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Natural products inspired synthesis of neuroprotective agents against H_2O_2-induced cell death

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ABSTRACT

Stroke is a debilitating disease and the third leading cause of death in the USA, where over 2000 new stroke cases are diagnosed every day. Treatment options for stroke-related brain damage are very limited and there is an urgent need for effective neuroprotective agents to treat these conditions. Comparison of the structures of several classes of neuroprotective natural products such as limonoids and cardiac glycosides revealed the presence of a common structural motif which may account for their observed neuroprotective activity. Several natural product mimics that incorporate this shared structural motif were synthesized and were found to possess significant neuroprotective activity. These compounds enhanced cell viability against H_2O_2 induced oxidative stress or cell death in PC12 neuronal cells. The compounds were also found to enhance and modulate Na^+/K^+-ATPase activity of PC12 cells, which may suggest that the observed neuroprotective activity is mediated, at least partly, through interaction with Na^+/K^+-ATPase.

Stroke is a major health concern in the industrialized countries. It is the third leading cause of death in the USA, ranking after heart disease and cancer.[1] It is also the leading cause of long term disability, resulting in enormous financial burden on affected families and on the health care system. Stroke-related healthcare costs are rising, with nearly 1 million hospitalizations each year in the U.S. and an estimated direct medical cost of 25.2 billion for the year 2007.[2] Currently available treatment options for stroke-related brain damage are limited. The only FDA approved drug for acute ischemic stroke, IV recombinant tissue plasminogen activator (rt-PA), unfortunately can be received by only 5–10% of acute ischemic stroke (AIS) patients because of the less than 3 h restrictive therapeutic time window within which it must be administered.[3] Therefore, there is an urgent need for identification of new neuroprotective drug candidates and drug targets for treatment of ischemic stroke.

Natural products are a prolific source of bioactive agents of diverse structure and varying biological activity. They are the single most productive source of lead molecules for development as clinically useful drugs for human disorders. In the search for neuroprotective agents from natural sources, a number of plant extracts and several natural products isolated from them have been reported to provide neuroprotection against ischemic stroke.[4–6] In addition, a wide range of natural product derivatives and natural product mimics of synthetic origin such as estrogen-like compounds,[7] kavalactone derivatives,[8] glucose-containing flavones,[9,10] arylidenepregnenolone derivatives,[11] lignophenol derivatives,[12] triazine derivatives,[13] pyrazolyl-2,4-thiazolidinedione derivatives,[14] phenolic thiazoles,[15] and indole derivatives[16] have been shown to protect neurons against oxidative damage induced by H_2O_2 or by other oxidative stress conditions. Among neuroprotective natural products, a group of naturally occurring limonoids dictamnusine **1**, dictamdiol **2**, fraxinellone **3**, calodendrolide **4**, obacunone **5** and limonin **6** (Fig. 1) isolated from *Dictamnus dasycarpus* were reported to have significant neuroprotective activity against glutamate-induced neurotoxicity in rat cortical cells.[5] Obacunone **5** was also reported to be protective against glutamate-induced oxidative damage in mouse hippocampal HT22 cells.[17] Further, it induced cell resistance to oxidative injury from glutamate-induced cytotoxicity in HT22 cells, presumably mediated through p38 MAPK pathway-dependent heme oxygenase-1 expression.[17]

The cardiac glycoside neriifolin **7** isolated from the yellow oleander *Thevetia neriifolia* and its 16-β-acetoxy derivative oleandrin,

Abbreviations: IV, intravenous; FDA, food and drug administration; AIS, acute ischemic stroke; rt-PA, recombinant tissue plasminogen activator; NMR, nuclear magnetic resonance; NOE, nuclear overhauser effect; *m*-CPBA, *meta*-chloroperoxybenzoic acid; ORTEP, oak ridge thermal ellipsoid plot; ROS, reactive oxygen species; GSH, glutathione; Pi, inorganic phosphate; TLC, thin layer chromatography; HRMS, high resolution mass spectrometry.

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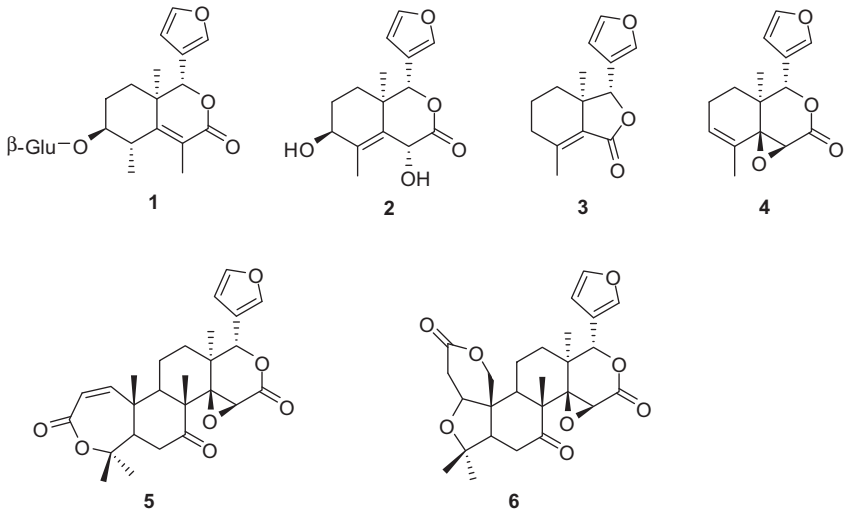

Figure 1. Neuroprotective limonoids.

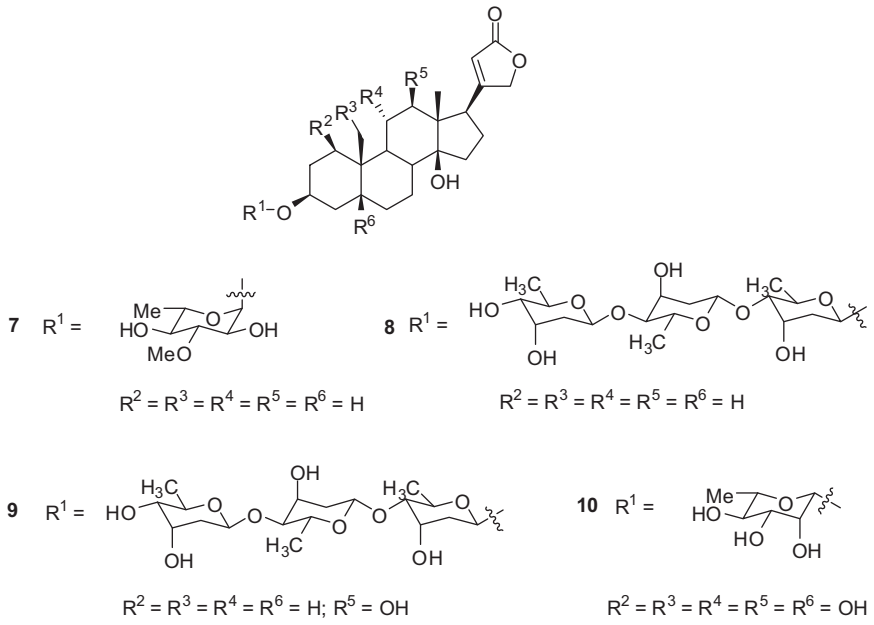

Figure 2. Neuroprotective cardiac glycosides.

and to a lesser extent the glycosides digitoxin **8**, digoxin **9** and ouabain **10** (Fig. 2), provided protection against neuronal cell death induced by oxygen and glucose deprivation in a brain slice assay, an effect believed to be mediated through their putative target the Na^+/K^+-ATPase.[6,18] Sublethal concentrations of ouabain has been shown to have neuroprotective activity against excitotoxicity mediated neuronal injury and this effect has been attributed to intracellular cascades linked to ouabain interaction with Na^+/K^+-ATPase leading to modulation of subcellular Bcl-2 levels.[19] However, cardiac glycosides are substrates for P-glycoprotein, which precludes their usefulness as therapeutics for ischemic stroke, as effective penetration of the blood brain barrier to build up therapeutic levels of the drugs within the CNS is inhibited.[6] Nevertheless, they may serve as lead molecules for designing more effective drug candidates.

A comparison of the structures of these naturally occurring neuroprotective molecules revealed that they share a common bicyclic ring system with a five-membered heterocycle (furanyl or γ-lactone) side chain and with or without an oxygen function at the ring junction (Fig. 3). Intrigued by the presence of this common structural motif in neuroprotective natural products, we designed and synthesized several structural congeners of this unit to explore their neuroprotective properties. The molecules designed for synthesis are shown in Figure 4.

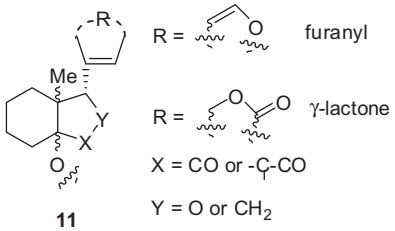

Figure 3. Structural motif shared by neuroprotective natural products.