Development of a new series of nitroimidazoles probes for oxygen tension (pO_2) measurement by ^{1}H magnetic resonance spectroscopy

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Introduction: Hypoxia is known to be an important physiological parameter in tumour development (1). A direct relationship between hypoxia and tumor aggressiveness, poor outcome (2,3) and resistance to different therapies (4,5) have been proved in different types of tumors. Consequently, a variety of methods have been proposed to measure tumour oxygenation (6). In particular, several nitroimidazole derivatives (SR-4554, EF5) have been used in combination with either optical methods or nuclear medicine approaches (7). This is based on the oxygen dependence of the reduction of these compounds *in vivo* (8). In this study we report the use of a novel series of nitroimidazoles for the measurement of oxygen tension in C6 astrocytoma cell cultures and the enzymatic reduction of these derivatives under normoxic and hypoxic conditions.

Materials and Methods: Nitroimidazolyl derivatives **1** and **2** (Figure 1b) were synthesized by Michael addition of the corresponding nitromidazol to the appropriate acceptor (Figure 1a). The Xantine/Xantine oxidase and NADPH:cytochrome P450 reductase enzymatic systems were used to investigate the *in vitro* reduction of derivatives **1** and **2** under normoxic (21% O_2) or hypoxic (1% O_2) conditions in aqueous media. C6 cells were grown to confluence in DMEM medium. At this stage the medium was changed to Krebs Ringer Bicarbonate buffer containing 2.5 mM of the imidazolic compound for 3, 6 and 24 hours. At the end of the incubation, the cells were harvested and a High Resolution Magic Spinning (HRMAS) ^{1}H NMR spectrum (Figure 1c) was taken from the cell pellet. (500.13 MHz, 4^{0}C, 4000 rpm).

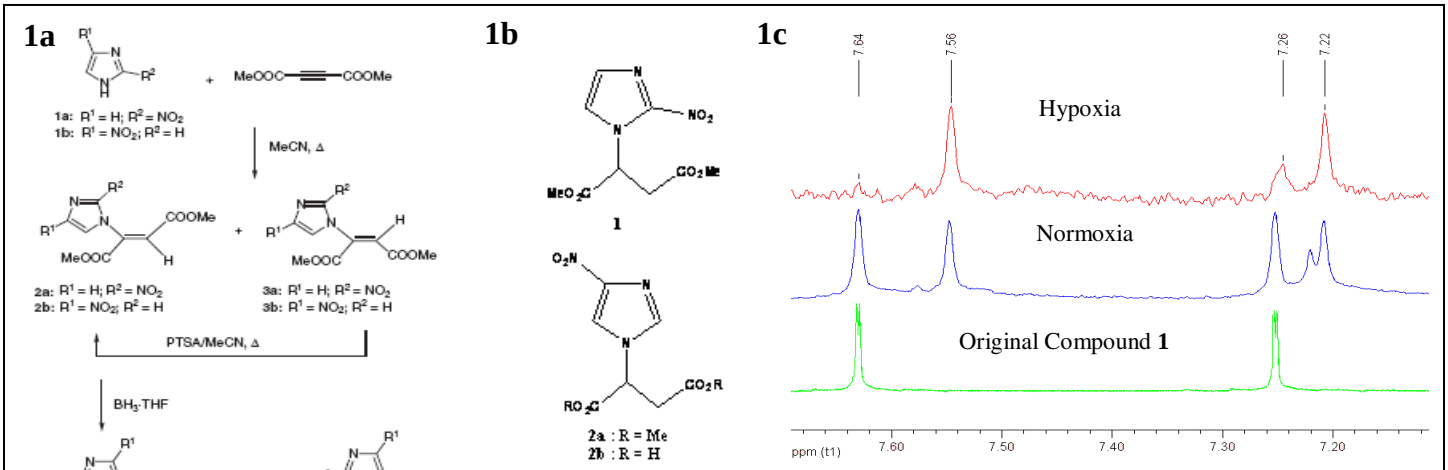

Figure 1. a) Synthetic pathway of the new generation of contrast agents **b)** The structure of Nitroimidazoles. c) ^{1}H-NMR spectra of C6 cell pellets containing the nitroiimidazol derivatives under different O_2 conditions.

Results and Discussion: Figure 1a shows the general synthetic approach used in the preparation of these new compounds and Figure 1b the chemical structure of the nitroimidazolyl compounds investigated in this study. Significant reduction of the compound **1** (Figure 1b) was observed with the NADPH:cytochrome P450 reductase system under anoxyc conditions. The ^{1}H HRMAS spectra of the cell pellets (Figure 1c) show in the aromatic region, the H2 (7.64 ppm) and H5 (7.26 ppm) resonances from the diester **1**, as well as the H2 (7.56 ppm) and H5 (7.22 ppm) resonances resonance from the corresponding hemiester **,** produced by intracellular hydrolysis. Notably, the H2 resonance from the diester in hypoxic cells (1% O_2) is significantly smaller than in normoxic cells, revealing its predominant disappearance during hypoxia. Based on that, we found a very good correlation between the ratio of H2 precursor's signal (7.64 ppm) and H2 hemiester's signal (7.56 ppm) vs the oxygen percentage.

Conclusions: We report the *in vitro* properties with C6 cells and the red-ox properties of a novel family of nitroimidazoles as pO_2 indicators for ^{1}H Magnetic Resonance Spectroscopic Imaging (^{1}H MRSI). Cytochrome P450 reductase appears to be the major enzyme involved in reduction of these nitroimidazolyl derivatives under anaerobic conditions. The incubation of C6 cells with the diester derivative, resulted in the intracellular production of the corresponding hemiester. This reaction appears to be oxygen level dependent, so the relation of the H2 precursor's and hemiester´s signal could be used as a quantitative parameter to measure tumoral hypoxia.

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