-proliferative effects on a panel of different tumour cell lines including adenocarcinoma and melanoma as well as glioma. The mechanism of action by which these compounds exert their anti-proliferative effects involves the generation of a reactive quinone methide intermediate which preferentially alkylates protein thiols.[5]

Taking into account the ability of such analogues to specifically alkylate biological thiols, it was of interest to investigate the possibility that they can modulate glutathione (GSH) levels in cells and potentially exhibit neuroprotective activity. In this manuscript, we report on the neuroprotective activity of these compounds using an in vitro model of oxidative stress-induced nerve cell death as well as their effects on GSH levels and their possible agonistic interactions with peroxisome proliferator-activated receptors



JLK 1472 $R^1 = CH_3$ $R^2 = H$ $R^3 = H$
JLK 1486 $R^1 = CF3$ $R^2 = H$ $R^3 = H$
JLK 1522 $R^1 = H$ $R^2 = CF3$ $R^3 = H$



JLK1535

Figure 1. Chemical structures of the *N*,*N*-bis-(8-hydroxyquinoline-5-yl methyl)-benzyl substituted amines (HQNBA): JLK1472, JLK1486, JLK1522 and JLK1535.

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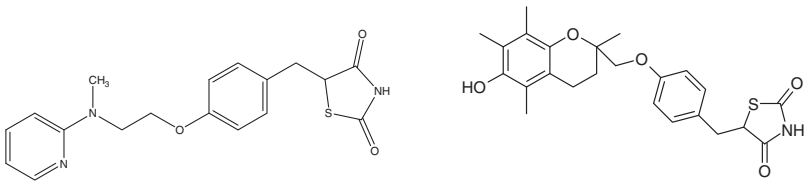

Figure 2. Glitazone structures.

(PPAR). Importantly, known PPAR-γ agonists such as glitazones (e.g., rosiglitazone, troglitazone) have been reported to have neuroprotective activity[6] and were used in this study for purposes of comparison (Fig. 2).

General methods for the preparation of the HQNBA derivatives and their identification data (NMR, mass spectrometry, centesimal analysis for compounds JLK1472, JLK1486, JLK1522 and JLK1535 have been reported and are described in Refs. 4,5. The PPARγ agonists rosiglitazone and troglitazone were from Cayman Chemical (SpiBio, France). Glutamic acid and the PPARγ antagonist GW9662 were from Sigma Chemicals (St. Louis, MO, USA).

The biological assays for the determination of neuroprotective effects including cell culture (HT22), cytotoxicity assay, total intracellular glutathione determination have been reported in Ref. 9 and references cited therein.

The HQNBA derivatives were tested in a cell culture-based model of neurodegeneration using the mouse hippocampal cell line HT22. Treatment of these cells with glutamate induces a sequence of biochemical events including modulation of cystine uptake, loss of intracellular glutathione (GSH), increased reactive oxygen species (ROS) production and, eventually, cell death via a well defined programmed cell death pathway.[7]

Half-maximal effective concentrations (EC_{50}) for protection for the HQNBA derivatives were determined by exposing the HT22 cells to different doses of each derivative in the presence of 5 mM glutamate for 24 h.[8] All of the derivatives had EC_{50}'s below 1 μM in this assay with maximal neuroprotective effects seen at 0.5–1 μM (Table 1 and Fig. 3). The neuroprotective effects of the HQNBA derivatives were found to be similar to that of glitazones such as troglitazone (Fig. 3), known to be PPARγ agonists with neuroprotective activity.[6]

For all of the derivatives, a monophasic response was observed such that increasing doses induced a level of cellular response which reached a plateau. It should be noted[11] that the most general schemes for compounds exhibiting this class of response often fall into one of three classes: (I) regulation of enzyme activity, (II) ligand interaction with one type of receptor or (III) ligand interaction with negatively cooperative receptors.

Since it is known from the literature that PPAR receptor agonists and, more specifically, isoform γ receptor agonists have neuroprotective effects against oxidative insults[12] and therefore could be useful for the treatment of neurodegenerative diseases,[13] we first investigated if the observed neuroprotective activities of the HQNBA derivatives were PPARγ-receptor dependent. For this purpose, HT22 cells were first treated with the known PPARγ antagonist GW9662[14] (Fig. 4) before exposure to glutamate. As shown in Figure 5, the neuroprotective effects of the derivatives were significantly reduced while the neuroprotective effects of a novel curcumin derivative, CNB001,[15] were unaffected. These results suggest that at least a portion of the neuroprotective effects of the HQNBA derivatives are dependent on their ability to act as PPARγ agonists. This finding is consistent with the neuroprotective effects of the known PPARγ agonists, troglitazone and rosiglitazone, in this same assay (Fig. 3 and Ref. 12).

Treatment of the HT22 nerve cells with glutamate, a known excitotoxic compound, results in the loss of GSH. Surprisingly, treatment of the HT22 cells with glutamate in the presence of the HQNBA derivatives but not troglitazone resulted in the maintenance of GSH levels (Fig. 6).

This observation is important for several reasons. First, it shows that the HQNBA derivatives have some neuroprotective activities that are distinct from those of known PPARγ agonists such as troglitazone.[12] Second, it suggests that the HQNBA derivatives can act as indirect antioxidants by increasing the levels of the major endogenous intracellular antioxidant GSH under conditions of oxidative stress, a mechanism distinct from that of classical antioxidant compounds.

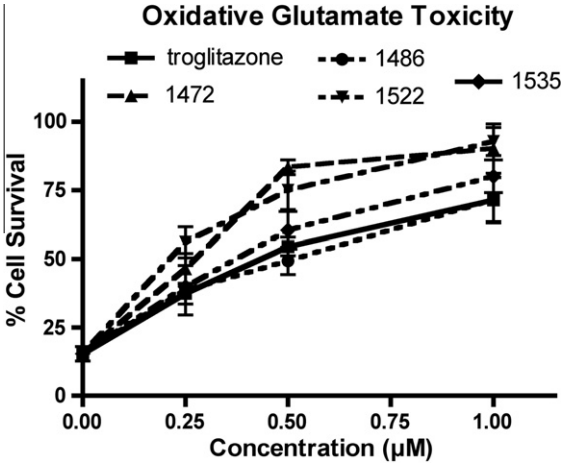

Figure 3. In vitro dose–response curves for the neuroprotective effects of HQNBA derivatives (see Ref. 10 for experimental details).

Table 1
Neuroprotective effects of HQNBA derivatives

	EC_{50} HT22/glutamate (nM)	% Max. protection	% Toxicity @ 1 μM
1472	125	100	39.4
1486	621	100	33.7
1522	168	99	40.7
1535	383	97	34.0

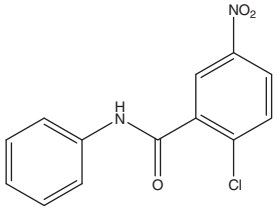

Figure 4. GW9662 (PPAR antagonist).

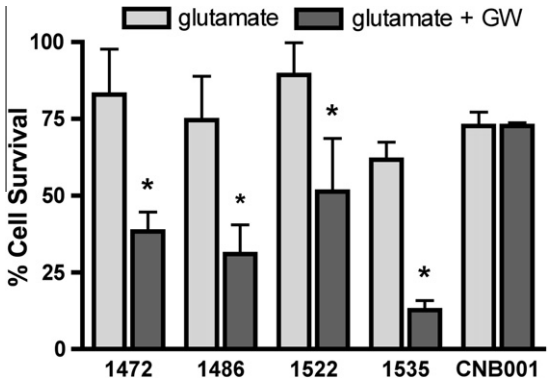

Figure 5. Effect of PPARγ antagonist GW9662 on HT22 cell survival (see Ref. 16 for experimental details).

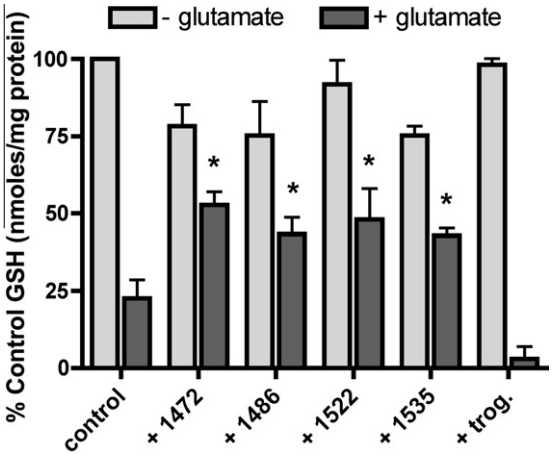

Figure 6. HQNBA derivatives maintain GSH levels in HT22 cells (see Ref. 17 for experimental details).

Together these data strongly suggest that the new derivatives, JLK1472, JLK1486, JLK1522 and JLK1535, do not act as direct anti-oxidants, but rather as inducers of anti-oxidant and/or neuroprotective protein synthesis. These analogues may be of particular therapeutic interest for the treatment of neurological disorders because they are very efficacious with EC_{50} values ranging from 125 to 621 nM, they are able to maintain intracellular GSH levels and they at least partly represent new PPARγ agonists. Although the mechanism by which these new derivatives exert their neuroprotective effects appears complex, this may be important for the treatment of diseases that are multi-factorial. Given their potent effects in cell-based assays, further exploration of the potential neuroprotective effects of these derivatives in animal models of neurological disorders is clearly warranted.

Acknowledgements

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10. Dose dependence of protection from oxidative glutamate toxicity. HT22 cells were treated with 5 mM glutamate for 24 h alone or in the presence of increasing concentrations of the HQNBA derivatives or troglitazone. The % survival was measured by the MTT assay. Similar results were obtained in 3–5 independent experiments. All derivatives provided significant protection (p <0.001) from oxidative glutamate toxicity.
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16. HT22 cells were treated with 5 mM glutamate for 24 h in the presence of 1 μM of the HQNBA derivatives (JLK1472, JLK1486, JLK1522 and JLK1535) or CNB001 or the compounds plus 10 μM GW9662. The % survival was measured by the MTT assay and compared to that obtained with untreated cells and with glutamate alone (10 ± 5%). GW9662 significantly (p <0.05) reduced protection by the HQNBA derivatives but not by CNB001. Similar results were obtained in 3–4 independent experiments.
17. HT22 cells were treated with 5 mM glutamate for 24 h alone or in the presence of 1 μM of the HQNBA derivatives or troglitazone. Cell extracts were prepared and assayed for GSH by a chemical assay. Similar results were obtained in three independent experiments. All derivatives significantly increased (p <0.05) GSH levels in the presence of glutamate.