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Conformational study of the electronic interactions and nitric oxide release potential of new *S* nitrosothiols esters derivatives of ibuprofen, naproxen and phenyl acids substituted (SNO-ESTERS): Synthesis, infrared spectroscopy analysis and theoretical calculations

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ABSTRACT

The conformational study on the new *S* nitrosothiols esters (SNO-ESTERS): *para*–substituted (X = H, OMe, Cl and NO_2) *S* nitrosothiol derivatives 2 methyl 2 (sulfanyl)propyl phenylacetates (**R1**), 2 (4 isobutylphenyl) propanoate (ibuprofen, **R2**), and 2 (4 isobutylphenyl)propanoate of 2 methyl 2 (nitrososulfanyl)propyl (naproxen, **R3**) was performed using infrared spectroscopy (IR) in solvents with increasing polarity (CCl_4, CH_3Cl, and CH_3CN), and theoretical calculations, to determine the preferential conformer and the potential of these compounds to release nitric oxide (NO). *S* Nitrosothiols were synthesized by esterification reactions, using chlorides of the corresponding carboxylic acids, with good yields (~60%). IR results showed that these compounds presented only one conformation, and the experimental data were supported by the theoretical results obtained by density functional theory (DFT) calculations using the 6311+G (2df, 2p) basis set. The calculations revealed that all *S* nitrosothiols presented one preferential *anticlinal* (*ac*) geometric conformation, which agrees with the data obtained experimentally in CCl_4. These conformers are stabilized by intramolecular hydrogen bonds. Examination of the geometry with regard to the R—SNO group revealed that these compounds are preferentially in the *trans* (*anti*) conformation. The calculation of the orbital interactions using the Natural Bond Orbital (NBO) method showed that the $n_{O(NO)} \rightarrow \sigma^*_{(S-N)}$ hyper-conjugative interaction increases the S—N bond length. The strong $n_S \rightarrow \pi^*_{(NO)}$ interaction and electronic delocalization induces a partial π character to the S—N bond. The weak σ_{S-N} bond indicates strong delocalization of the electron pair in O (NO) by the $n_{O(NO)} \rightarrow \sigma^*_{(S-N)}$ interaction, thereby increasing the capacity of NO release from SNO-ESTERS.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are effective in the treatment of pain, fever, and inflammation. NSAID–based therapy effectively reduces the symptoms of many painful arthritic syndromes. However, side effects involving damage to the patient's gastrointestinal tract during NSAID therapy are frequently reported. The most common clinical manifestation of NSAID-related damage is a combination of gastroduodenal erosions and ulcerations, often called NSAID-induced gastropathy, affecting at least 15% of chronic NSAID users [1–3].

During the 1990s, several structurally diverse compounds derived from NSAID and displaying gastro sparing properties in rodents were synthesized. The important strategy used to improve the NSAID safety profile was coupling a nitric oxide (NO) donor group to the NSAID [4–6]. NO released from NO-derivatives of NSAID, promotes vasodilatation of veins and arteries, inhibits platelet aggregation, and reduces the formation of blood clots [6,7]. In addition, it has been shown that NO exerts beneficial effects to the gastrointestinal (GI) tract, maintaining the integrity of the gastric mucosal barrier [4,5,8].

Different chemical strategies have been used to provide NSAIDs with the capacity to release NO. Nitrooxybutyl ester derivatives of NSAID display anti-inflammatory activity comparable to the respective parental NSAID against acute inflammation in rodent models, but with reduced GI toxicity [9].

NCX-4016, also called NO aspirin, is the prototype of an NO-releasing NSAID and consists of the parental aspirin molecule linked via an ester to an NO moiety. Both aspirin and NO moieties of NCX-4016 contribute to its effectiveness, with the effect of the latter

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occurring via both cyclic GMP-dependent and -independent mechanisms. Human studies have shown that, while NO aspirin maintains its anti-thrombotic activity, it spares the GI tract. The use of NCX-4016 results in a 90% reduction of the gastric damage caused by equimolar doses of aspirin [10,11].

S Nitrosation, the NO-mediated modification of sulfhydryl residues of peptides and proteins has been associated with the anti-inflammatory actions of NO aspirin and NO naproxen [12,13]. Furthermore, recent studies have shown that *S* nitrosation of the signaling proteins Src, EGFR, and Ras generating their *S* nitrosothiols derivatives, regulates pathways associated with cell survival and proliferation (reviewed in [14]).

In addition to NO-NSAIDS, *S* nitrosothiols are a class of NO donors that under appropriate conditions decompose to liberate NO. They decompose by thermal and photochemical reactions to give the corresponding disulfide and NO. It was proposed that the photolytic decomposition of *S* nitrosothiols is a two-step process: the first step being the homolytic dissociation of the S—N bond to form •NO and RS• radicals, followed by dimerization of RS• forming the disulfide RSSR [15]. On the other hand, in aqueous buffers at physiological pH, *S* nitrosothiols decompose in the presence of trace transition metal ions generating NO and RSSR. In this process RS• radicals are not formed [16].

By the reasons explained above, addition of an *S* nitrosothiol moiety to NSAIDs may prove to be advantageous to these molecules. However, syntheses of these derivatives have been scarcely reported [17].

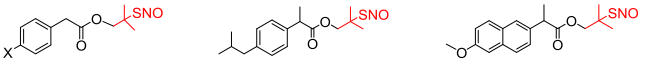
Theoretical approaches combined with experimental approaches, provide a new and consistent picture of the conformational behavior of *S* nitrosothiols. The combination of methodologies also provides information on how the conformational behavior influences *S* nitrosothiols spectroscopic and chemical properties. For instance, to assign the structure of *S* nitroso methanethiol, reported calculations showed for B3LYP/6-311+G* calculation minima for the *syn* and *anti* conformations (the former possessing an eclipsed C—H bond). MP2/6-311+G* geometry optimizations and QCISD (T)/6-311+G* single-point calculations further confirm the *syn* preference. Also of note is the preferred eclipsing of an R-CH and *anti-clinal* orientation of the alkyl chain with respect to the SNO moiety in *syn* exclusively in the *anti*-orientation [18].

The reliability and accuracy of the conventional electron correlation methods MP2 and QCISD, and the density functional theory methods B3LYP and B3P86, to obtain optimized structures and homolytic bond dissociation energies S—N of a range of *S* nitrosothiols, has been investigated. A variety of model RSNOs (HSNO, CH_3SNO, C_2H_3SNO, C_2H_5SNO, C_6H_5SNO, and CysSNO (*S* nitroso cysteine) have been used. For all methods considered, optimized S—N bond lengths were found to be highly dependent on the basis set being employed. In general, to obtain convergence in the r(S—N) values of RSNO for a given method, the 6-311$^+$(2df,p) or larger basis set was required [19].

The combination of IR spectroscopy and theoretical calculations has been extensively employed in conformational studies of carbonyl compounds. Analysis of the carbonyl stretching frequencies (ν_{CO}) in solution determines the different conformations assumed by the compounds. The unusual solvent effect and ν_{CO} shifts for the conformers as compared to other α-hetero-substituted carbonyl compounds are interpreted in terms of a decrease in polarity [20–22].

The presence of carbonyls in the structure of an *S* nitrosothiol might interfere with its conformational behavior. However, potential interference of these groups on the conformational behavior of *S* nitrosothiols has not been evaluated.

In this work we synthesized the *S* nitrosothiols 2 methyl 2 (nitrososulfanyl) propyl phenylacetate *para* substituted **R1**, 2 methyl 2 (nitrosothio) propyl 2 (4 isobutylphenyl) propanoate **R2** (derivative of ibuprofen), and 2 methyl 2 (nitrosothio) propyl 2 (6 methoxynaphthalen 2 yl)propanoate (derivative of naproxen) **R3**. A conformational study of the compounds was carried out using IR spectroscopy and theoretical calculations. This combination of experimental and theoretical approaches, allowed us to determine the most stable conformation they can assume in relation to the carbonyl group and their potential as NO releasing compounds.



R1 (X=OMe, H, Cl) **R2 (Ibuprofen derivative)** **R3 (Naproxen derivative)**

The compounds showed in solvents of different dielectric constants (CCl_4, CH_3Cl and CH_3CN) only one band in the carbonyl region in most cases. We carried out the conformational search for the studied compounds and stable conformations geometries were theoretically optimized using the B3LYP/DFT/G-6311+ (2df, 2p) basis set. Results obtained in the IR analysis were compared with the theoretical data showing good agreement with experimental results in CCl_4 for isolated molecules. Calculations of orbital interactions using the method of *Natural Bond Orbital* (NBO) showed no electronic interactions capable of stabilizing the SNO-ESTERS conformations, potentially enhancing their ability in releasing NO.

2. Materials and Methods

2.1. S Nitrosothiols Synthesis

S Nitrosothiols (**R1**–**R3**) were prepared using the method presented in Scheme 1. The reaction of intermediate **2**, obtained from the reaction of compound **1** with potassium thioacetate (KSAc) in acetone, for 2 h with reflux and with excess of $LiAlH_4$, led to the total reduction of the thioacetate group (-SAc) to thiol (-SH), along with the reduction of the ester group to alcohol (**3**). The *S* nitrosylation reaction using *t* butylnitrite (*t*-BuONO) led to the intermediate **4**, which underwent coupling with the carboxylic acids chlorides, precursors of the compounds of interest. All *S* nitrosothiols, **R1**–**R3**, were obtained in moderate to good yields (45–60%) [23].

Each compound was purified by column chromatography on silica gel using hexane/ethyl acetate as eluent. The structures of all compounds were determined by ^{1}H NMR, ^{13}C NMR, MS (ESI) and IR (Supporting information).

Compounds **R1**–**R3** are stable green oils at room temperature for more than one month. Compounds should be kept in closed vials and protected from light.

2.2. Infrared Spectroscopy

Infrared measurements on the solutions were performed using the FT-IR Michelson Bomem™ MB100 spectrometer, with 1.0 cm^{-1} resolution, between 4000 and 600 cm^{-1}. The solutions were properly prepared with 0.02 $mol \cdot L^{-1}$ concentration in CCl_4, CH_3Cl, and CH_3CN. For the measurements of carbonyl stretch band, we used NaCl cell with a 0.5 mm optical path. The determinations in the first harmonic region were obtained in a quartz cell with 1.0 cm optical path in CCl_4. We used the GRAMS/4.04 program for analyzing the bands [24]. The population of conformers was estimated from the maximum of each component of the resolved carbonyl doublet or triplet. The population of conformers was expressed as a percentage of the absorbance, assuming equimolar absorptivity coefficients for the referred conformers.

2.3. Computational Methods

Calculations were obtained using the Gaussian 09 program [25], in Linux environment, 64-bits Ubuntu, with three servers, two of which contained 16 processors in two Intel® Xeon eight-core E5-26770 sockets of 2.6 GHz, 128 GB RAM, and 9-TB disk, while the other contained two Intel® Xeon® six-core 5560 sockets, 12 processors with 96 GB of RAM, and 3-TB disk.