Consiglio Nazionale delle Ricerche
Istituto di Chimica Biomolecolare

Pozzuoli (Napoli), 05th August 2014

SCIENTIFIC REPORT Prof. MARIA JESUS OSET GASQUE
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Avarol and avarone, two sesquiterpenes (hydroquinone and quinone, respectively) possessing a rearranged drimane skeleton, were isolated from the marine sponge *Dysidea avara* Schimdt. Previous studies have revealed that these secondary metabolites show a wide variety of biological activities such as antibacterial, antifungal, antiviral, cytotoxic, antioxidant, anti-inflammatory and anti-psoriatic effects. Recent findings indicate that some thio-avarol derivatives exhibit acetylcholinesterase (AChE) inhibitory activity (1). The abnormal activity of this enzyme is one important factor responsible for Alzheimer's disease, the most common cause of senile dementia in later life. The multiple pharmacological properties of **avarol, avarone** and/or their derivatives prompted us to continue with the in vitro screening of the bioactivity noted focusing on AChE inhibitory. During the scientific stay of Prof. M.J.Oset Gasque we performed the synthesis of new compounds starting from the chemical structure of avarol. In particular, we synthesized **N.4 prenyl-hydroquinons** (Fig.1) having a lateral carbon chain with 5, 10, 15 and 20 carbon atoms and an hidroquinone ring with a thio-functional group (thiosalicylic acid). All synthetic compounds were tested according the spectrophotometric method of Ellman using AChE (from *Electrophorus electricus*, Sigma-Aldrich, Milan, Italy). The final volume of reaction was 0.5 mL, containing 0.875mM of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and 0.035 U of AChE in 0.1M phosphate-buffered solution pH 8. The mixture was incubated with eight different concentrations of compounds (from 1 μM up to 50 μM) for 10 min. After this time, the substrate (0.35 mM AcTCho) was added to the mixture. The absorbance was registered at 405 nm in a spectrophotometer plate reader. Galanthamine was used as a standard, while the mixture without compounds tested was used as a control (100% enzymatic activity).

Preliminary EeAChE inhibition results with the assayed compounds suggest that the most potent inhibitory compound was Q20Phy, with a similar cholinesterase inhibitory capacity to that of AvaTio (Fig. 2), followed by Q5 and Q10 at approximately the same potency. Q15 and Q20GG were found to be the least potent molecules.

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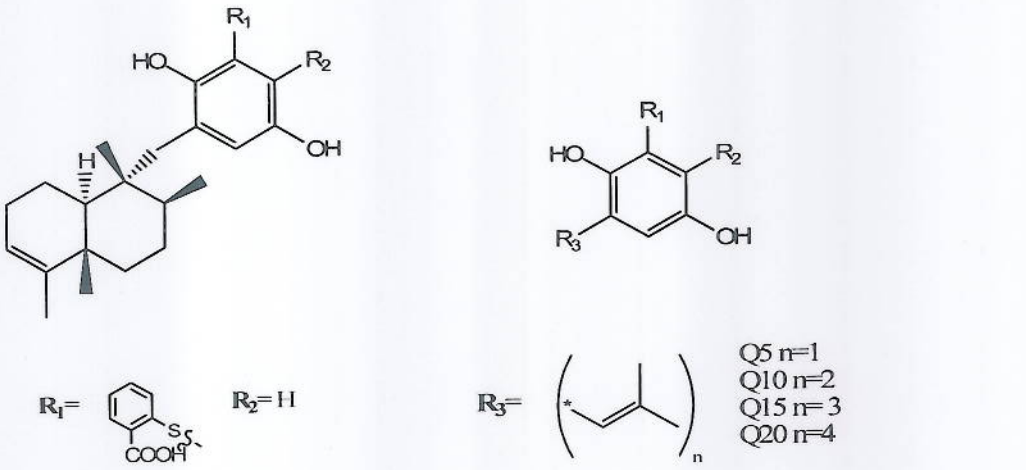

Thiosalicylic Avarolo (AvaTio) Thiosalicylic Prenyl hydroquinones

Figure 1: Chemical structures of tested synthetic compounds

However, further experiments are currently being performed in Dr. María Jesús Oset's laboratory in order to test the anti-cholinesterase activity of these compounds against hAChE and buAChE, as well as to determine their IC50 and anti-cholinesterase mechanism. After describing and quantifying the anti-cholinesterase activity of these compounds, neuroprotection experiments will be undertaken in the aforementioned laboratory in the context of Alzheimer's disease, namely primary cortical cell cultures exposed to B-amyloid (AB 1-40), okadaic acid (tau phosphorylation) or a cocktail of oligomycine and rotenone (oxidative stress). Thus, neuronal survival will firstly be assayed by means of the XTT test (2,3). Accordingly to the obtained results, the anti-apoptotic and anti-necrotic properties of the selected compounds will be assayed through the caspase-3 activity and the LDH release tests, respectively (3,4). Simultaneously, experiments looking at the neurotoxicity and hepatotoxicity of the compounds will be conducted in human SH5YSY neuroblastoma cells and human hepG2 hepatoma cells by means of techniques established in Dr. María Jesús Oset's laboratory. Taken together, these results will enable us to characterise the neuroprotective potential of these molecules in neurodegenerative diseases, and correlate their effect with their anti-cholinesterase activity. This project will allow Dr. Oset's and Dr. Tommonaro's groups to collaborate with each other for the benefit of both laboratories in the field of pharmaceutical enterprises.

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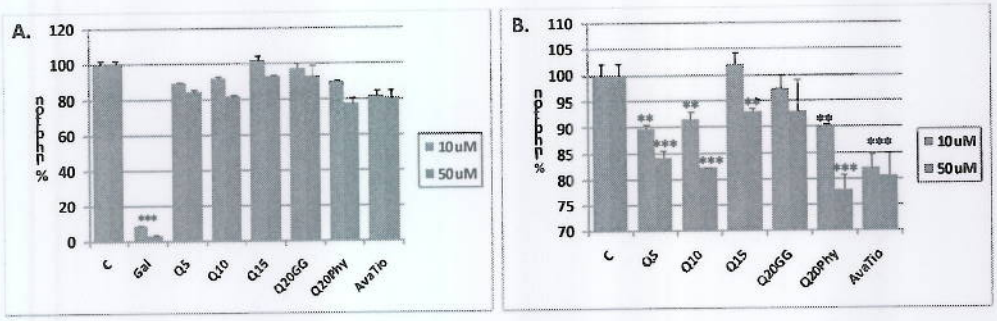

Figure 2: Inhibition of EeAChE by N4 Prenyl-Hydroquinones.

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