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SYNTHESIS OF ACTIVE METABOLITES OF INDOLE PYRROLOTHIAZOLE PAF ANTAGONISTS

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Abstract: The major metabolites of indole pyrrolothiazole PAF antagonist **1** were shown to be sulfoxide **2** and pyridine *N*-oxide **3**. These compounds were generated *in vitro* using either isolated hepatocytes or liver microsomes. The synthesis of *N*-oxide **3** was accomplished by heating pyridinium salt **5** with hydroxylamine. Pyridine *N*-oxide **3** was determined to be a potent PAF antagonsist both *in vitro* and *in vivo*.

Platelet activating factor (PAF) has been implicated as a mediator of the pathologic effects observed in several inflammatory and allergic diseases.[1] PAF antagonists have consequently been targeted as potential new therapeutics for the treatment of asthma, allergic rhinitis, sepsis and ischemia reperfusion injury.[2] Our efforts in these area have culminated in the identification of indole pyrrolothiazole **1** which blocks the action of PAF both *in vitro* and *in vivo*.[3] The characterization and synthesis of the principle metabolites of **1** is the subject of this communication.

The pharmacokinetic properties of **1** have been evaluated in a number of species including rat, monkey and dog. In each case, elimination of **1** is coupled with the appearance of significant blood levels of two metabolites. This is illustrated in Figure 1 which shows the blood concentration of **1** and its metabolites after a 10 mg/kg dose in dogs.[4] One of these metabolites (Metabolite 2) exhibits a similar elimination half-life as **1**. In order to determine whether the *in vivo* activity of **1** is influenced by the presence of these metabolites, we sought to isolate, identify, synthesize and characterize both compounds.[5]

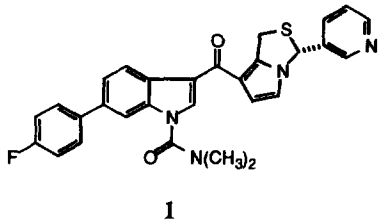

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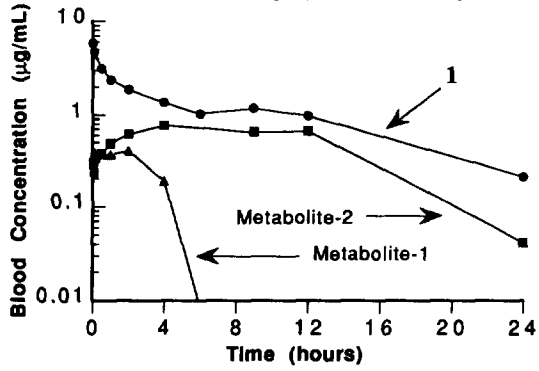
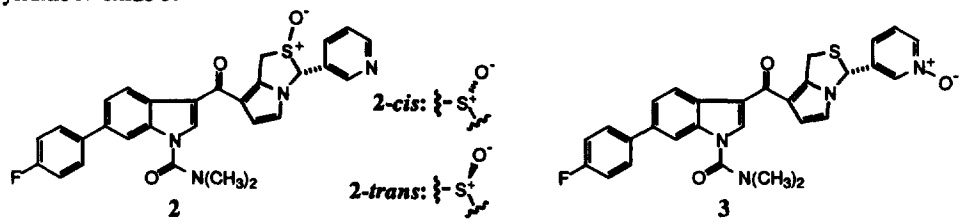
Figure 1. Blood Concentration of **1** and Metabolites After a 10 mg/kg iv Dose in Dog

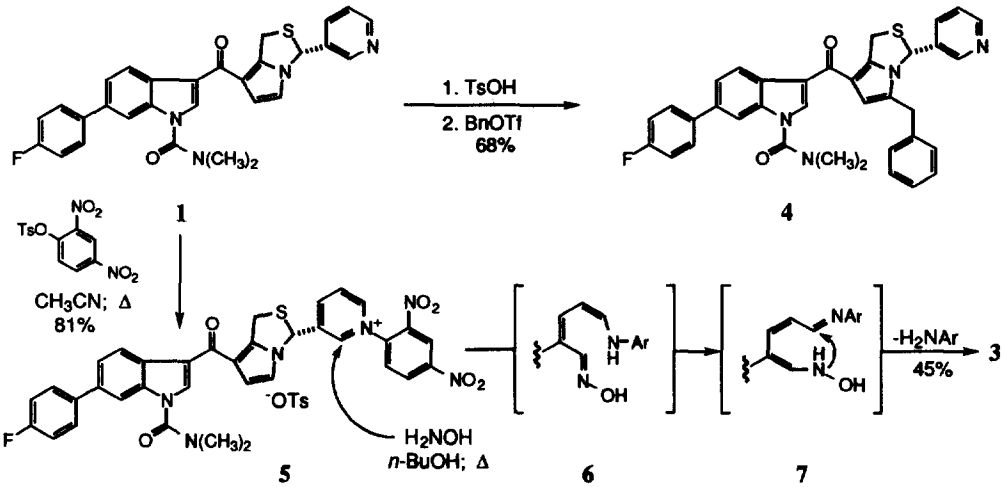


An *in vitro* system for the generation of the metabolites of **1** was established so that sufficient amounts could be isolated for structural studies. Incubation of **1** with isolated rat hepatocytes resulted in extensive conversion to compounds which coeluted on HPLC with the metabolites generated *in vivo*.[6] The same metabolites were also generated upon incubation of **1** with rat, monkey, and human hepatic microsomes in the

presence of NADPH.[7] Using either system, milligram quantities of Metabolite-1 and Metabolite-2 could be produced. The metabolites were isolated by HPLC and analyzed by PMR, CMR and mass spectroscopy. Metabolite-1 was identified as a mixture of diastereomeric sulfoxides **2**[8] while Metabolite-2 was shown to be the pyridine *N*-oxide **3**.



The next objective was to synthesize enough of both metabolites to allow for their *in vivo* characterization. Treatment of **1** with *m*-chloroperbenzoic acid gave a 72% yield of diastereomeric sulfoxides.[9] Preparation of **3** was less obvious given that oxidation of **1** under a variety of more forcing conditions gave either a mixture of sulfoxide and sulfone or merely decomposition. This was not surprising since it is known that sulfides are more prone to oxidation than pyridines.[10] Rapoport has described a method for the protection of sulfides from oxidation by conversion to benzyl sulfonium salts.[11] Unfortunately, treatment of the pyridinium salt of **1** with benzyl triflate produced the pyrrole-substituted analog **4** rather than the desired benzyl sulfonium triflate.



A method for the preparation of pyridine *N*-oxides which avoids oxidative conditions was inspired by the research of Zincke and König who studied the ring opening of pyridinium salts by primary amines nearly 100 years ago.[12] Tamura and others subsequently demonstrated that dinitrophenylpyridinium salts are cleaved and recyclize upon exposure to nucleophiles such as hydroxylamine.[13] Application of these procedures to the present example required conversion of pyrrolothaizole **1** to pyridinium salt **5** by treatment with dinitrophenyltosylate in acetonitrile. A suspension of **5** in *n*-butanol was heated in the presence of aqueous hydroxylamine which immediately produced a dark red solution indicative of conjugated intermediates **6** and **7**. Progress of the cleavage/recyclization reaction was monitored by the production a

yellow non-polar spot by TLC (dinitroaniline) and a 45% yield of **3** was obtained after flash chromatography on silica gel.[14]

The PAF antagonist activity of indole pyrrolothiazole **1** and metabolites **2** and **3** is shown in Table 1. *Trans*-sulfoxide (**2-*trans***) is more than ten-fold less potent than **1** both with respect to receptor binding and inhibition of PAF-induced cutaneous vascular permeability in the rat.[15] The *cis*-isomer (**2-*cis***) is clearly more active than **2-*trans***, but regardless of potency, neither sulfoxide achieves the blood concentrations necessary to contribute substantially to the bioactivity of **1** (Figure 1). An unsubstituted sp^2 nitrogen has been hypothesized as a crucial requirement for receptor binding among 3-pyridyl containing PAF antagonists.[16] It is therefore very interesting that *N*-oxide **3** maintains the intrinsic potency of **1**. Pyridine *N*-oxide **3** also exhibits *in vivo* potency and an elimination half-life similar to **1**. Considering these factors it seems likely that at least part of the *in vivo* activity of **1** may be attributed to the formation of *N*-oxide **3**.

Table 1. PAF Antagonist Activity of **1** and Metabolites

Compound	Receptor Binding K_i (nM)	PAF-Induced Vascular Permeability ED_{50} (mg/kg; iv)
1	3.8	0.006
2-*trans*	68.0	0.1
2-*cis*	8.5	0.025
3	2.3	0.018

The metabolites of indole pyrrolothiazole **1** in a number of species were identified as sulfoxides **2** and *N*-oxide **3**, the latter of which is a potent PAF antagonist. The synthesis of **3** involves a rearrangement reaction of pyridinium salt **5** which avoids sulfur oxidation. This reaction has been applied to the synthesis of water-soluble analogs of **1** which is the subject of the following letter.

Reference and Notes:

1. Braquet, P. *Handbook of PAF and PAF Antagonists*; CRC: Boca Raton, 1991.
2. For recent reviews see: (a) Summers, J. B.; Albert, D. H. *Adv. Pharmacol.* **1995**, *32*, 67. (b) Summers, J. B.; Davidsen, S. K.; Sheppard, G. S. *Current Pharmaceutical Design* **1995**, *1*, 161.
3. See previous paper in this journal and references cited therein.
4. The pyrrolothiazole indole **1** was administered in dimethylsulfoxide (DMSO), polyethylene glycol 400, 45% hydroxypropyl-beta-cyclodextrin, and 0.9% saline (2.5:20:30:47.5, vol/vol) via a foreleg vein. Venous blood samples were collected at various times following dosing into heparinized tubes containing an internal standard in 0.1 vol. methanol. Plasma proteins were precipitated by the addition of two volumes ice-cold acetonitrile (adjusted to pH 4.0 with acetic acid). Precipitated materials were removed by centrifugation and the supernatants assayed by HPLC. Extracts were injected onto a C18 reverse column (5 x 0.46 cm, 3 μ Spherisorb S30DS2, Regis, Morton Grove, IL) and eluted with a linear gradient of 10 mM trimethylammonium perchlorate and 10% methanol in acetonitrile (30:70-50:50) over 20 minutes. Elution was monitored at 270 nm and the concentration determined by comparison of peak areas to an external calibration curve.
5. For a description of the *in vivo* properties of **1** see: (a) Summers, J. B.; Albert, D. H.; Davidsen, S. K.; Conway, R. G.; Holms, J. H.; Magoc, T. J.; Luo, G.; Tapang, P.; Rhein, D. A.; Carter, G. W. *Adv. Prostaglandin Thromboxane Leukot. Res.* **1995**, *23*, 475. (b) Davidsen, S. K.; Summers, J. B.; Albert, D.

H.; Holms, J. H.; Heyman, H. R.; Magoc, T. J.; Conway, R. G.; Rhein, D. A.; Carter, G. W. *J. Med. Chem.* **1994**, *37*, 4423.

6. Rat hepatocytes were isolated and used as described by Moldeus, see: Moldeus, P.; Hogberg, J.; Orrenius *Methods in Enzymology*; Fleisher, S.; Packer, L.; Eds.; Academic: New York, 1978, pp 60-71.
7. Hepatic microsomes were prepared by standard centrifugation techniques. Metabolism studies were performed at 37°C using: 100mM Tris (pH 7.4), a microsomal protein concentration of 2 mg/mL and an NADPH-generating system consisting of 1 mM NADP+, 8 mM glucose-6-phosphate and 1 unit glucose-6-phosphate dehydrogenase/mL.
8. While the HPLC conditions used to analyze blood samples from pharmacokinetic studies of **1** in the dog did not distinguish between the diastereomeric sulfoxides, conditions were subsequently developed which separated the *cis* and *trans* isomers (YMC ODS-AQ column (150 x 4.6 mm) using a mobile phase of 50% CH_3CN/0.05% TFAA and 100 mM tetramethylammonium perchlorate at a flow rate of 1.3 mL/min). A 5:1 ratio in favor of the *trans* sulfoxide was generated by rat liver microsomes which is entirely consistant with studies of enzymatic sulfur oxidation in a similar system. See: Cashman, J. R.; Olsen, L. D. *Mol. Pharma.* **1990**, *38*, 573.
9. A solution of **1** (432 mg; 0.85 mmol) in CH_2Cl_2 (0.1M) at room temperature was treated with *m*-CPBA (110 mol %) and stirred over 15 min. The reaction mixture was partitioned between saturated aqueous $NaHSO_3$ and CH_2Cl_2. The organic phase was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated. The resulting tan foam was purified by preparative HPLC using a sg Ranin 60A 10 mm column eluting with CH_2Cl_2:MeOH (95:5 v/v) at 3.5 mL/min. Two fractions were collected, concentrated and the resulting foams were triturated with diethyl ether which gave 241 mg of the *trans* sulfoxide and 79 mg of the *cis*-sulfoxide both as white solids. The stereochemical assignments are based on the similarity of the ^1H NMR data with a related analog (6-phenoxy indole instead of 6-*p*-fluorophenyl indole) for which an X-ray crystal structure was obtained for the *trans* sulfoxide.
10. For methods of preparing pyridine *N*-oxides see: Katritzky, A. R.; Lam, J. N. *Heterocycles* **1992**, *33*, 1011. For an additional example of the oxidation of sulfur in the presence of a pyridine heterocycle see: Brown, T. J.; Chapman, R. F.; Cook, D. C.; Hart, T. W.; McLay, I. M.; Jordan, R.; Mason, J. S.; Palfreyman, M. N.; Walsh, R. J.; Withnall, M. T.; Aloup, J.; Cavero, I.; Farge, D.; James, C.; Mondot, S. *J. Med. Chem.* **1992**, *35*, 3613.
11. Roemmele, R. C.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1866.
12. (a) Zincke, Th. *Ann. Chim* **1903**, *330*, 361. (b) Zincke, Th.; Heuser, G.; Möller *Ann. Chim* **1904**, *333*, 296. (c) König, W. *J. Prakt. Chem. [2]* **1904**, *69*, 105. (d) Kost, A. N.; Gromov, S. P.; Sagitullin, R. S. *Tetrahedron* **1981**, *37*, 3423.
13. (a) Tamura, Y.; Tsujimoto, N. *Chem. Ind.* **1970**, 926. (b) Tamura, Y.; Tsujimoto, N.; Mano, M. *Chem. Pharm. Bull.* **1971**, *19*, 130. (c) Genisson, Y.; Mehmandoust, M.; Marazano, C.; Das, B. C. *Heterocycles* **1994**, *39*, 811. (d) Genisson, Y.; Marazano, C.; Das, B. C. *J. Org. Chem.* **1993**, *58*, 2052.
14. A heterogeneous mixture of **1** (10.00 g; 19.6 mmol) in CH_3CN (0.1M) was treated with 2,4-dinitrophenyltosylate (125 mol %) and the mixture heated at reflux for 16 h then cooled and poured into diethyl ether. The resulting solid was collected by filteration and triturated with hot methanol which gave 13.51 g (81%) of **5** as a tan solid. A portion of this material (8.48 g; 10.0 mmol) was suspended in *n*-butanol (0.05M) and aqueous H_2NOH (150 mol % of a 1.0M solution prepared from H_2NOH•HCl and NaOH) was added and the mixture heated at reflux over 1.5 h. The reaction mixture was then cool, concentrated and the resulting solid was chased with diethyl ether. The orange solid was purified by flash chromatography on silica gel eluting with CH_2Cl_2:MeOH (95:5 v/v) which gave 2.39g (45%) of **3** was an tan solid.
15. For assay protocols, see: Sheppard, G. S.; Pireh, D.; Carrera, G. M. Jr.; Bures, M. G.; Heyman, H. R.; Steinman, D. H.; Davidsen, S. K.; Phillips, J. G.; Guinn, D. E.; May, P. D.; Conway, R. G.; Rhein, D. A.; Calhoun, W. C.; Albert, D. H.; Magoc, T. J.; Carter, G. W.; Summers, J. B. *J. Med. Chem.* **1994**, *37*, 2011. The dose-response curves were generally measured twice, with the K_i reported as the mean of the observed values. Values differing by less than 2-3 fold cannot be reliably differentiated with this number of data points. For comparison, reference compound WEB 2086 was found to have a K_i of 98 nM.
16. Hodgkin, E. E.; Miller, A.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 597. For an example of the requirement for an unsubstituted sp^2 nitrogen among 3-pyridyl containing PAF antagonists, see: Tilley, J. W.; Burghardt, B.; Burghardt, C.; Mowles, T. F.; Leinweber, F.-J.; Klevans, L.; Young, R.; Hirkaler, G.; Fahrenholtz, K.; Zawoiski, S.; Todaro, L. J. *J. Med. Chem.* **1988**, *31*, 466.

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