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Sodium *N*-(Methylsulfonyl)-*N*-(4-nitro-2-phenoxyphenyl)sulfamate: A Water-Soluble Nimesulide Prodrug for Parenteral Use

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Abstract: Several nimesulide preparations (i.e., tablet form, gels) have been marketed, but no parenteral solution has achieved the market because of their low wettability and unsatisfactory chemical−physical properties required for parenteral use. In this paper we describe the synthesis of the nimesulide prodrug **1** and its anti-inflammatory and antihyperalgesic properties. Pharmacological studies, carried out to evaluate the *in vivo* anti-inflammatory and analgesic activities of compound **1** and nimesulide, showed that sodium sulfamate **1** is an effective nimesulide prodrug that can be administered by parenteral route, undergoing a satisfactory absorption and an extensive transformation into the active nimesulide compound. Moreover, the evaluation of the plasma concentrations of nimesulide after rat treatment with compound **1** showed an increased and dose-dependent release of nimesulide. In contrast, the plasma concentrations of nimesulide, after "native" drug administration, still remain substantially unchanged. These preliminary results prompt further investigations on this prodrug as a possible candidate for parenteral use.

Keywords: Anti-inflammatory prodrug; analgesic prodrug; COX2 preferential drug; analgesia; nimesulide

Introduction

In the 1990s encouraging biochemical, pharmacological and clinical data prompted researchers to develop a new generation of anti-inflammatory drugs endowed of high selectivity toward COX2 isoenzyme. Although initially COX1 and COX2 were considered as the main isoenzymes responsible for the production of cytoprotective (COX1) and pro-inflammatory (COX2) prostaglandins, nowadays the physiological role of these isoenzymes is recognized as much more complex. Thus, there is a possibility that prostaglandins produced as a result of COX1 expression may also contribute to inflammation, pain and fever. Moreover, also prostaglandins produced by COX2 have been shown to play important physiological functions, including the regulation of bone resorption,[1] and the contribution to mucosal protection.[2,3] Recently, the detrimental role of COX2 enzyme in cardiovascular homeostasis has been underlined. Brief periods of ischemia increase the myocardial resistance against a sub-

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sequent and more severe ischemia. This "ischemic preconditioning" (IPC), articulated in two phases, is ensured by several different biological mechanisms involving many triggers and/or end-effectors,[4,5] and COX2 may be included in this plethora of mediators.[6,7] In particular, COX2 activity is upregulated during the last phase of IPC and it seems to be implicated in cardioprotection.[7,8] Therefore the complete inhibition of this pathway may not always be beneficial. As a matter of fact, highly selective COX2 inhibitors showed a better gastrointestinal tolerability, but their long-term use produced severe cardiac side effects. The fair compromise seems to be a right balance between the inhibitory activity on both isoenzymes. This target seems to be fulfilled by the so-called "preferential COX2 inhibitors", namely, having from 5- to 20-fold greater potency against COX-2 than COX-1 such as nimesulide and meloxicam. In particular, nimesulide met with great success due to its excellent efficacy in painful and inflammatory conditions, especially when a fast onset of analgesic activity is required. Also, its tolerability profile was considered generally good. It is mainly indicated for joint inflammation, osteoarthritic pain, fever, musculoskeletal conditions, acute pain including that from perioperative conditions, and dysmenorrhea.

More recently, in some countries the use of this drug has been suspended or strongly limited because of its liver toxicity. However, this toxicity seems not related to the mechanism of action, but rather to unpredictable, idiosyncratic hepatic reactions. Its uniqueness is due to its fair balance of its inhibitory activity toward the two COXs. From the pharmaceutical point of view, nimesulide is characterized by reduced bioavailability. Indeed, the very poor aqueous solubility (about 0.01 mg/mL) and wettability of this drug give rise to difficulties in the design of some pharmaceutical formulations for oral and in particular parenteral use. The parenteral administration is of particular interest because the exclusion of the first-pass or presystemic metabolism could contribute to reduce the liver exposure.

To overcome these limitations a variety of nimesulide preparations (i.e., tablet form, syrup, gels) have been marketed, but no parenteral solution has achieved the market. In order to improve the solubility of nimesulide solid dispersions, cyclodextrin complexes and conglobation in nano- or microspheres have been studied.[9−13] However, the only advantage of the aqueous solutions obtained with these approaches is that they have a pH that can be physiologically tolerated, whereas there is little increase in water solubility so that in no case do they allow parenteral administration of the drug.

Nimesulide has acidic properties, thus it can form salts with inorganic or organic bases, but due to the relatively high pK_a value (6.5), a substantial solubility can be achieved only at very high pH values. Solubility of nimesulide lysine salt[14] is only 5.42 mg/mL. To increase this value, complex technological interventions are required. Higher water solubilities are reported for *N*-methyl glucamine[15] and choline[16] salts (160 and 500 mg/mL, respectively), but their pH values must be maintained between 8 and 9, thus compromising the tolerability of these aqueous solutions.

In order to enhance the bioavailability of nimesulide, thus improving its pharmacokinetic and pharmacodynamic profiles, we believed it important to study a new nimesulide prodrug, soluble in water at neutral pH and then useful for parenteral administration, able to release in vivo the parent drug without toxicological liability connected to the chemical transformation.

We conceived that a salt of the *N*-sulfamate derivative of nimesulide could have the requisites of wettability and solubility and relative instability in vivo which could be useful to reach our purposes.

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Therefore, in this paper we describe the synthesis of the nimesulide prodrug (**1**, sodium salt of *N*-(methylsulfonyl)-*N*-(4-nitro-2-phenoxyphenyl)sulfamic acid) and its anti-inflammatory and analgesic properties compared to those of nimesulide.

Experimental Section

Materials and Methods. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Mass spectra were recorded on a VG 70-250S mass spectrometer or an HP-5988 A spectrometer. The elemental compositions of the compounds agreed to within $\pm$0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040−0.063 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was used as the drying agent. Commercially available chemicals were purchased from Sigma-Aldrich.

Preparation of Sodium *N*-(methylsulfonyl)-*N*-(4-nitro-2-phenoxyphenyl)sulfamate (1). To a solution of 2-picoline (2.22 g, 23.86 mmol) in CH_2Cl_2 anhydrous (10 mL) cooled at −5 °C was added dropwise chlorosulfonic acid (1.38 g, 11.83 mmol) maintaining the temperature at 5 °C. The resulting solution was stirred for 15 min at temperature of −5°/5 °C, then was treated dropwise with a solution of nimesulide (1.00 g, 3.25 mmol) in CH_2Cl_2 (6 mL) and refluxed for 24 h. After this period the reaction mixture was cooled and washed with a buffer solution at pH = 4.6, and the organic layer was evaporated. The residue was chromatographed on a silica gel column eluting with AcOEt, and then the product was diluted in EtOH and treated with AcONa. The resulting solution was evaporated and the residue crystallized by i-PrOH to afford compound **1** (387 mg; 0.87 mmol; 26.7%). Mp:152−155 °C (decomp). ^{1}H NMR (D_2O): δ 3.49 (s, 3H, Me); 7.20 (d, 2H, J = 8.4 Hz, Ar); 7.27−7.35 (m, 1H, Ar); 7.46−7.54 (m, 2H, Ar); 7.75 (dd, 1H, J = 8.8, 2.5 Hz, Ar); 7.82 (s, 1H, Ar); 8.04 (dd, 1H, J = 8.8, 2.5 Hz, Ar) ppm. ^{13}C NMR (DMSO-d_6): δ 155.36; 155.32; 146.87; 135.66; 132.64; 130.34; 124.99; 120.11; 117.43; 111.99; 41.49 ppm.

MS (ES, *m*/*z*): 316 (M^+ − PhOH); 288 (M^+ − SO_3Na − H_2O). MS (FAB, *m*/*z*): 307 (M^+ − SO_3Na); 289 (M^+ − SO_3Na − H_2O). Anal. ($C_{13}H_{11}N_2O_8S_2Na\cdot 2H_2O$) C, H, N, S [% calcd, 34.98 (C), 3.39 (H), 6.98 (N), 14.37 (S); % found, 35.18 (C), 3.50 (H), 6.87 (N), 14.22 (S)].

Stability of Sodium *N*-(methylsulfonyl)-*N*-(4-nitro-2-phenoxyphenyl)sulfamate (1). The stability of the tested salt was evaluated in water and in phosphate buffer (pH 7.4). The solution of compound **1** was stirred at 25 °C for 1 day. The analyzed probes were collected at definite time intervals (30 min, 1 h, 6 h, 12 h and 24 h) and mixed with deuterated water (D_2O). In these conditions, the NMR analysis does not reveal the formation of any decomposition products thus indicating a high degree of stability of prodrug **1** in the above-mentioned experimental conditions.

Pharmacological Method. *Animals.* Male Sprague−Dawley albino rats (Charles River, Calco, Italy) weighing between 200 and 250 g were used for these studies. The animals were housed 4 to a cage, at 22 °C $\pm$ 2 °C with a light−dark cycle of 12/12 h and free access to water and food. The rats were allowed to habituate to the housing facilities for 1 week before the experiments began. Behavioral studies were carried out in a quiet room between 8:00 and 12:00. Eight rats were used in each experimental group. All procedures were approved by the Department of Pharmacology of the University of Milan Animal Care and Use Committee and followed the ethical guidelines for the treatment of animals of the International Association for the Study of Pain.[17] All efforts were made to minimize the number of animals and their suffering.

Induction of Paw Inflammation and Evaluation of Edema and Hyperalgesia. Paw inflammation was induced by subcutaneous injection into the midplantar region of the left hindpaw of 100 μL of 1% carrageenan (type IV, Sigma, St. Louis, MO) prepared in saline.

The intensity of inflammatory edema and mechanical hyperalgesia were measured 3 h after the intraplantar injection of carrageenan. The paw swelling (edema) was assessed by measuring the volume of both hind paws by a plethysmometer (7150 plethysmometer, Basile, Comerio, Italy). The results are expressed as the algebraic difference between the volume (mL) of inflamed (carrageenin-injected) and normal hind paw.[18,19]

The Randall−Selitto paw-withdrawal test, which uses mechanical force as nociceptive stimulus, was used to measure inflammatory hyperalgesia.[18,19] The stimulus was applied with an analgesymeter (Basile, Comerio, Italy) which generates a linearly increasing mechanical force, applied by a conical piece of plastic with a dome-shaped tip on the dorsal surface of the rat's hind paw. The animals were gently held, and incremental pressure (maximum 250 g) was applied onto the dorsal surface of the hind paw. The thresholds represent the pressure (expressed in grams) at which the animal withdrew its hind paw. To avoid tissue damage, only one trial was performed at this time point. The observer was

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blind to treatment allocation of the animals. For blinding completeness, both nimesulide and prodrug were labeled with a code and no dose adjustment for molecular weight was requested.

Pharmacological Treatment Drugs. Nimesulide and the parent compound **1** were dissolved in a vehicle composed of metocell 0.5%/saline and injected by intraperitoneal route (ip) in a volume of 0.2 mL/100 g of body weight. Control animals were treated ip with the same volume of vehicle. All drugs were administered 15 min before the induction of paw inflammation.

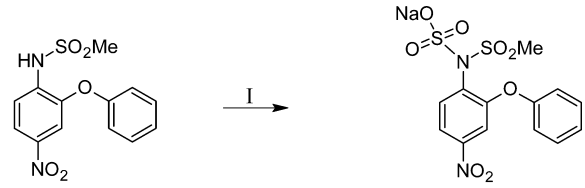
HPLC Analysis of Nimesulide Plasma Concentrations. Nimesulide plasma concentrations were measured by HPLC with UV detection, using a method previously described and validated.[20,21] Immediately after the behavioral evaluations the animals were sacrificed by decapitation and blood was collected in heparinized polypropylene tubes (containing 45 USP units sodium heparin) and centrifuged at 2500*g* for 10 min. Plasma was transferred in polypropylene tubes and immediately frozen. Frozen plasma samples were thawed at room temperature for about 30 min then vortexed for 30 s. Plasma (250 μL) was pipetted into a 5 mL polypropylene tube, and this was followed by addition of 1 mL of ice-cold 50 mM $Na_2HPO_4 \cdot 12H_2O$, pH 3.00 buffer, containing 2500 ng of the internal standard. This mixture was vortexed for 30 s and then extracted. Solid-phase extraction of plasma was performed on disposable C_{18} cartridges primed with 1 volume of methanol and 1 volume of 50 mM $Na_2HPO_4 \cdot 12H_2O$, pH 3.00 buffer. Plasma, prepared as described above, was loaded on the conditioned cartridges. After washing with two volumes of the same phosphate buffer, the elution was carried out with two fractions of 0.5 mL of methanol. The eluate was evaporated to dryness at 40 °C under nitrogen flow, and the residues were reconstituted in 100 μL of mobile phase. Aliquots (50 μL) of this solution were injected into the HPLC system.

Nimesulide standard was kindly provided by Helsinn Healthcare (Pambio-Noranco, Switzerland). Internal standard, 2′-(cyclohexyloxy)-4′-nitrophenyl methanesulphonanilide NS39, was purchased from Inalco (Milan, Italy) while disodium orthophosphate dodecahydrate, sodium citrate and orthophosphoric acid, all of analytical grade, were obtained from Sigma (St. Louis, MO). Methanol and acetonitrile (LC grade) were purchased from Merck (Darmstadt, Germany).

A Millipore Waters model 590 liquid chromatograph (Waters Milford, MA), with a variable wavelength LC75 Spectrophotometric UV detector (Perkin-Elmer Norwalk, CT) was employed. The injection valve was a model 7125 rheodyne (Cotati, CA). The system was connected to a Hitachi-Merck D-200 chromato-integrator (Merck). A Waters Symmetry column C18 3.5 mm (150 × 4.6 mm internal diameter), coupled to a Waters Sentry Symmetry C18 guard column (10 × 2.1 mm internal diameter), was operated at room temperature. The mobile phase was acetonitrile:50 mM sodium citrate buffer, pH 3.00 (53:47). The buffer was prepared fresh daily by dissolving 16.8 g of citric acid and 2.66 g of sodium hydroxide in 1000 mL of bidistilled water and adjusting the solution to pH 3.00 with orthophosphoric acid. The flow rate was 1.1 mL/min, and the elution was monitored at 240 nM.

Statistical Analysis. The data were analyzed by one way ANOVA followed by Bonferroni *t* test for multiple comparison. *P* values <0.05 were considered statistically significant.

Scheme 1[a]



Nimesulide **1**

[a] I: (a) 2-picoline, chlorosulfonic acid, anhydrous DCM, −5 °C/5 °C, then refluxed; (b) AcONa, EtOH.

Results and Discussion

Compound **1** (Scheme 1) was obtained by reaction of nimesulide and chlorosulfonic acid in the presence of picoline. After the workup, the treatment of the crude product with AcONa in EtOH afforded the corresponding sodium salt **1**. The stability of the sodium salt **1** was evaluated in water and in phosphate buffer (pH 7.4) (see Experimental Section). Any decomposition product was detected after stirring for 24 h at room temperature of both aqueous and buffer solutions. Studies concerning the preparation of injectable solutions and their stability are beyond the scope of this work.

Pharmacological studies were carried out to evaluate the in vivo anti-inflammatory and analgesic activities on compound **1** and nimesulide. As expected, 3 h after the injection of carrageenan into the hind paw a marked increase in paw volume (edema), associated with a decrease in the paw withdrawal latency to noxious mechanical stimulation, were observed. The effect of rat treatment with either vehicle, compound **1**, or nimesulide injected ip 15 min before the induction of inflammation is reported in Figure 1. The upper panel shows that the prodrug **1** inhibited the edema formation in a dose-dependent manner: a slight reduction was already present at the dose 1.25 mg/kg, and the edema inhibition reached statistical significance at the doses of 2.5 mg/kg and 5.0 mg/kg. In contrast, parental nimesulide was able to modestly decrease edema formation only at the highest dose of 5.0 mg/kg.

The effect of the two drugs on inflammatory hyperalgesia is shown in the lower panel. The carrageenan-induced

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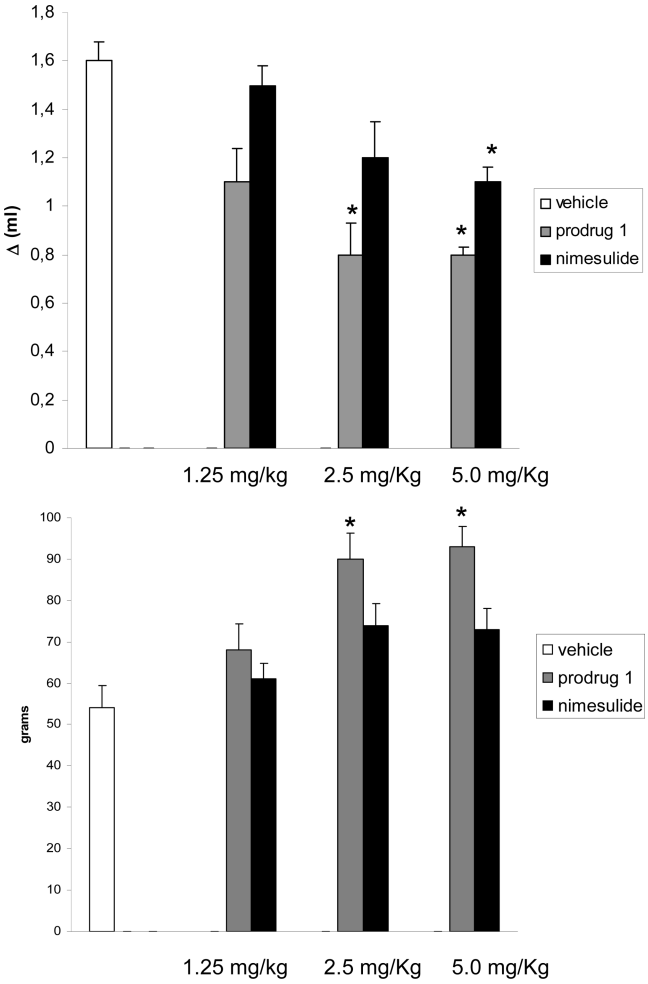

Figure 1. Upper panel: Effect of prodrug **1** and nimesulide (1.25, 2.5, and 5.0 mg/kg ip) on the inflammatory edema induced by the hindpaw injection of carrageenan. Drugs or vehicle were administered 15 min before carrageenan injection, and edema measurement was performed 3 h after induction of inflammation. Data are expressed in mL, as mean ± SEM of the algebraic difference between the volume of inflamed (carrageenan-injected) and uninflamed hind paw. Lower panel: Effect of prodrug **1** and nimesulide (1.25, 2.5, and 5.0 mg/kg ip) on the inflammatory hyperalgesia induced by the hindpaw injection of carrageenan. Drugs or vehicle were administered 15 min before carrageenan injection, and the evaluation of hyperalgesia was performed by Randall−Selitto test 3 h after carrageenan. Data are the thresholds to mechanical stimulation, expressed in grams, as mean ± SEM. * = $P < 0.05$ vs carrageenan + vehicle.

mechanical hyperalgesia was significantly reduced by the parent compound **1** at doses of 2.5 and 5.0 mg/kg, while no significant difference was identified between the values measured in animals treated with **1** (1.25 mg/kg) and those measured in rats treated with vehicle. Moreover ip nimesulide did never affect inflammatory hyperalgesia, since thresholds to mechanical stimulation were not significantly different from those of vehicle treated inflamed animals.

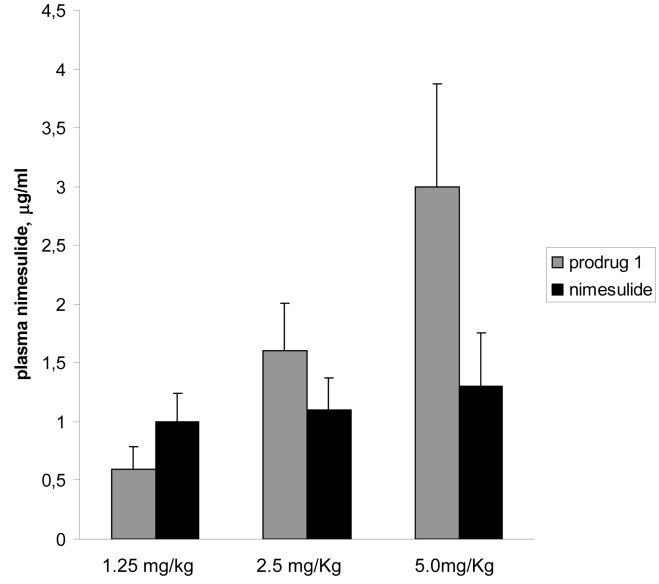

Figure 2. Plasma concentrations of nimesulide measured by HPLC method 3.5 h after ip treatment of rats with either nimesulide or its prodrug **1** at the doses of 1.25, 2.5, and 5.0 mg/kg. Values are means ± SEM of 8 animals/group.

A highly sensitive HPLC method was used in order to measure plasma concentrations of nimesulide after ip rat treatment with the prodrug **1** or with nimesulide itself (Figure 2). The drugs were administered at the doses of 1.25, 2.5, and 5 mg/kg and plasma concentrations evaluated at the end of behavioral experiments, corresponding approximately to 3.5 h after drug administration. The concentrations of plasma nimesulide measured after ip nimesulide treatment do not appear to change in a significant way depending on the dosage utilized. In contrast after rat treatment with the nimesulide prodrug **1** the presence of a dose related increase of plasma nimesulide concentrations is evident.

The results presented in this study confirm that compound **1** has many characteristics that make it a reliable nimesulide prodrug. The HPLC analysis clearly shows that when "native" nimesulide is administered by ip route an erratic and unpredictable absorption is present, since the plasmatic concentrations that are measured are not dose dependent and remain low. Therefore, as well as for most drugs, also for nimesulide a clear relationship between plasma concentrations and pharmacological effect does not exist. From our study it can be suggested that in order to have a significant anti-inflammatory effect in vivo (Figure 1) the minimum necessary nimesulide plasma level should be higher than 1 μg/mL (Figure 2). In contrast, after the parental administration of the prodrug **1**, a dose related increase of nimesulide concentrations can be measured in plasma, reaching much higher plasma concentrations in comparison with the same doses of nimesulide. The better solubility of prodrug **1** at neutral pH can explain its higher and more regular absorption. Moreover, the relevant plasma concentrations of nimesulide that are reached indicate that the parent drug is significantly released in vivo.

The doses of nimesulide that were used in the in vivo experiments have been previously shown to be frankly anti-inflammatory when administered by oral route.[20,22] In contrast, in our work, the same nimesulide dosages injected ip only slightly affected the inflammatory edema and were never able to prevent hyperalgesia, thus further confirming that the parenteral route is not adequate for the drug. As concerns the parental administration of **1**, it exerted both anti-inflammatory and anti-hyperalgesic activities which proved to be of statistical significance since the dose of 2.5 mg/kg.

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Conclusion

The in vivo study demonstrated that sodium sulfamate derivative **1** is an effective nimesulide prodrug since, in contrast to the "native" drug, it can be administered by the parenteral route, undergoes satisfactory absorption and an extensive transformation into the active nimesulide compound and, most importantly, exerts significant and possibly clinically relevant anti-inflammatory and antihyperalgesic effects.

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