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procedures and their copper(II) complexes were prepared. These ligands present high stability constants for copper(II), as determined by potentiometric methods. The superoxide scavenging activity of the complexes was studied using two different methods: the nitroblue tetrazolium reduction and the dihydroethidium oxidation. Cu(II)-L1 and Cu(II)-L3 have shown the ability to scavenge $O_2^{\cdot -}$, with IC_{50} values in the low micromolar range. Cu(II)-L3 presented the lowest IC_{50}. The cytotoxicity profiles of the complexes were evaluated in V79 Chinese hamster cells, using the MTT assay. The complexes were not considerably toxic up to 100 μM, with the exception of Cu(II)-L2. Among the complexes studied, Cu(II)-L3 presents a number of important characteristics. It has an effective superoxide scavenging activity, a high stability constant and a low cytotoxicity, appearing to be a promising superoxide scavenger.

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P-044

Decrease of GLUT1-mediated glucose uptake in endothelial cells in response to oxidative stress

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Increased oxidative stress is implicated in the pathogenesis of diabetic retinopathy. The goal of this study is to assess the regulation of glucose transport by oxidative stress. Retinal endothelial cells were subjected to oxidative stress by incubation with glucose oxidase. Protein carbonyl formation was used as an indicator of oxidized proteins. GLUT1 mRNA levels were determined by real-time RT-PCR. GLUT1 protein levels were detected following biotinylation of the membrane proteins. The glucose transport activity was measured by 3H-DOG uptake. Incubation of endothelial cells with glucose oxidase leads to an accumulation of oxidized proteins. Oxidative stress induces a decrease in the GLUT1 mRNA and protein levels. Significantly, glucose transport is decreased in oxidative stress. This result is in agreement with the decreased expression of the protein at the plasma membrane as well as with its decreased halflife. The inhibition of proteasome upon oxidative stress restores glucose transport to basal levels. In conclusion, the data suggest that sub-cellular redistribution of GLUT1 under conditions of oxidative stress contribute to disrupt glucose homeostasis in diabetes.

P-045

Determination of 3-N-tyrosine in human saliva by high-performance liquid chromatography (HPLC) with electrochemical detection.

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3-Nitrotyrosine (Ntyr) is considered as a biomarker of the generation of reactive nitrogen species (RNS). However, it is still difficult to determine its concentration in biological samples, in particular in saliva. Saliva is the first barrier against free radicals in the human organism and the determination of salivary Ntyr could tell us how saliva deals with nitric oxide-mediated damage. High performance liquid chromatography with electrochemical detection (HPLC-ECD) offers an attractive alternative to measurement of protein oxidation and nitration products. To develop a reliable and high-throughput method, we optimized the conditions for HPLC-ECD. The preparation of human saliva samples consisted of incubation with Fenton reagent, protein precipitation, enzymatic digestion and Ntyr determination by HPLC-ECD. The best separation of Ntyr was achieved using a highly acidic mobile phase (pH 3.1). Our protocol is suitable for analysing saliva samples to study RNS production.

P-046

Lipid peroxidation inhibition, free radical scavenging activity and electrochemical behaviour of a dihydroxylated di(hetero)arylamine

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The skin provides the first line of defence against oxidative damage induced by environmental factors, having an elaborated antioxidant system designed to deal with free radicals and oxidative stress. However, under severe stress conditions this biological response is not sufficient, leading to oxidative damage and, in consequence, to skin disorders, immunosuppresion, premature skin ageing and ultimately cancer. In these circumstances, antioxidants may play an essential role in enhancing the antioxidant system and thus preventing carcinogenesis. Considering treatment limitations and the high number of cancer patients, the development of new therapeutic strategies are urgently required. In this study, the antioxidant properties of ethyl 3-(2,4-dihidroxyphenylamino)benzo[*b*]thiophene-2-carboxylate, synthesized by us, were evaluated through their lipid peroxidation inhibition capacity, free radical scavenging activity and electrochemical behaviour. The chemical assays gave the following EC_{50} values: 211 μM for reducing power and 145 μM for radical scavenging activity of DPPH radicals (under the same conditions, the EC_{50} values for α-tocopherol were 158 and 92 μM). The biochemical assays used as models for the lipid peroxidation damage in biomembranes revealed the following EC_{50} values: 44 μM for inhibition of β-carotene bleaching in the presence of linoleic acid radicals (6 μM for α-tocopherol), 99 μM for inhibition of erythrocytes haemolysis mediated by peroxyl radicals (16 μm for α-tocopherol) and 63 μm for inhibition of thiobarbituric acid reactive substances (TBARS) formation in brain cells (11 μM for α-tocopherol). Cyclic voltammetry of the compound in acetonitrile/Pt electrode, at fast scan rates, showed an irreversible oxidation system with three anodic peaks at $E_{a1} = 0.82$ V, $E_{a2} = 1.59$ V and $E_{a3} = 1.77$ V. After the first scan a new oxidation/reduction system appears at lower potentials, $E_{a4} = 0.16$ V and $E_{c4} = 0.05$ V, that increases in intensity with the first five scans. At slow scan rates, below 0.1 V/s, this new system is not observed, pointing out a slow homogenous reaction after the first electron transfer.

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P-047

Electrochemical study of diarylamines in the benzo[*b*]thiophene series

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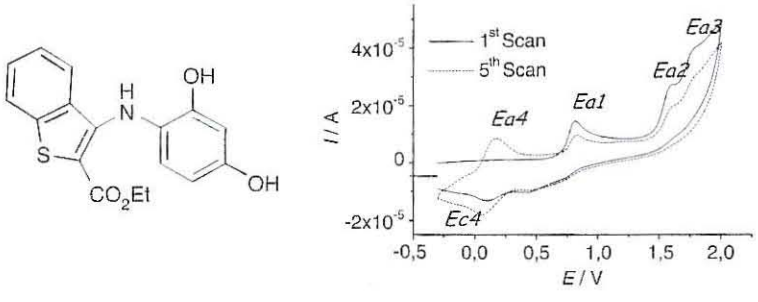

Figure 1.