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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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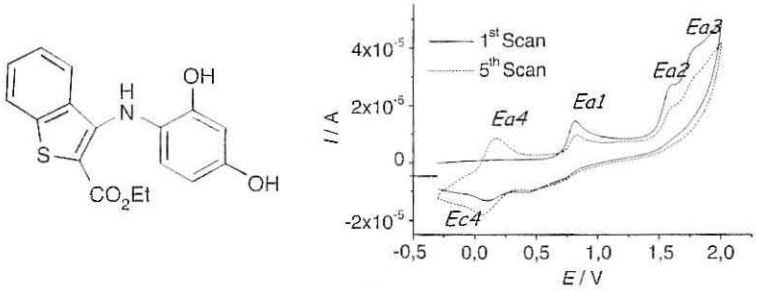

Figure 1.

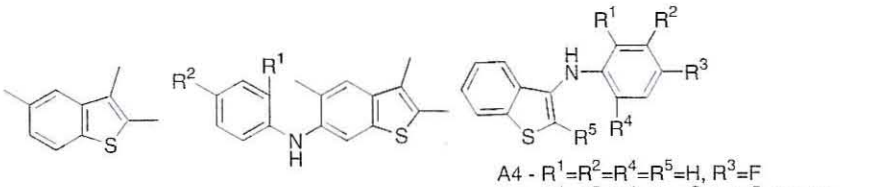

Figure 2.

The search for new molecules with antioxidant properties is a very active domain of research, since they can protect the human body from free radicals and retard the progress of many chronic diseases, such as vascular diseases, some forms of cancer and oxidative stress responsible for DNA, protein and membrane damage. Differently substituted diarylamine derivatives of benzo[*b*]thiophene were synthesized using C–N palladium-catalysed cross-couplings [1,2]. Recently, we have described the structure–activity relationship of some diarylamines in the benzo[*b*]thiophene series as antimicrobial [3] and antioxidant agents [4]. Here we extend this evaluation to the study of their electrochemical behaviour, regarding specially the influence of the compound structure, such as the position of the arylamination, the presence of different groups on the phenyl ring (Br, F, OMe) and on the thiophene ring (H, CO_2Et). The electrochemical studies of the above compounds were achieved in acetonitrile/TBAP, with a platinum electrode. At low scan rate, the cyclic voltamogram of benzo[*b*]thiophene *A1* presents two typical irreversible oxidation processes at $E_{a1} = 1.49$ V, $E_{a2} = 1.84$ V, controlled by the diffusion of the substract. The same pattern is observed in most of the compounds studied, however at different potentials. For example, the arylamination on the electron rich thiophene ring decreases the oxidation potential (*A2* vs *A4*) and the introduction of an electron withdrawing group in the thiophene moiety, CO_2Et, increment 0.3 V in the first oxidation potential (*A4* vs *A5*). This changes will influence the reducing power, with the highest effect found for compounds with lower oxidation potentials [5].

Research Project POCI/QUI/59407/2004 (FCT-Portugal).

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P-048

The thioredoxin TRX-1 is involved in ASJ-dependent starvation stress responses in *C. elegans*

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The *C. elegans* gene *trx-1* encodes a thioredoxin that is expressed in the ASJ ciliated chemosensory neurons. We have previously shown that *trx-1* deletion mutants are short-lived, whereas animals expressing a translational *trx-1::gfp* fusion transgene are long-lived relative to wild type (WT). We report here that trx-1 participates in ASJ-dependent starvation stress responses. We find that *trx-1::gfp* expression in ASJ neurons is up-regulated in WT animals under conditions of starvation and in the developmentally arrested dauer larval stage, implicating *trx-1* in ASJ-dependent stress resistance mechanisms. Interestingly, in *daf-11* guanylyl cyclase mutants, *trx-1::gfp* expression in ASJ neurons is constitutively up-regulated, indicating that depletion of cGMP up-regulates *trx-1* expression in ASJ. Similarly, deletion of *trx-1* alters the dauer formation constitutive (Daf-c) phenotype of *daf-11* mutants, supporting the involvement of *trx-1* in ASJ-dependent starvation stress responses (e.g. dauer arrest). In addition, we observe that deletion of *trx-1* partially suppresses the Daf-c phenotype of *daf-28* insulin-like mutants, suggesting that TRX-1 affects the insulin/IGF-like signalling (IIS) pathway in ASJ neurons to promotes dauer arrest. Taken together, it seems likely that the up-regulation of *trx-1* under conditions of starvation forms part of a stress response elicited to protect *C. elegans* in harsh food-deprived environments. As a consequence, this ASJ-dependent stress response would promote longevity and dauer formation at the cost of reproduction and growth. Current efforts are directed towards studying the genetic interactions of *trx-1* with genes involved in ageing and dauer pathways to identify the upstream regulators of *trx-1* expression.

P-049

Measurement of total antioxidant status in biological fluids as indicator of the activity of the antioxidant system

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Oxidative damage has been implicated in the aetiology or pathogenesis of a large number of diseases, in tissue injury as well as in the ageing process. To reduce free radical damage, control mechanisms are present, the most important is the antioxidant system. Antioxidants are a heterogeneous group of substances such as enzymes, other proteins and a range of compounds such as vitamins, uric acid, glutathione, flavonoids, phenols and carotenoids. The antioxidant defenses interact to form an integrated system and it is of great interest to measure the total antioxidant status (TAS) as an indicator of the functioning of the entire system. We report here examples of the applicability of an assay kit to the quantitative determination of total antioxidant status in serum or plasma samples. Serum/plasma samples were collected, stored and assayed with a two-reagent colourimetric total antioxidant status assay kit. Measurements were taken at 600 nm. The linearity of the assay was 2.5 mmol/L. Measurement of TAS in geriatric ($n = 77$) and normal working ($n = 156$) populations data indicated significant reduction of serum total antioxidants in geriatrics when compared with the working age group (1.284 mmol/L vs 1.536 mmol/L, $p < 0.05$). In both groups the values were higher in males when compared with female subjects. Measurement of TAS in plasma of normal volunteers ($n = 16$) before and after 60 days of vitamin supplementation showed a statistically significant increase post-supplementation (from 1.56 mmol/L to 1.62 mmol/L). In conclusion, data show applicability of this total antioxidant status kit to the assessment of the integrated antioxidant system.

P-050

Oxidation of Tyr-Leu and Leu-Tyr: Identification of spin-trapped free radicals by tandem mass spectrometry

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Reactive oxygen species formed during oxidative stress are implicated in diseases and ageing. Being proteins, one of the oxidation targets resulting in their structural modifications and loss of function. $HO^{\bullet}$, the most reactive radical, when generated by different sources (radiolysis or metal-catalysed oxidation) leads to different intermediate free radicals and oxidation products. These free radicals have short-living time, making them difficult to detect. Spin traps, namely DMPO, form stable adducts, usually identified by EPR, with these radicals. Mass