Brief Articles

Spectral and Crystallographic Study of Pyridinic Analogues of Nimesulide: Determination of the Active Form of Methanesulfonamides as COX-2 Selective Inhibitors

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Compound **7**, *N*-(3-phenoxy-4-pyridinyl)trifluoromethanesulfonamide, showed in vitro (whole blood assay) a strong inhibitory activity on the two cyclooxygenase (COX) enzymes (IC_{50}(COX-1) = 2.2 μM and IC_{50}(COX-2) = 0.4 μM), being more active but less COX-2-selective than nimesulide. Physicochemical studies and structural analyses indicated that the anionic sulfonamidate species seemed to be the active form of methanesulfonamides, which optimally interacted with the COX enzymes' active sites.

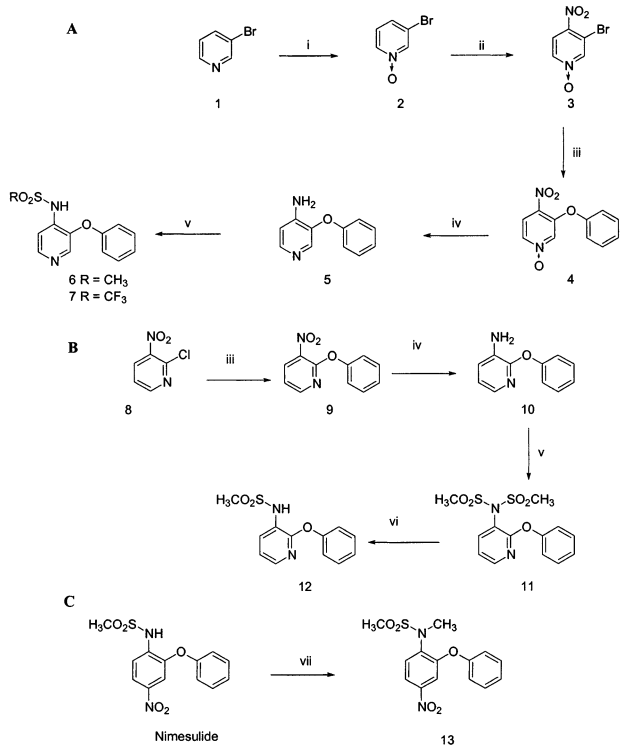
Introduction

Prostaglandins (PGs) are key mediators involved in the inflammation processes. They are synthesized by cyclooxygenases (COXs) from arachidonic acid.[1] COX-1 is a constitutive enzyme responsible for the physiological production of PGs. This enzyme is involved in several homeostatic processes and is thus considered as a "housekeeping" enzyme. In contrast, COX-2 is an inducible enzyme, which is mainly produced during inflammation processes. The inhibition of COX-1 is thought to be responsible for the side effects of the nonsteroidal antiinflammatory drugs (NSAID).[2] Antiinflammatory drugs inhibit the synthesis of PGs by inhibiting these enzymes. Following the discovery of the second isoform of cyclooxygenase,[3] the search for new inhibitors of the COX-2 isoform has led to the development of selective drugs such as celecoxib.[4]

At the present time, nimesulide is marketed as a NSAID and is reported as a COX-2-selective methanesulfonamide.[5] The methanesulfonamide class has been widely developed in the literature.[6,7] All compounds of this class present the same chemical skeleton: a benzene ring substituted by an electron-withdrawing group at the 4-position.

To determine the ionic state of drugs from the pyridinic methanesulfonamide class expected to interact with the active site of the COX enzymes, four derivatives have been synthesized. Their chemical structures have been determined from X-ray, IR, and NMR experiments. Their inhibitory effects on the COXs and their selectivity have been evaluated by a whole blood assay. The antiinflammatory effects of these drugs have also been determined in vivo by a carrageenan-induced rat paw oedema test.

Scheme 1[a]



[a] (i) H_2O_2, CH_3COOH, Δ; (ii) HNO_3, H_2SO_4, Δ; (iii) $C_6H_4O^-Na^+$, Δ; (iv) Fe, H_2O/CH_3COOH, Δ; (v) $R-SO_2Cl$, K_2CO_3/CH_3CN; (vi) KOH, Δ; (vii) CH_3I, K_2CO_3/CH_3CN.

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Table 1. Estimated IC_{50} Values from Whole Blood Assay and Effect on Rat Paw Oedema for Compounds **6**, **7**, **12**, and **13** and Nimesulide[a]

	whole blood assay			antiinflammatory effect on rat paw oedema		
compd	IC_{50}(COX-1) (μM)	IC_{50}(COX-2) (μM)	IC_{50}(COX-1)/ IC_{50}(COX-2)	10 mg/kg dose	30 mg/kg dose	control
6	4.9	37.5	0.13	NT	toxic	
7	2.2	0.4	5.28	74.7 ± 7.2	54.1 ± 17.5	96.0 ± 8.7
12	ni	ni		NT	NT	
13	ni	ni		84.6 ± 4.7	65.6 ± 16.8	97.3 ± 13.7
nimesulide	22.0	1.3	16.92	58.0 ± 6.0	54.0 ± 7.0	97.0 ± 3.0

[a] ni: no inhibition at 100 μM. NT: not tested. Results on rat paw oedema are expressed as percentage of growth of the paw after injection of carrageneen (mean ± standard deviation; $n = 6$).

Chemistry

The synthesis of compound **6** has been previously described[8] but has been optimized in order to easily obtain compounds **6** and **7**. Those two compounds were synthesized in five steps. The first four steps of Scheme 1A led to the aminopyridine **5**. The synthesis started with oxidation of 3-bromopyridine **1** by hydrogen peroxide in the presence of acetic acid. This oxidation was followed by nitration at the 4-position of the pyridine *N*-oxide **2** by a mixture of nitric acid and sulfuric acid at 90 °C. After that, the bromine atom was substituted with sodium phenolate to give compound **4**. The nitro and the *N*-oxide groups were then reduced by iron in the presence of acetic acid and water to afford the aminopyridine **5**. Compounds **6** and **7** were obtained by reaction of **5** with the appropriate sulfonyl chloride.

The synthesis of compound **12**, a geometric isomer of compound **6**, was achieved in four steps (Scheme 1B). This scheme presents an alternative pathway to the previous process used for the synthesis of **10**.[9] First, the chlorine atom of 2-chloro-3-nitropyridine was substituted with sodium phenolate. In the second step, the nitro group of compound **9** was reduced by iron in the presence of acetic acid and water to produce the aminopyridine **10**. Compound **10** reacted with an excess of mesyl chloride to form the sulfonimide **11**. This sulfonimide was hydrolyzed by KOH in aqueous medium under reflux to afford compound **12**.

Nimesulide reacted with iodomethane in the presence of potassium carbonate in acetonitrile to produce compound **13** (*N*-methyl-*N*-(4-nitro-2-phenoxyphenyl)-methanesulfonamide, *N*-methylnimesulide) in good yield.

Pharmacological Evaluation

Compounds **6**, **7**, **12**, and **13** and nimesulide have been evaluated as COX inhibitors in vitro and as NSAID in vivo. For the in vitro evaluation (whole blood assay), each drug was studied in triplicate at drug concentrations ranging from 100 to 0.01 μM and the IC_{50} values were calculated. In this assay, the COX-1 activity was measured as TXB_2 production after blood coagulation, and the COX-2 activity was measured as PGE_2 production after stimulation by lipopolysaccharide (LPS). Table 1 reports the results obtained with compounds **6**, **7**, **12**, and **13** and nimesulide. As observed, compound **6** inhibited both COX-1 and COX-2 but was a COX-1 preferential inhibitor. In contrast, the results with compound **7** and nimesulide showed that the two drugs were COX-2 preferential inhibitors, compound **7** being more potent on COX-2.

Those compounds, except **12**, were also evaluated in a rat paw oedema study. In this test, compound **6** was found to be toxic after an intraperitoneal injection at a dose of 30 mg/kg (proconvulsant). Compound **7** presented a dose-dependent antiinflammatory effect. At 30 mg/kg, the antiinflammatory effect of compound **7** was similar to that of 10 mg/kg nimesulide. Compound **13**, which was inactive in vitro, presented an antiinflammatory effect at a dose of 30 mg/kg. This activity could be explained by a metabolization of the product to generate nimesulide. It is hypothesized that compound **13** acts as a prodrug of nimesulide.

Structural Analysis

To determine the ionic state of the compounds in solution at the physiological pH of 7.4, we have evaluated the pK_a values of compounds **6**, **7**, and **12** by spectrophotometry. Compound **6** has two pK_a values of 2.98 and 8.13. Compounds **7** and **12** were found to have a first pK_a value under 1 and a second of 6.1 and 7.85, respectively. Nimesulide has a pK_a value of 6.56.[10] By this method, the pK_a cannot be associated with the species in equilibrium.

To elucidate the ionic state of the molecules in the solid state, we have studied the compounds by IR spectrometry and crystallography. The IR spectra of compounds **6** and **7** revealed that the two drugs appear to be pyridinium ions in the solid state. Those compounds present three broad absorption bands between 2800 and 2650 cm^{-1} expected for the presence of a N^+-H bond. In contrast, compound **12** presents a strong absorption band at 3226 cm^{-1} that can be associated with a N−H bond. This strong absorption band is also present in the spectrum of nimesulide at 3284 cm^{-1}. Finally, compound **13** is devoid of any absorption band near 3200 cm^{-1} as well as between 2800 and 2650 cm^{-1}. A previous crystallographic study of **6** confirmed that this drug was a zwitterionic pyridinium compound substituted in the 4-position by an anionic sulfonamidate group.[11] Figure 1 reports an ORTEP view of the crystallographic structures of compounds **7** and **12**. Compound **7** was also found to be in a comparable zwitterionic pyridinium form in the solid state. In contrast, the X-ray structure of **12** revealed that the drug was present in the crystal as an un-ionized pyridine compound substituted with a nonanionic methanesulfonamido group. Furthermore, this analysis revealed that in **7** the $C_9-N_{10}-C_{11}$ angle was 121.1° and that in **12** the $C_8-N_9-C_{10}$ angle was 116.4°. This information also led to the conclusion that **7** was crystallized as a pyridinium compound and **12** as an un-ionized pyridine compound. Finally the structure of compound **13** has been confirmed by a recently published crystallographic study.[12]

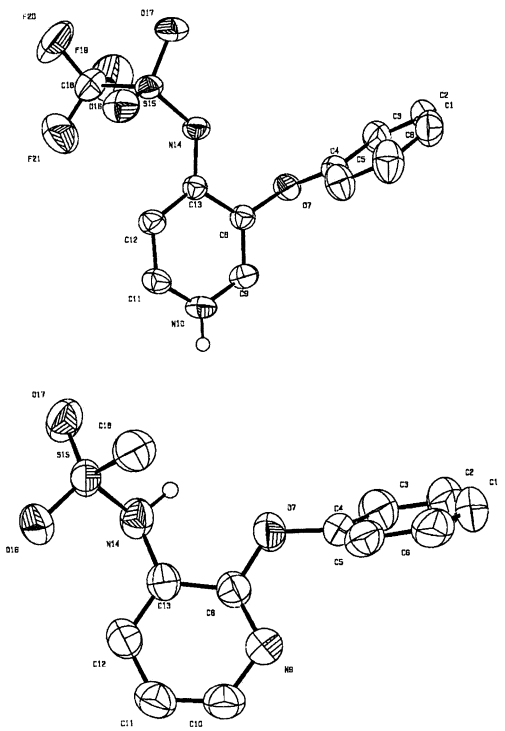

Figure 1. ORTEP view of compounds **7** (top) and **12** (bottom).

Compounds **6**, **7**, and **12** were also studied by ^{1}H NMR in deuterated DMSO. All compounds presented an exchangeable proton. For nimesulide, the proton of the sulfonamide function was found at a chemical shift of 10.13 ppm. For compounds **6**, **7**, and **12** in the same solvent, the chemical shift of the exchangeable proton appeared at 11.87, 13.90, and 9.64 ppm, respectively. By comparison of the respective chemical shifts of the N−H proton, it can be hypothesized that nimesulide and compound **12** present a protonated sulfonamide function (−SO_2NH−; expected chemical shift around 10 ppm) and that compounds **6** and **7** are zwitterions with a pyridinium N^+−H proton found in a more deshielded position (around 12−14 ppm), and in the para position, a sulfonamidate (SO_2N^-−) function is present. Such a structural feature is confirmed by the chemical shift of the CH_3 group, which was found at 2.88, 3.15, and 3.17 for **6**, **12**, and nimesulide, respectively. Because of the proximity of the negative charge in **6**, the methyl group appeared to be shielded in compound **6** compared to **12** and nimesulide.

For compounds **6** and **7**, two hypotheses can be proposed for the attribution of the pK_a values to the equilibrium involved (Figure 2). In the first hypothesis, the lowest pK_a value should be associated with the equilibrium pyridinium/pyridine and the highest with the equilibrium sulfonamide/sulfonamidate. In the second hypothesis, in contrast, the more acidic pK_a value should be associated with the equilibrium sulfonamide/sulfonamidate and the second with the equilibrium pyridinium/pyridine. The spectral and X-ray analyses of compounds **6** and **7** show that in the solid state and in solution in DMSO-d_6 those compounds are in the zwitterionic pyridinium sulfonamidate form. We can then conclude that for **6** the pK_a value of 2.98 could be attributed to the sulfonamide/sulfonamidate equilibrium and that the pK_a value of 8.13 could be attributed to

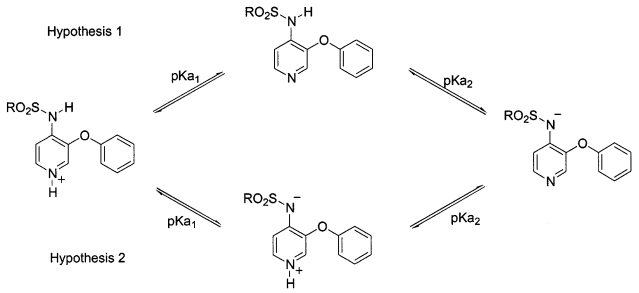

Figure 2. Hypotheses for the attribution of the pK_a values for compounds **6** and **7**.

the pyridinium/pyridine equilibrium. For compound **7**, the pK_a value of the equilibrium sulfonamide/sulfonamidate could be estimated as being less than 1 and the pK_a value of 6.81 could be associated with the equilibrium pyridinium/pyridine. For compound **12**, in contrast, the spectral and structural analyses show that in the solid state and in solution this compound is present as an un-ionized pyridine compound substituted by an un-ionized sulfonamide group. The protonated pyridinium nitrogen atom of compound **12** in acidic medium is expected to exhibit a pK_a value less than 1, and the sulfonamide function has a pK_a value of 7.85.

Conclusion

At the physiological pH of 7.4, compound **6** is expected to be mainly present as a positively charged pyridinium species substituted with an anionic sulfonamidate moiety (84.9%), compound **7** a nonprotonated pyridinic compound substituted by an anionic sulfonamidate moiety (79.5%), compound **12** a nonprotonated pyridinic compound substituted by a nonionic sulfonamide moiety (73.8%), and nimesulide an anionic sulfonamidate (87.0%). By comparison of the activity of nimesulide and compound **13**, we can conclude that inhibition of the COX enzymes probably requires the deprotonation of the sulfonamide function. Furthermore, the weak activity of compound **6** compared to **7** leads to the conclusion that the molecules should not bear a positively charged nitrogen atom on the pyridine ring. By comparison of the activity of compounds **6** and **12**, we can also conclude that the substitution at the 2- and 3-position of the pyridine ring is unfavorable for obtaining a COX inhibitor. Thus, optimal binding site interactions are obtained with anionic sulfonamidate compounds devoid of a positively charged nitrogen atom on the aromatic ring.

Finally, in the in vitro study, compound **7** was found to be more potent than nimesulide but less COX-2-selective. Furthermore, this compound presented an interesting in vivo antiinflammatory activity. Thus, this compound will serve as a lead structure in the design of new potent and selective nonsteroidal antiinflammatory drugs.

Experimental Section

Human Whole Blood Assay. The compounds have been evaluated by a whole blood assay performed on human blood as described by de Leval.[13]

Carrageenan-Induced Rat Paw Oedema. Wistar rats (250 g) were treated with an intraperitoneal injection of the drug at the appropriate concentration (solution at 10 mg/mL in DMSO). Lambda carrageenan (0.1 mL, 1%) was injected 1 h later in the plantar region of the right-hand paw. Three hours thereafter, the rats were euthanized by injection of

nembutal (100 mg/kg) and the paws were cut at the ankle. The swelling was calculated as a percentage increase in the weight of the control paw.

pK_a Determination. The procedure for the determination and calculation of the pK_a value was essentially the same as that described by Albert and Serjeant.[14]

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Supporting Information Available: Procedure for the synthesis of compounds **6**, **7**, **12**, and **13**, crystallographic data, and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dannhardt, G.; Kiefer, W. Cyclooxygenase Inhibitors—Current Status and Future Prospects. *Eur. J. Med. Chem.* **2001**, *36*, 109−126.

(2) Crofford, L.; Lipsky, P.; Brooks, P.; Abramson, S.; Simon, L.; van de Putte, L. Basic Biology and Clinical Application of Specific Cyclooxygenase-2 Inhibitors. *Arthritis Rheum.* **2000**, *43*, 4−13.

(3) Xie, W.; Chipman, J.; Robertson, D.; Erikson, R. L.; Simmons, D. Expression of a Mitogen-Responsive Gene Encoding Prostaglandin Synthase Is Regulated by mRNA Splicing. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, 2692−2696.

(4) Amgad, G.; Praveen Rao, P. N.; Knaus, E. E. Design and Synthesis of 4,5-Diphenyl-4-isoxazolines: Novel Inhibitors of Cyclooxygenase-2 with Analgesic and Antiinflammatory Activity. *J. Med. Chem.* **2001**, *44*, 2921−2927.

(5) Griswold, D. E.; Adams, J. L. Constitutive Cyclooxygenase (COX-1) and Inducible Cyclooxygenase (COX-2): Rational for Selective Inhibition and Progress to Date. *Med. Res. Rev.* **1996**, *16*, 181−200.

(6) Talley, J. Selective inhibitors of Cyclooxygenase-2 (COX-2). *Prog. Med. Chem.* **1999**, *36*, 201−234.

(7) Inaba, T.; Tanaka, K.; Takeno, R.; Nagaki, H.; Yoshida, C.; Takano, S.; Synthesis and Anti-inflammatory Activity of 7-Methanesulfonylamino-6-phenoxychromones. Antiarthritic Effect of the 3-Formylamino Compound (T-614) in Chronic Inflammatory Disease Models. *Chem. Pharm. Bull.* **2000**, *48*, 131−139.

(8) Cignarella, G.; Viaello, P.; Berti, F.; Rossoni, G. Synthesis and Pharmacological Evaluation of Derivatives Structurally Related to Nimesulide. *Eur. J. Med. Chem.* **1996**, *31*, 359−364.

(9) Eatough, J.; Fuller, L.; Good, R.; Smalley, R. Synthesis of Polynuclear Heterocycles. Part II. Cyclisations of 2- and 4-substituted 3-Amino and 3-Nitro-pyridines. *J. Chem. Soc. C* **1970**, 1874−1878.

(10) Singh, S.; Shardra, N.; Mahajan, L. Spectrophotometric Determination of pKa of Nimesulide. *Int. J. Pharm.* **1999**, *176*, 261−264.

(11) Michaux, C.; Charlier, C.; Julemont, F.; Dogne, J.-M.; de Leval, X.; Norberg, B.; Pirotte, B.; Durant, F. The *N*-(3-phenoxy-4-pyridinyl)methanesulfonamide, Strict Pyridinic Analogue of Nimesulide, a Selective Inhibitor of Cyclooxygenase-2. *Acta Crystallogr.*, in press.

(12) Michaux, C.; Charlier, C.; Julemont, F.; Norberg, B.; Dogne, J.-M.; Pirotte, B.; Durant, F. FJ6, *N*-methyl-(4-nitro-2-phenoxyphenyl)methanesulfonamide. *Acta Crystallogr.* **2001**, *E57*, o1012−o1013.

(13) de Leval, X.; Delarge, J.; Devel, P.; Neven, P.; Michaux, C.; Masereel, B.; Pirotte, B.; David, J.-L.; Henrotin, Y.; Dogne, J.-M. Evaluation of Classical NSAID and COX-2 Selective Inhibitors on Purified Ovine Enzymes and Whole Blood. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2001**, *64*, 211−216.

(14) Albert, A.; Serjeant, E. P. Refinements of Potentiometric Titration: Apparatus and Calculation. In *The Determination of Ionization Constants: A Laboratory Manual*; Ed Chapman & Hall Ltd.: London, 1971; pp 342−369.

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