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Nitric oxide-donating non-steroidal anti-inflammatory drugs: the case of nitroderivatives of aspirin

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Abstract

Nitric oxide (NO) acts as a key signalling mechanism in a number of cells and tissues in the mammalian organism. Modulation of the biosynthesis of NO has emerged to be relevant to the treatment of a variety of human diseases. In the attempt to reduce the serious side effects of non-steroidal anti-inflammatory drugs (NSAIDs), especially in the gastrointestinal tract, a NO-releasing moiety has been linked to conventional NSAIDs. A prototypical example is that of NO-releasing derivatives of aspirin. Thanks to the cytoprotective action of NO such compounds do not produce gastric damage and are emerging as an interesting novel group of drugs for their unique pharmacological properties.

Keywords: Organic nitrates; Nitric-oxide donors; Nitric esters; Non-steroidal anti-inflammatory drugs; Aspirin derivatives

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used of all therapeutic agents. Currently there are more than 50 different NSAIDs available which share certain therapeutic actions and side effects. Chemically, they are a heterogeneous group of compounds. The prototype is aspirin. In addition to salicylic acid derivatives, there are para-aminophenols, indole and indene acetic acids, arylpropionic acids, to name a few chemical classes [1]. NSAIDs share common therapeutic properties like the reduction of inflammation and the production of analgesic and antipyretic effects. The degree of anti-inflammatory activity varies with the different chemical classes. Unwanted side effects are also common to most NSAIDs. These drugs are responsible for a large number of adverse drug reactions which range from effects on gastrointestinal tract to effects on the liver, kidney, spleen, blood and bone marrow [1]. The most common side effect is the propensity of NSAIDs to induce gastric or intestinal ulceration. Thus, the patients who use NSAIDs on a chronic basis have about three times greater relative risk for serious adverse gastrointestinal events compared to the population of non-users.

NSAIDs act by inhibition of the biosynthesis of prostaglandins, specifically through the enzyme cyclooxygenase (COX). The discovery that there are two isoforms of COX, namely COX-1 and COX-2, has stimulated the search of compounds possessing selectivity for one COX versus the other. Specifically, based on the knowledge that COX-1, but not COX-2, is constitutively expressed in the stomach [2], new COX-2 selective inhibitors have been synthesized [3]. The consequence of this effort has been the development of compounds such as rofecoxib and celecoxib that are now widely prescribed. However the initial expectation that such new generation of NSAIDs would lack gastrointestinal side effects has been challenged by reports showing that these side effects also occur. Indeed, there are now data showing that celecoxib is not better than classic NSAIDs such as ibuprofen regarding gastrointestinal lesions [4].

Moreover, other side effects of these new generation of NSAIDs, regarding the cardiovascular and renal

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system, have been brought to the attention of the medical community [5].

Therefore, despite the recent efforts made, currently there is need for new anti-inflammatory agents showing good activity in combination with a high degree of safety. One interesting innovative approach relies on the incorporation of a nitric-oxide (NO) releasing moiety into the structure of established NSAID molecules. Such NO-donating NSAIDs are emerging as a new interesting class of effective anti-inflammatory compounds without the side effects which accompany the other NSAIDs used thus far [6,7]. The rationale behind this drug design is based on the biological proprieties of NO. This small molecule acts as a key signalling molecule in various cells and tissues [8]. Interestingly, NO possesses some properties of prostaglandins within the gastric mucosa, therefore leading to cytoprotection, possibly through the increase of mucosal blood flow and mucous fluid secretion by the gastric epithelial cells. The combination of two mechanisms within the same drug, i.e., NO release and COX inhibition, may lead to new safe therapies for inflammatory-associated disorders. In this short review article we will describe the chemistry of NO-donating NSAIDs with a special focus on the derivatives of aspirin.

1.1. Traditional nitric oxide donors and hybrid nitrates

Organic nitrates and nitrites are the most commonly used NO donor drugs in cardiovascular therapy. Glyceril trinitrate (GTN) and amyl nitrite were proposed in the nineteenth century as antianginal drugs (Fig. 1). A more recent drug discovery effort (1950s) lead to the development of isosorbide dinitrate (ISDN) (Fig. 1), which is a stable nitrate that has a longer duration of action that GTN. Likewise, sodium nitroprusside (Fig. 1) was introduced as therapeutic agent more than 50 years ago and has been used for the treatment of hypertension.

However, these classical NO donors are characterised by side effects such as marked hypotension, reflex tachycardia and headache. Moreover, they undergo tolerance over a repeated administration regimen.

During the last decade, the search for new NO donors with reduced side effects and improved oral bioavailability has greatly intensified. Several reviews have been published illustrating the various chemical approaches which have been used to improve the pharmaceutical profile of NO donors [9]. In this review article we will describe a class of new chemical entities synthesized in our laboratories through the hybridisation of NO donor moieties with established NSAIDs (Figs. 2 and 3).

With such an approach we have synthesized many NO-donating NSAIDs, including derivatives of ibuprofen, flurbiprofen, diclofenac, naproxen, ketoprofen and aspirin [10]. Fig. 2 shows the example of two NO-releasing derivatives of flurbiprofen (HCT 1026 and NCX 2216).

Compounds such as HCT 1026 display anti-inflammatory properties similar to those of the parent drugs (flurbiprofen, in this case) and, thanks to their NO-donating moieties, they also show a marked reduction of gastrointestinal side effects [7,11].

In this paper, we describe several compounds synthesized by esterification of the carboxylic moiety of acetylsalicylic acid with a NO-donor linker.

1.2. Nitric oxide-donating aspirins

A variety of nitric esters have been synthesized by coupling the nitrate group to the carboxylic moiety of acetyl salicylic acid. The nitrate group has been linked using different spacers conferring different pharmacological and pharmacokinetic properties to the final compounds. Thus, a product with an aliphatic chain such as NCX 4018 (Fig. 3) has been synthesized. Likewise, other compounds containing an aromatic spacer, such as the three isomers NCX 4040, NCX 4016, NCX 4060 have also been prepared (Fig. 3). A heterocyclic spacer, i.e., the pyridine ring, has been introduced with the synthesis of NCX 4050 (Fig. 3).

These structural changes lead to compounds with new pharmacological properties which combine the biology of NO and acetyl salicylic acid. Unlike the classical NO donors, the new compounds do not produce rapid vasodilation with the consequent fall of blood pressure typically associated with NO donor administration;

Glyceryl trinitrate GTN | Amyl nitrite | Isosorbide dinitrate ISDN | Sodium nitroprusside SNP

Fig. 1. Example of classical NO donors used clinically.

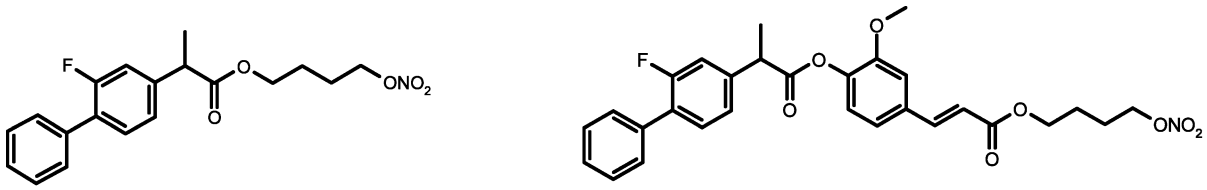


Fig. 2. Example of hybrid NO donors containing a nitric ester linked to an established NSAID; (a) HCT 1026 is a NO-releasing derivative of flurbiprofen. (b) NCX 2216 is a NO-releasing derivative of flurbiprofen containing a spacer (ferulic acid) having anti-oxidant properties.

these unique properties have been mainly attributed to the slow release of NO in the various body compartments [7,12,13].

NO aspirins possess antiaggregating effects because of inhibition of COX by aspirin and formation of soluble guanylate cyclase by NO; NCX 4016 also appears to retain the antithrombotic and anti-inflammatory actions of the COX inhibitor with the added benefit of protection against ulceration of the gastric mucosa [6,7,13].

2. Chemistry

The synthesis of NCX 4016, NCX 4060 and NCX 4040 is represented in Scheme 1.

The acetyl salicylic acid **1** is converted to acyl halide **2** with thionyl chloride in dichloromethane in presence of pyridine. The acyl halide **2** is condensed with *p*-HOPhCHO, *m*-HOPhCHO and *o*-HOPhCHO in dichloromethane in presence of triethylamine to obtain the three positional isomers **3**.

The aldehydic function of these isomers **3** is reduced to benzyl alcohol **4** by H_2/Pd/C in AcOEt and halogenated by $SOCl_2$. The final products are obtained by nitration with $AgNO_3$ in CH_3CN.

The synthesis of NCX 4050 is represented in Scheme 2. 2,6-Bis-(hydroxymethyl)pyridine **6** is converted to 2,6-bis-(dichloromethyl)pyridine **7** with thionyl chloride. The mixture is stirred at room temperature for 2 h and concentrated under vacuum. The residue is treated with chloroform and water and the organic layers are dried with sodium sulphate, and concentrated under reduced pressure. The product **7** is obtained as solid.

The sodium salt of acetyl salicylic acid **8** is prepared adding at room temperature sodium ethoxide to a solution of acetyl salicylic acid **1** dissolved in dimethylformamide and the resulting mixture is stirred for 2 h.

To a solution of 2,6-bis-(chloromethyl)pyridine **7** in dimethylformamide the previous mixture is added.

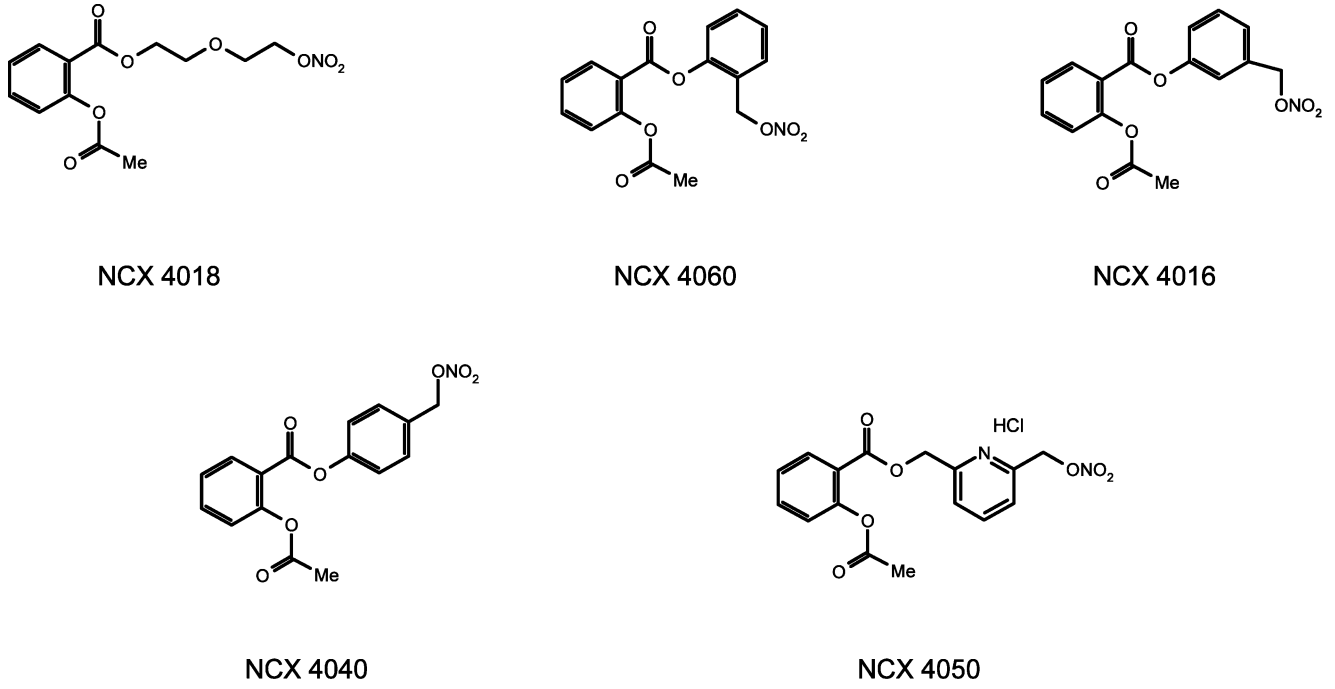
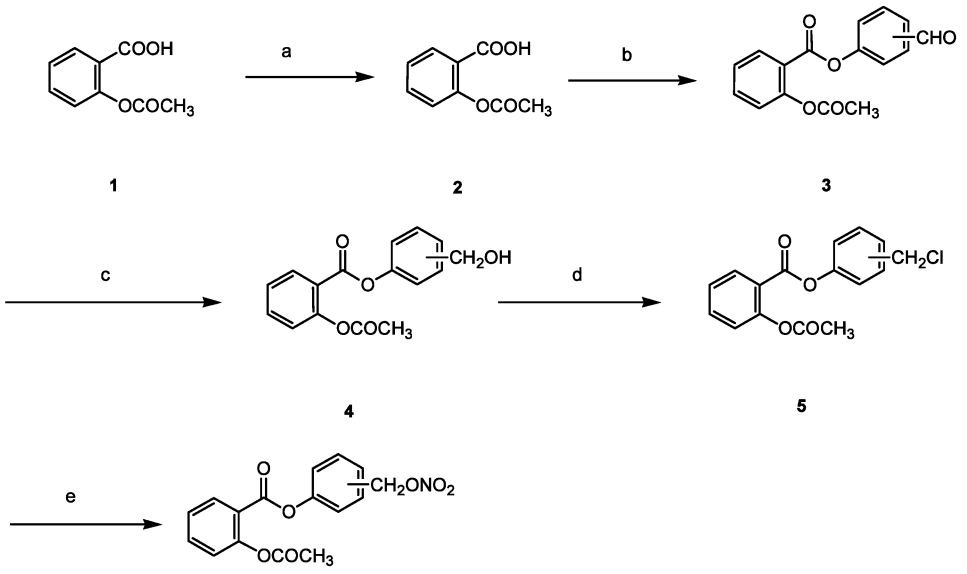


Fig. 3. Example of five hybrid NO donors derived from aspirin. The nitrate group is linked to acetylsalicylic acid through different types of spacer.



Scheme 1. (a) $SOCl_2$/Py/CH_2Cl_2, 0–37 °C/2 h; (b) *o*,*m*,*p*-HOPhCHO/Et_3/CH_2Cl_2, 0 °C-r.t./2 h; (c) H_2/Pd/AcOEt, r.t.; (d) $SOCl_2$, 0 °C/1 h-r.t./1 h; $AgNO_3$; (e) $AgNO_3$/CH_3CN, 75 °C/8 h.

The resulting solution is stirred at room temperature. After 24 h a new mixture of acetyl salicylic acid in dimethylformamide, sodium ethoxide is added at room temperature. The resulting mixture is stirred for 2 h, diluted with diethyl ether and washed with water; the organic layers are dried with sodium sulphate and the solvent is evaporated under vacuum.

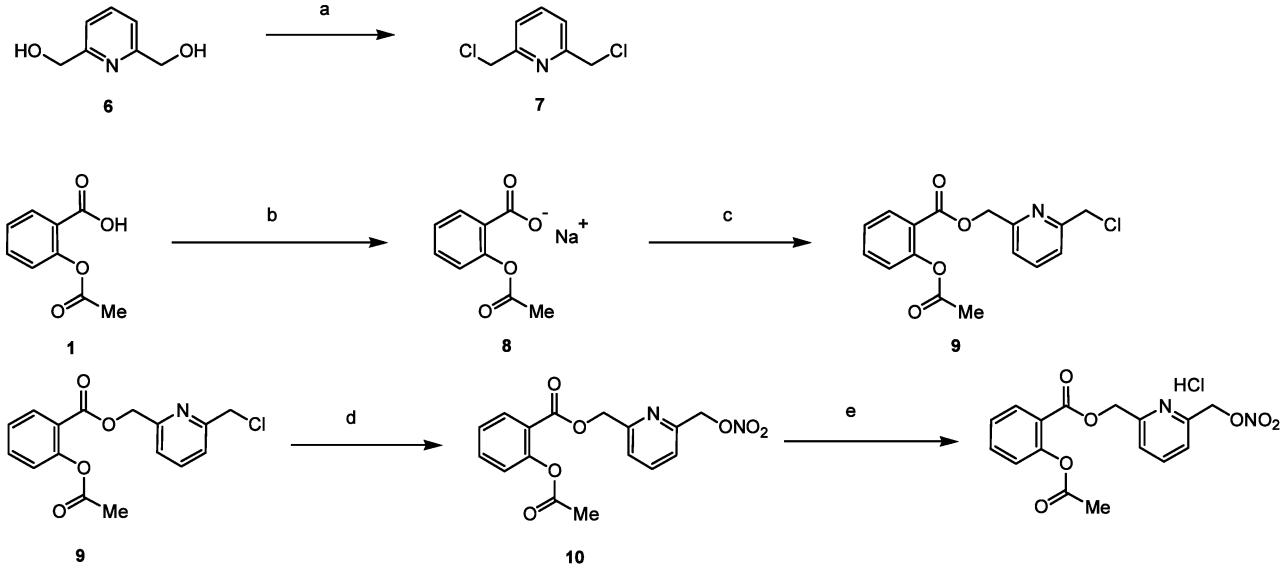
The residue is purified by silica gel chromatography and the product **9** is obtained as a solid.

A solution of the product **9** and silver nitrate in acetonitrile is refluxed in the dark for 15 h.

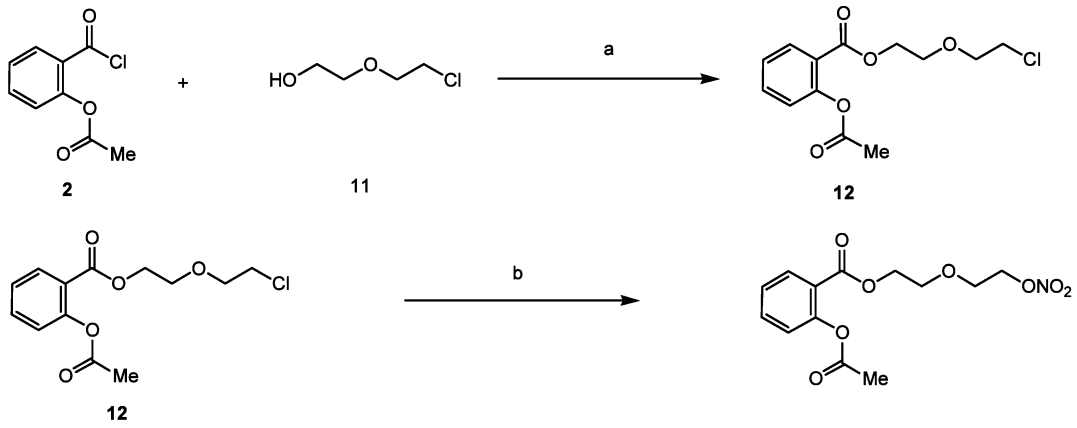
The precipitated (silver salts) is filtered off and the solvent is evaporated under vacuum.

The product **10** is then purified by silica gel chromatography, using as eluent methylene chloride/methanol 20/0.1.

To a solution of this purified product **10** in ethyl acetate, cooled at 0 °C, HCl/AcOEt (3.1 N) in ethyl acetate is added. The solution is stirred at 0 °C for 1 h and then at room temperature for 2 h. The white precipitate is filtered off and washed with diethyl ether. The product is obtained as a white solid.



Scheme 2. (a) $SOCl_2$, 0 °C-r.t./2 h; (b) EtONa/DMF, r.t./2 h; (c) 2,6-bis-(chloromethyl)pyridine/DMF, r.t./24 h+24 h+2 h; (d) $AgNO_3$/CH_3CN, 80 °C/12 h; (e) HCl/AcOEt 3.1 M, 0 °C/20 min-r.t./1 h.



Scheme 3. (a) TEA/THF r.t./24 h; (b) $AgNO_3/CH_3CN$/80 °C/48 h.

The synthesis of NCX 4018 is represented in Scheme 3.

Acetyl salicylic acid chloride **2**, 2-(chloroethoxy)ethanol **11** and triethylamine are dissolved in tetrahydrofuran and the resulting mixture is stirred at room temperature for 24 h.

The precipitate (triethylammonium chloride) is filtered off and the solvent is evaporated under vacuum.

The residue is dissolved in dichloromethane and washed with water; the organic layers are dried with sodium sulphate and the solvent is evaporated under vacuum.

The product **12** is purified by silica gel chromatography, using as eluent hexane/ethyl acetate 8/2. A solution of the product **12** and silver nitrate in acetonitrile is refluxed in the dark for 48 h. The precipitate (silver salts) is filtered off and the solvent is evaporated under vacuum. The product is purified by silica gel chromatography, using as eluent hexane/ethyl acetate 7/3. The spectroscopic, physical and chemical data of the compounds are reported in Table 1.

3. Pharmacology and discussion

The results obtained with one lead compound, NCX 4016, offer an opportunity to illustrate that the new NO-donating NSAIDs have an interesting pharmacological profile, significantly different than that of the parent drug. Indeed, NCX 4016 can be viewed as a new chemical entity showing unique activities clearly different than those of the simple combination between aspirin and a conventional NO donor. Several reviews and papers have been published describing the profile of NCX 4016 like the recent review by Wallace et al. [13].

Briefly, NCX 4016 is effective when given orally in animals and humans. In the body, the ester linkage is cleaved by esterases so as to release salicylate and the nitrate species [14]. Unlike conventional NO donors, there is a low rate of NO release associated with NCX 4016 [8,15]. Thus, the drug does not produce changes of blood pressure while the biological activity of NO occurs in parallel with COX inhibition by salicylate [15,16]. Among the many relevant pharmacological effects it is worth mentioning that NCX 4016 reduces the peripheral vascular tone induced by endogenous vasoconstrictor such as phenylephrine [17]; this is a unique property which is not shared by either aspirin or NO donors. Thanks to NO release, NCX 4016 has been found to protect vascular endothelium in diabetic rats, a condition where the endothelial function is impaired [18]. In another study NCX 4016 was found to prevent restenosis and inhibit the proliferation of vascular smooth cells, especially in the mouse model of hypercholesterolemia [19] and aging rats [20]. In models of cardiac ischemia NCX 4016 reduced the infarct size in rats [16] and pigs [21]. Unlike aspirin, NCX4016 was effective also in a variety of thromboembolic models in mice [22].

Moreover, following the evidence that NSAIDs decrease the incidence of mortality from colon cancer, NCX 4016 and other NO-releasing derivatives have been examined in cultured colon cancer cells [23]. Here, the NO-releasing aspirins have shown a superior activity compared with aspirin itself. The data confirm previous results obtained in in vivo studies [24].

Altogether, a variety of data in disease models have shown that NCX 4016 possesses unique pharmacological properties. Considering the relevance of animal models to human disorders, it may be inferred that NCX 4016 has a potential for treatment of diseases associated with impairment of endothelial and vascular functions. Thus, in perspective, NCX 4016 can be a candidate drug to be tested in a variety of human diseases including peripheral vascular disease and problems associated with atherosclerosis such as coronary angioplasty. Moreover, chemoprevention of colon cancer is another interesting perspective [25], with the added possibility to prevent concomitantly both cardiovascular diseases and cancer.

Table 1

Compound	Melting point	^{1}H-NMR ($CDCl_3$) (ppm)
3 (meta subst)	80–84 °C	10.04 (1H, s), 8.25 (1H, dd), 7.82 (1H, dd), 7.74–7.60 (3H, m), 7.45 (2H, m), 7.21 (2H, dd), 2.32 (3H, s)
4 (meta subst)	77–79 °C	8.23 (1H, dd), 7.66 (1H, dt), 7.40 (2H, m), 7.30–7.15 (3H, m), 7.10 (1H, dd), 4.71 (2H, d), 2.32 (3H, s), 2.12 (1H, t)
5 (meta subst)	71–73 °C	8.24 (1H, dd), 7.67 (1H, t), 7.42 (1H, dd), 7.33–7.14 (5H, m), 4.61 (2H, s), 2.33 (3H, s)
NCX 4016	63–64 °C	8.22 (1H, dd), 7.67 (1H, dt), 7.48 (1H, dt), 7.42 (1H, td), 7.33 (1H, dt), 7.26 (1H, d), 7.20 (2H, m), 5.45 (2H, s), 2.33 (3H, s)
3 (orto subst)	80–81 °C	10.01 (1H, s), 8.20 (1H, dd), 7.98 (2H, m), 7.65 (1H, dt), 7.40 (3H, m), 7.21 (1H, dd), 2.31 (3H, s)
4 (orto subst)	115–115.2 °C	8.20 (1H, d), 7.83 (1H, t), 7.55 (1H, t), 7.42 (2H,d), 7.38 (1H, dd), 7.23(2H, m), 5.29 (1H, dt), 4.58 (2H, d); 2.30 (3H, s)
5 (orto subst)	108–110 °C	8.22 (1H, dd), 7.85 (1H, dt), 7.59 (3H, m), 7.30–7.45 (3H, m), 4.86 (2H, s), 2.31(3H, s)
NCX 4040	86–88 °C	8.18 (1H, dd), 7.77 (1H, dt), 7.58 (3H, m), 7.32 (3H, m), 5.61 (2H, s), 2.26 (3H, s)
3 (para subst)	78–80 °C	10.19 (1H, s), 8.25 (1H, dd), 7.96 (1H, dd), 7.68 (2H, t), 7.45 (2H, m), 7.22 (2H, m), 2.29 (3H, s)
4 (para subst)	70–73 °C	8.22 (1H, dd), 7.64 (1H, dt), 7.51 (1H, dd), 7.40–7.25 (3H, m), 7.14 (2H, m), 4.61 (2H, s), 2.28 (3H, s), 2.05 (1H, s)
5 (para subst)	91–93 °C	8.23 (1H, dd), 7.68 (1H, dt), 7.50(1H, dd), 7.40 (2H, t), 7.32–7.18 (3H, m), 4.56 (2H, s), 2.30 (3H, s)
NCX 4060	52–54 °C	8.27 (1H, dd), 7.51 (1H, dt), 7.43–7.20 (6H, m), 5.45 (2H, s), 2.32 (3H, s)
9	39–42 °C	8.08 (1H, dd), 7.72 (1H, t), 7.58 (1H, dt), 7.40 (1H, d), 7.30 (2H, m), 7.08 (1H, d), 5.40 (1H, d), 7.30 (2H, m), 7.08 (1H, d), 5.40 (2H, s), 4.65 (2H, s), 2.21 (3H, s)
NCX 4050	109–110 °C	8.15 (1H, dd), 7.80 (1H, t), 7.60 (1H, t), 7.43–7.25 (3H, m), 7.06 (1H, d), 5.55 (2H, s), 5.42 (2H, s), 2.25 (3H, s)
12	oil	8.02 (1H, dd), 7.59 (1H, dt), 7.30 (1H, dd), 7.07(1H, dd), 4.43 (2H, t), 3.79 (4H, m), 3.63 (2H, t), 2.36 (3H, s)
NCX 4018	oil	8.00 (1H, dd), 7.53 (1H, dt), 7.29 (1H, dt), 7.07 (1H, dd), 4.58 (2H, t), 4.38 (2H, t), 3.75 (4H, t), 2.31 (3H, s)

While clinical trials currently ongoing will provide more information on the action of NCX 4016 in patients it is noteworthy that, unlike aspirin, in phase I studies NCX 4016 has been found to spare the stomach at doses producing clear COX inhibitory activity [26], therefore providing further support to the concept of NO cytoprotection.

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