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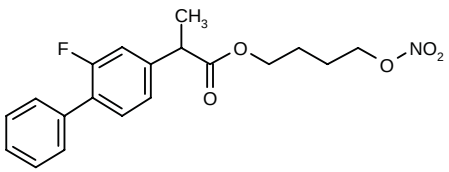
CCC: 0377-8282/99

HCT-1026

Treatment of Septic Shock
Treatment of Urinary Incontinence
Treatment of Osteoporosis
Nitric Oxide Donor

Flurbiprofen Nitroxybutyl Ester

2-Fluoro-α-methyl[1,1'-biphenyl]-4-acetic acid 4-(nitrooxy)butyl ester



$C_{19}H_{20}FNO_5$ Mol wt: 361.3730

CAS: 158836-71-6

EN: 224707

Synthesis

The adopted procedure for the preparation of HCT-1026 consisted in the reaction of flurbiprofen sodium salt (I) and 1,4-dibromobutane (II) in acetonitrile at reflux temperature to obtain HCT-1031 (III), the 4-bromobutyl ester of flurbiprofen. HCT-1031 was then converted into HCT-1026 by reaction with silver nitrate in acetonitrile at reflux temperature. The crude HCT-1026 thus obtained showed 85% HPLC purity with the presence of one major impurity (15%) that was identified as the bis-compound HCT-1028. The crude product was then purified by silica gel column chromatography using toluene as eluent to give a product with >99% HPLC purity in 57% overall yield. After cooling, pure HCT-1026 was obtained as a thick oil (Scheme 1).

Introduction

As characterized a few decades ago, the antiinflammatory action of NSAIDs (also commonly referred to as aspirin-like drugs) is associated with the inhibition of a key enzyme called cyclooxygenase (COX). This enzyme converts the arachidonate freed from the plasma membrane by the action of phospholipase A_2 into several types of eicosanoids (prostaglandins, prostacyclin, thromboxanes, *etc.* However, this molecular mechanism of action of NSAIDs is also the basis for their major side effects, which indeed are manifested in organs or cells where COX-derived prostaglandins play an important physiological role. Such an example is the gastrointestinal tract, where, for instance in the stomach, prostacyclin inhibits acid secretion. Other examples are the kidney and the coagulation system.

NO, an atmospheric gas and free radical, is an important biological mediator in maintaining the physiological conditions in animals and humans. NO can be quickly formed by producing cells via an enzymatic synthesis operated by a distinct class of enzymes, called nitric oxide synthase (NOS). These include a constitutive non-neuronal NOS (also termed endothelial NOS, eNOS or NOS-III), a neuronal NOS (also termed nNOS, bNOS or NOS-I) and an inducible NOS (also referred to as iNOS or NOS-II). These enzymes have different catalytic activities but the endpoint is identical in the sense that they transform L-arginine and atmospheric oxygen into L-citrulline and NO. These reactions are now relatively well characterized as well as the interaction between NO and other biological molecules such as reactive oxygen species (1).

In pathological conditions, the host defense and immunological systems are fully activated. As a consequence, large amounts of NO can be generated, mainly by the action of iNOS (although the formation of other cofactors for the NOS class of enzymes can also be induced, such as tetrahydropterin). Such an intense NO generation was first observed in activated macrophages where this mediator contributed to macrophage-mediated cytotoxicity against tumor cells, bacteria and viruses. The cytotoxic actions of NO result from its inhibitory actions on enzymes in the respiration of target cells (2). NO may

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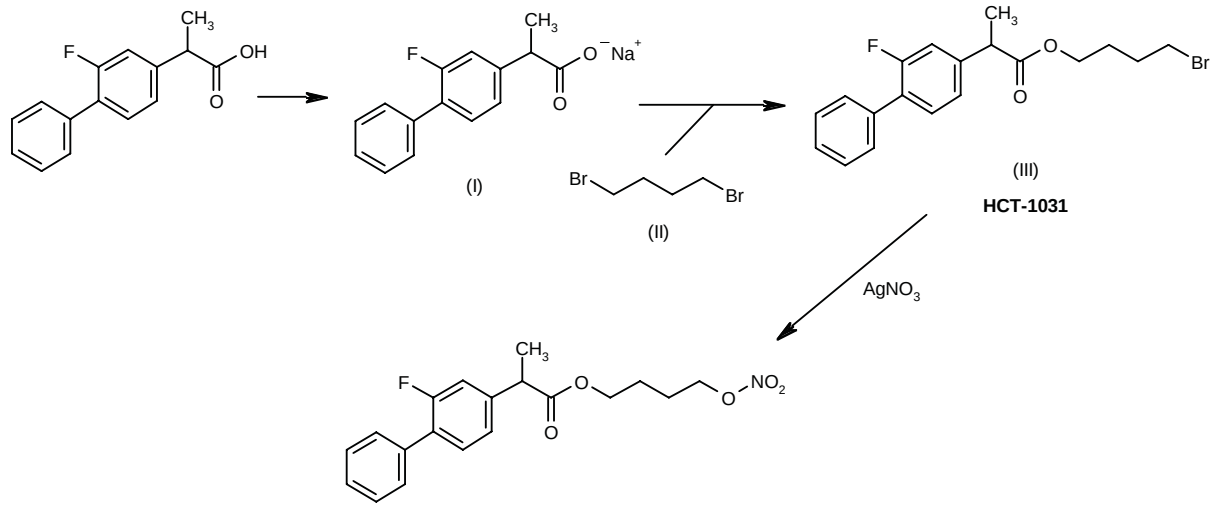
Scheme 1: Synthesis of HCT-1026



also interact with oxygen-derived radicals to produce other toxic substances such as peroxynitrite which is a powerful oxidant. Thus, NO plays a role in immunological host defense and is also involved in the pathogenesis of conditions such as septic shock and inflammation.

In contrast to the above, the generation of small amounts of NO in vascular endothelial cells by the action of eNOS maintains a vasodilator tone that is essential for the regulation of blood flow and systemic pressure. Endothelium-derived NO plays a crucial role in the local regulation of vascular homeostasis and platelet aggregation (3). An apparent decrease in the bioavailability of NO is a characteristic feature in patients with coronary artery disease (4) and recent studies have shown that the generation of NO by endothelial cells has a potent antiatherogenic effect (5). Moreover, NO has been shown to protect endothelial cells from apoptosis induced by proatherosclerotic factors (6-8). In addition to its role in preventing atherosclerotic lesion formation, NO also beneficially modulates remodeling following injury (9, 10) and inhibits smooth muscle cell proliferation and restenosis.

The addition of the NO releasing moiety to the structure of flurbiprofen may satisfy the dual aim of achieving a better pharmacological profile (lower ED_{50}) and reducing its gastrointestinal side effects. We propose to use these tools to unravel previously unrecognized biological actions of flurbiprofen but in which its presence may potentiate or facilitate the activities due to the NO releasing portion of the molecule.

Pharmacological Actions

The biological activity of nitroflurbiprofen (HCT-1026) has been evaluated in different experimental models to characterize its antiinflammatory and analgesic effects in chronic neuroinflammation and urinary incontinence.

The effects of HCT-1026, compared to the parent compound, were determined in an experimental model of endotoxic shock. Intravenous administration of endotoxin from *Salmonella typhosa* to rats pretreated with flurbiprofen produced a significant decrease in systemic arterial blood pressure, an increase in hematocrit and extensive gastric and small intestinal damage (11). In rats pretreated with flurbiprofen 4-nitroxybutylester, endotoxin produced changes in blood pressure and hematocrit comparable to those seen in flurbiprofen-treated rats; however, the severity of gastrointestinal damage was significantly reduced. Gastric blood flow was significantly decreased following endotoxin administration but was higher in rats pretreated with nitro-flurbiprofen than in rats pretreated with flurbiprofen. These results demonstrated that, although it did not affect the acute systemic effects of endotoxin administration, flurbiprofen 4-nitroxybutylester is capable of protecting the gastrointestinal mucosa from injury, possibly through preservation of mucosal blood flow.

Daily peripheral administration of HCT-1026 (15 mg/kg) for 30 days significantly reduced lipopolysaccharide (LPS)-induced neuroinflammation in rat brain as demonstrated by the decreased reactive state of both microglia and astrocytes (12). The results demonstrated that HCT-1026 could reduce brain inflammation. This could lead to the development of new antiinflammatory therapy of CNS diseases in which chronic inflammation is associated with selected neurodegenerative changes, such as Alzheimer's disease.

Futhermore, treatment with HCT-1026 improved the performance of LPS-infused young rats, but not LPS-infused adult or old rats. LPS infusions increased the

number of activated microglia in young and adult rats but not in old rats. HCT-1026 treatment attenuated the effects of LPS upon microglia activation in young and adult rats but not in old rats. Taken together, these data suggest that NSAID therapies designed to affect the onset of Alzheimer's disease should be initiated in adults before age-associated inflammatory processes within the brain have a chance to develop (13).

HCT-1026 was compared to the parent compound in two models of bladder function, namely, cystometry in conscious rats with bladder infused with saline or with diluted acetic acid solution (14).

For intravenous (i.v.) injection the compounds were dissolved using pure *N*,*N*-dimethylformamide (4% final concentration), adding Tween 80 (58% final concentration) and distilled water to a total volume of 1 ml/kg. The administered dose of HCT-1026 was calculated equimolar (mg.eq/kg) to the parent compound flurbiprofen. The changes in bladder volume capacity (BVC) were evaluated and $AUC_{(0\text{-}60min)}$ were calculated after intravenous administration versus time 0 value (pretreatment) for the two compounds with both saline solution and acetic acid infusion. Data showed a statistically significant positive increase in the BVC for the two compounds with both saline solution and acetic acid infusion. HCT-1026 was more potent than flurbiprofen in the saline infusion tes and much more potent in the acetic acid infusion test at a dose of 0.3 eq/kg.

When considering the effects on BVC, HCT-1026 was slightly more active than equimolar doses of flurbiprofen in increasing the BVC in saline-infused rats. It was also more potent in conteracting the reduction in BVC induced by the noxious stimulus than the corresponding acid. The nonconsistent and slight effect on micturition pressure exerted by the two compounds suggests that they could increase the bladder capacity by acting on bladder afferences (or by interfering with the regulatory activity of the micturition center) without impairing bladder contractility at the peripheral level. This may be confirmed, in part, by the data obtained in the rhythmic bladder voiding contractions model in anesthetized rats.

Gastric damage associated with hemorrhagic shock appears to occur, at least in part, through a neutrophil-dependent mechanism. Since NO can inhibit neutrophil adherence, we compared the effects of HCT-1026 and flurbiprofen (5-20 mg/kg p.o.) on gastric mucosal injury and leukocyte adherence. HCT-1026 was much better tolerated than flurbiprofen (15). Flurbiprofen at a dose of 5 mg/kg provoked dose-dependent gastrointestinal damage, whereas HCT-1026 was well tolerated up to a dose 20 mg/kg. Histological evaluation confirmed these findings. Futhermore, unlike flurbiprofen (10 mg/kg), HCT-1026 (15 mg/kg) did not induce significant leukocyte adherence. In the flurbiprofen group, vessel diameter decreased by an average of 6.7% during 1 h after administration, whereas in the HCT-1026 group, vessel diameter increased by an average of 16.6%.

We recently studied the effects of HCT-1026 on osteoclast formation in the murine osteoclast/bone marrow coculture assay. This experiment showed that HCT-1026 strongly inhibited interleukin-1-stimulated osteoclast formation at concentrations of 10-100 mM. Unlike flurbiprofen, HCT-1026 also inhibited basal bone resorption. Thus, HCT-1026 appears to be a potent inhibitor of osteoclast formation, with effects that are qualitatively and quantitatively different from the parent compound flurbiprofen (16).

Toxicity

The toxicological profile of HCT-1026 was evaluated *in vitro* and *in vivo*. Mutagenicity was assessed in the Ames test, with and without metabolic activation (17). There was no appreciable increase in the number of reversions with HCT-1026 in comparison with the negative control in any of the experiments at all doses tested (50-5000 μg/plate) for all strains of *Salmonella typhimurium*, in both the presence and absence of metabolic activation. Toxicity *in vivo* was also evaluated in a single-dose toxicity study. Sprague-Dawley rats were treated with HCT-1026 either orally (100, 150, 200, 300 and 400 mg/kg) or intraperitoneally (100, 150 and 200 mg/kg) and kept under observation for 14 days. No toxicity was observed in animals treated with oral or intraperitoneal doses of 100 mg/kg, except for piloerection and hunched posture which lasted for 1-2 days. The LD_{50}s in rats were 341 mg/kg p.o. and 162 mg/kg i.p. (18).

Clinical Studies

A pharmacodynamic and pharmacokinetic study of HCT-1026 (100 mg) in healthy volunteers has been completed. The results showed an excellent gastrointestinal and systemic tolerability. The bioavailability of HCT-1026 capsules was good. HCT-1026 was detected within 1 h after administration and flurbiprofen was present in plasma at later times. Full inhibition of platelet aggregation was found at all time points for HCT-1026 and flurbiprofen (19).

A crossover, single-dose administration of HCT-1026 (50 or 100 mg) and flurbiprofen (100 mg) demonstrated that HCT-1026 was absorbed and well tolerated. There were no adverse events and all subjects had regular blood pressure and heart rate measurements (20).

In a 7-day gastroscopic clinical trial, HCT-1026 was found to cause less gastric damage than the parent compound, while exerting the same inhibitory effects on gastric mucosal prostaglandin synthesis and serum thromboxane levels (21).

Manufacturer

NicOx, S.A. (FR).

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