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**[A023]**

# Synthesis of new oxygen tension ($pO_2$) probes for $^1H$ Magnetic Resonance Spectroscopy Imaging

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## INTRODUCTION

Hypoxia has been known for a long time to be an important physiological parameter in tumour development. Hypoxia arises from an imbalance between oxygen supply and oxygen consumption[1]. Both human and animal tumours have been reported to contain regions of low oxygen tension[2].

A wide variety of methods have been developed to measure tumour oxygenation[3, 4] and, in recent years, several nitroimidazoles derivatives (e.g., SR-4554, EF5) have been used for this aim[5,6]. These nitroimidazoles undergo a one-electron reduction catalysed by cellular reductases, resulting in reactive intermediates which form adducts with cellular components[7-9] under anaerobic conditions. Presence of molecular oxygen hinders this process[10].

Our group has developed a non-invasive functional probe, IEPA (2-imidazole-1-yl-3-ethoxycarbonylpropionic acid) (see figure 1), for measurement extracellular pH ($pH_e$) and cell volume using $^1H$ Magnetic Resonance Spectroscopy Imaging[11]. It has allowed us to report the first vascular and extracellular pH map in solid tumours[12] and compared them with maps of metabolites[13].

In regard of likeness of nitroimidazoles derivatives (e.g., SR-4554, EF5) and IEPA, and as part of our interest in the development of new molecular probes for non-invasive studies of physiological events using the MRSI, we have prepared the nitroimidazolyl derivatives **1** y **2**.

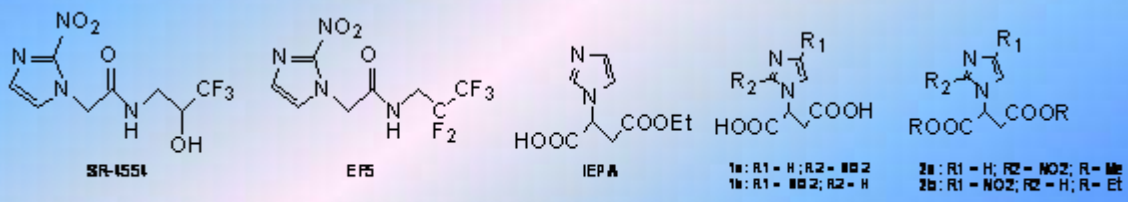


**Figure 1**

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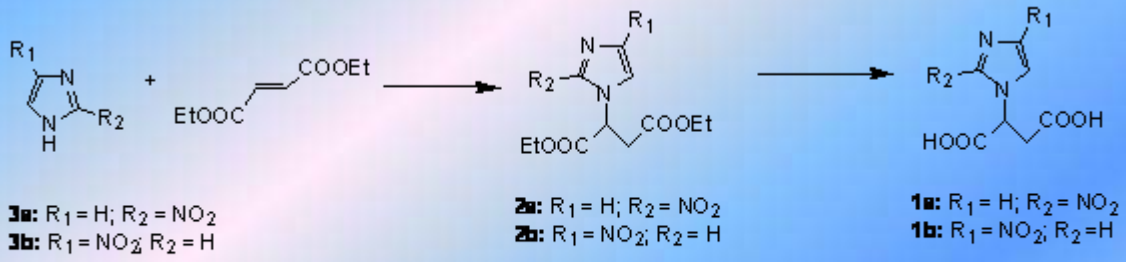
## RESULTS AND DISCUSSION

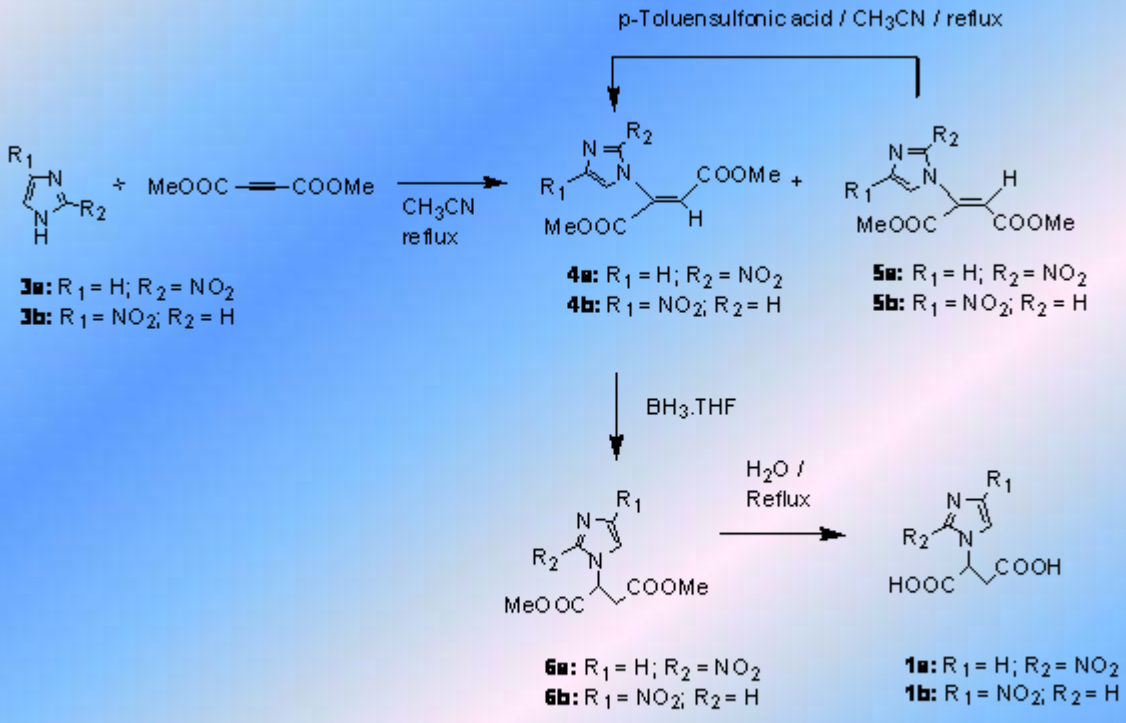
1. Synthetic pathway

Several synthetic approaches have been used to obtain these compounds (both classical techniques combines with a new ones like microwaves activation). Firstly, we tried to synthesize derivatives **1** and **2** by reaction between the corresponding nitroimidazole with different electrophiles such as, diethyl maleate, dimethyl maleate, maleic anhydride, diethyl fumarate, monoethyl fumarate and bromosuccinic acid. Michael's addition product **2b** was only obtained when diethyl fumarate was employed in DMF at 100 °C (50 % chemical yield, scheme 1). This addition resulted a regioselective reaction leading compound **2b** as unique reaction product. Subsequently, hydrolysis of diesther **2b**, under neutral conditions, yielded the corresponding acid **1b** in 46 % as a white solid.




**Scheme 1.** Addition of nitroimidazoles to diethyl fumarate under different conditions

However, when 2-nitroimidazole was used compound **2a** was not obtained. In this regard, we tried another synthetic approach, which is depicted in scheme 2.




**Scheme 2**. Synthetic pathway

We came out the reaction between 2-nitroimidazole and diethyl acetylendicarboxylate obtaining traces of addition's compounds. Surprisingly, when we used dimethyl acetilendicarboxylate, the chemical yield increased considerably, obtaining a mixture of **4a** and **5a** (78%) as Z/E isomers found in a ratio 65/25. Structures of the compounds **4a** and **5b** were confirmed by X-ray (see figure 2)

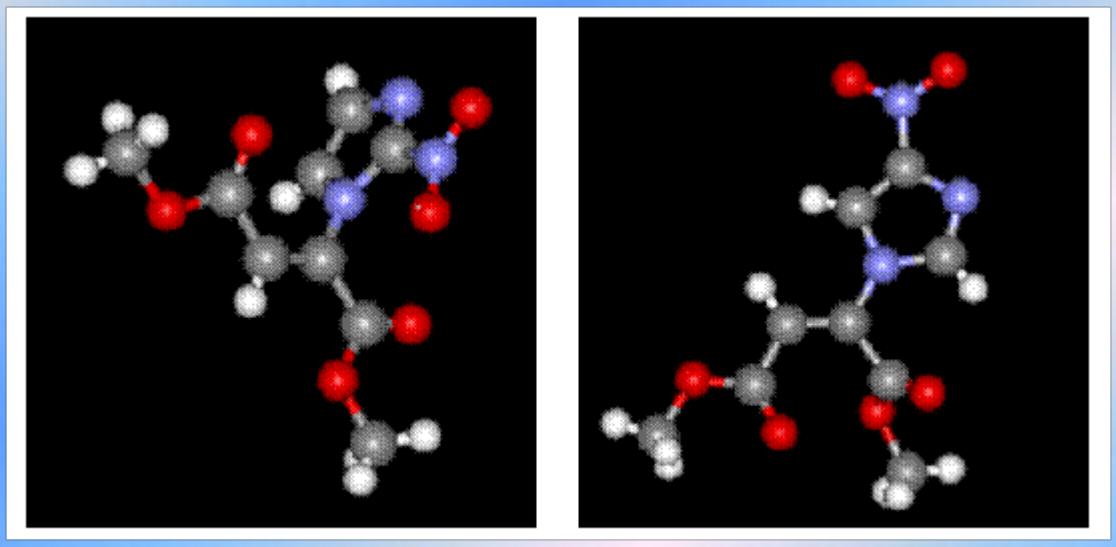


**Figure 2**. X-ray structures of **4a** and **5b**

The selective reduction of the double bound of **4a** was carried out using 1 equivalent of BH3·THF in THF at 0 ºC, giving the compound **6a** in a 80% yield. It is important to remark that, under this reaction conditions, no evolution of **5a** was observed, being only the Z isomer transformed in **6a**. When temperature was raised to 40ºC or excess of borane was used, solely degradation products were isolated. For this reason, we tried to convert compound **5a** to **4a**, employing different reaction conditions. The better results were found when *p*-toluensulfonic acid was used, achieving selective and almost complete transformation of **5a** in **4a** by refluxing in acetonitrile. Finally, hydrolysis of **6a** to **1a** was unsuccessful in acidic or basic conditions, or even when the reaction was carried out at high temperatures. Retro-Michael reaction products was observed under basic conditions. Finally, compound **1a** was only obtained when **4a** is maintained in water at room temperature during more than 30 days.

According to this synthetic pathway, similar results were found when 4(5)-nitroimidazole was employed as nucleophile. Exclusively the regioisomer at position 4 (**1b**) was obtained.

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## 2. Theoretical calculations.

### 2.a. Addition reaction to diethyl fumarate

Theoretical calculations were carried out, in order to rationale the experimental results.

| Compound | NPA charge over N (B3LYP/6-31+G) | HOMO energy (eV) HF/6-31 G* | HOMO-LUMO energy (eV) HF/6-31 G* | HOMO energy (eV) HF/6-31+G* | HOMO-LUMO energy (eV) HF/6-31+G* |
|---|---|---|---|---|---|
| Imidazole | -0.497 | -8.692 | 10.698 | -9.467 | 11.014 |
| 2-$NO_2$ | -0.425 | -9.851 | 11.857 | -10.006 | 11.553 |
| 4-$NO_2$ | -0.445 | -9.965 | 11.971 | -10.128 | 11.675 |
| 5-$NO_2$ | -0.484 | -10.086 | 12.092 | -10.262 | 11.809 |

**Table 1**. Electronic properties of different imidazoles calculated at different levels

In table 1 are represented the electronic properties of different imidazoles used. The first column gives us an idea of nucleophility of the heterocycles used. The obtained values are in good agreement with experimental results where the reaction with diethyl fumarate was only take place with imidazole and 5-nitroimidazole, in this last case, giving the corresponding 4-nitroimidazolyl derivative **1b**.

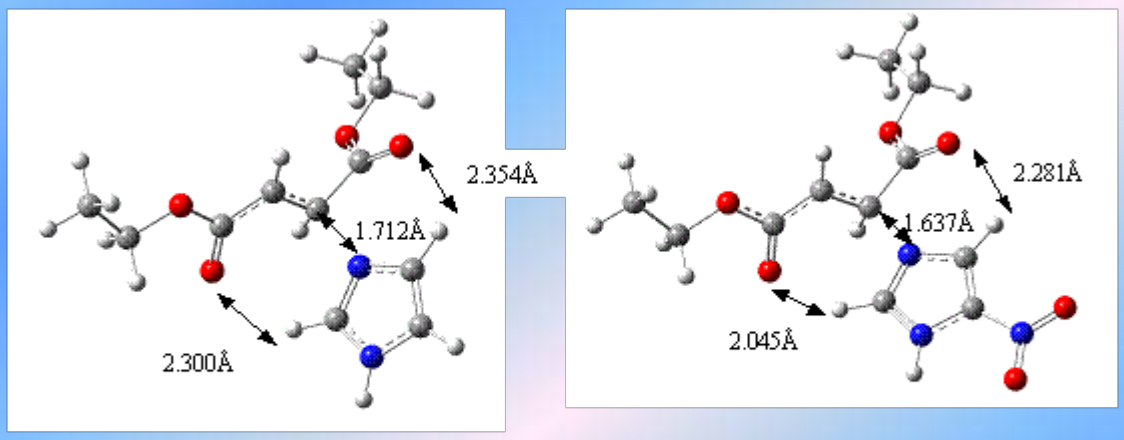


**Figure 3.** Transition structures for imidazole and 5-nitroimidazole addition to diethyl fumarate calculates at HF/6-31G* level

On the other hand, the transition structures for imidazole and 5-nitroimidazole addition to diethyl fumarate are shown in figure 3. In both cases, the imidazole ring and the diethyl fumarate are sited at enough distance for the reaction take place by attack of pyridinic nitrogen of the heterocycle. This circumstance could justify the only formation of the 4-nitroimidazolyl regioisomer **2b**.

In same way, figure 4 depicts the hypothetical transitions states when 4 and 2-nitroimidazoles were used. In both cases, strong steric interactions occurs between the oxygen atoms of the carbonyl and nitro moieties. In order to minimize the unfavourable interaction of those, the

distance between the starting materials is increased and consequently the addition reaction do not occur.

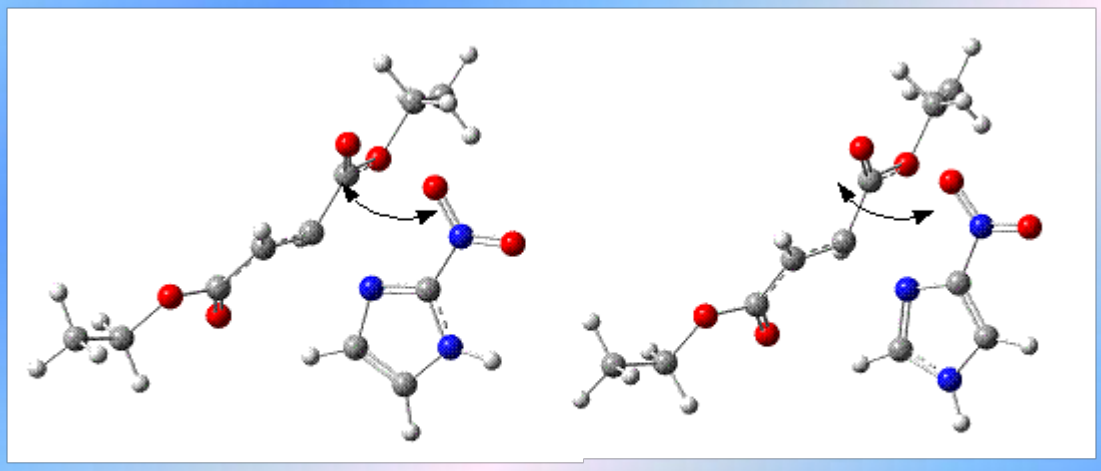

**Figura 4.** Hypothetical transitions structures for 2- and 4-nitroimidazole additions to diethyl fumarate

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2.b Addition reaction to diethyl acetilendicarboxylate

As part of the experimental results, the corresponding 2-nitroimidazolyl derivative **2a** was prepared by reaction of the 2-nitroimidazole to dimethyl acetylendicarboxylate. In figure 5 it is represented the transition structure for the addition of 2-nitroimidazole to the triple bond. In this case, although dimethyl acetylendicarboxylate is an less efficient acceptor, the steric interactions appears reduced making possible this reaction.

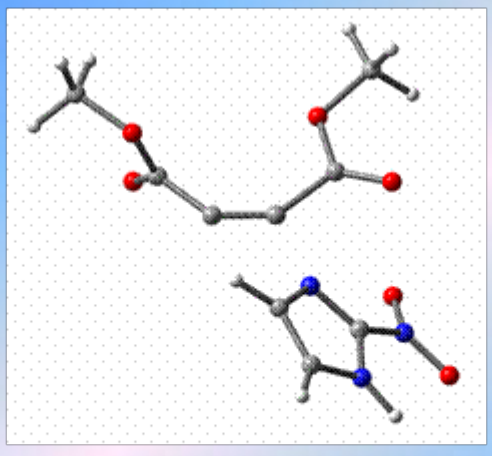

**Figura 5.** Transition structure for 2-nitroimidazole addition to dimethyl acetylendicarboxylate calculates at HF/6-31G* level

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3. Redox potentials

Evaluation of the compounds **1b**, as $pO_2$ indicators, was carried out by determination of their

redox potential using cyclic voltammetry methods (figure 6). The corresponding nitroimidazole and 4-imidazolylacetic acid were taken as base in determination of redox potential of **1b**. The reduction of **1b** take place through three steps as follow: i) two of them are irreversible processes and, ii) the reduction of nitro group was a reversible process corresponding to a monoelectronic reduction ($DE_{1/2}$ = 85 mV), being the catodic peak redox potential value, ($E_p^c$ = -1.55 V).

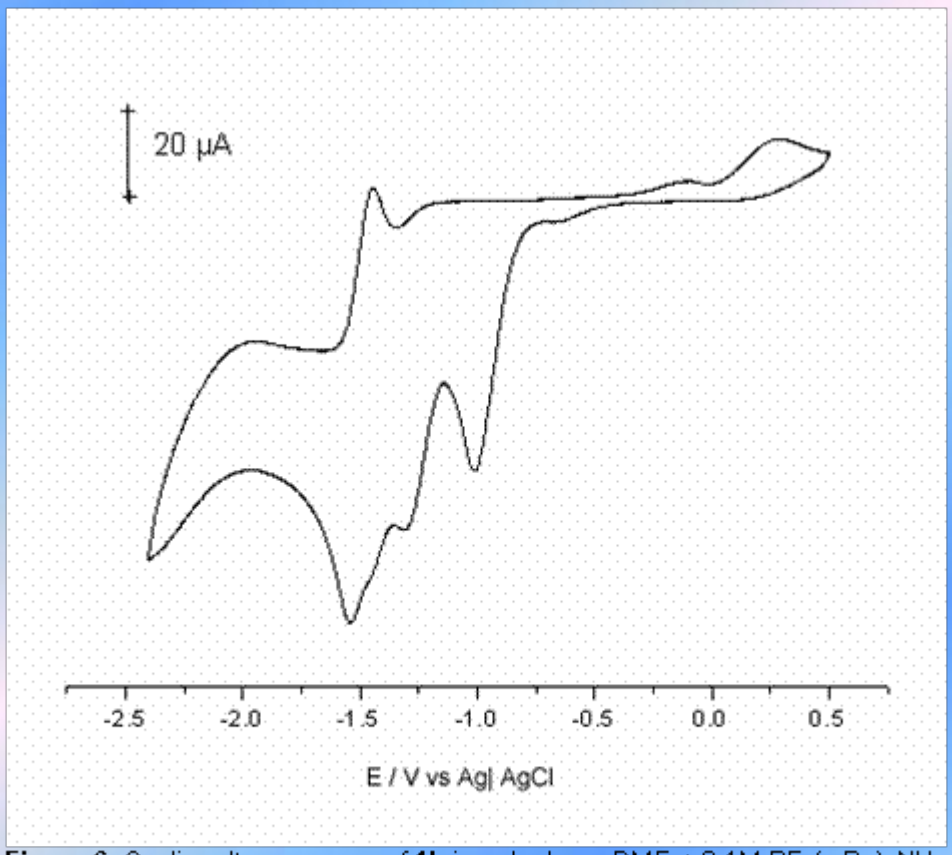


**Figure 6.** Cyclic voltammogram of **1b** in anhydrous DMF + 0,1M $BF_4(n\text{-}Bu)_4NH_4$. Sweep rate 100 mV $s^{-1}$

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## CONCLUSIONS

We have communicated the first synthesis of a new family of nitroimidazoles as $pO_2$ indicators for $^1H$ Magnetic Resonance Spectroscopy Imaging ($^1H$ MRSI).

The experimental study of the reactivity of nitroimidazoles to electrophiles, including multiples bonds, such as, diethyl fumarate and dimethyl acetylendicarboxylate was presented. Theoretical calculations, using *ab initio* methods, were shown in this communication which

justified the experimental results.

Measurement of redox potential of compound **1b** is described, making it a good candidate for measurement of hypoxia in tumours using ($^1$H MRSI).

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