Table IV. Electrostatic Potentials (in kcal/mol) for Compound 2, 6, and 7 in Conformation a (p1 and P2) and in Conformation b (p3)[a]

	electrostatic potential at point p1				electrostatic potential at point p2				electrostatic potential at point p3			
compd	STO-3G	contribution from Ph[b]	AM1	contribution from Ph[b]	STO-3G	contribution from Ph[b]	AM1	contribution from Ph[b]	STO-3G	contribution from Ph[b]	AM1	contribution from Ph[b]
2	-11.30	-0.99	-10.60	-2.98	-10.12	-0.41	-8.79	-1.41	-19.59	2.21	-11.71	6.44
6	-10.31		-7.62		-9.71		-7.38		-21.80		-18.15	
7	-9.71		-7.16		-9.30		-7.34		-23.03		-18.02	

[a] Points p1 and p2 have a positive unit charge. Point p3 has a negative unit charge. [b] The electrostatic potential for 2 minus the electrostatic potential for 6.

stituted 1-phenyl is most probably not the cause of the low biological activity of 7.

The calculations suggest that the electrostatic interactions between 2 and receptor sites corresponding to p1 and p2 should be more favorable than the interactions between the same receptor sites and compounds 6 and 7. The calculations performed for 2, 6, and 7 in conformation b predict that 6 and 7 should have higher biological activity than 2 due to electrostatic interactions. Thus, the results obtained for conformation a, but not for b, are in agreement with observed relative biological activities for the D-1 receptor (Table II).

As shown above for 1, the phenyl rotamer of 2 giving the strongest electrostatic interaction with p1 and p2 is the one in which the two aromatic ring planes are orthogonal, corresponding to the global energy minimum of 2 and to the proposed biologically active conformation.[10] Since receptor binding data for the agonists are not available, the conclusions above should be treated with caution.

Conclusions

Calculations of molecular electrostatic potentials for the compounds studied in this work suggest that the phenyl ring interacts with the DA D-1 receptor by electrostatic forces. For antagonists as well as agonists, maximal electrostatic interactions with receptor sites at p1 and p2 are obtained for a phenyl ring rotamer in which the two phenyl ring planes are orthogonal. This corresponds to the global energy minimum of 1 and 2 and to our previously proposed biologically active conformation for these compounds.[11] Only in conformation a, in which the oxygen atom of the 8-hydroxy group is a hydrogen bond acceptor with respect to our proposed receptor sites, does the phenyl ring give a favorable contribution to the electrostatic interaction with these sites. In the alternative conformation b, in which the 8-hydroxy is a hydrogen bond donor with respect to our proposed receptor site (p3), the contribution from the phenyl ring to the electrostatic interaction and thus to the binding energy is repulsive.

The results above indicate that the electrostatic potential field of substituents in compounds related to 1 and 2 should be taken into account in the design of new DA D-1 agonists and antagonists.

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3-Carboxy-5-methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide, a New Prodrug for the Antiarthritic Agent 2-Cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide[1]

John W. Patterson,* Paul S. Cheung, and Michael J. Ernest

Institutes of Organic Chemistry and Biological Sciences, Syntex Research, Palo Alto, California 94304. *Received June 10, 1991*

The title compound 3-carboxyisoxazole 3 was synthesized by cycloaddition of carbethoxyformonitrile oxide to *N*-[4-(trifluoromethyl)phenyl]-3-pyrrolidino-2-butenamide (6) with spontaneous elimination of pyrrolidine followed by hydrolysis of the ethyl ester. Compound 3 was shown to be absorbed intact after oral administration to rats. Over 24 h, the compound was metabolized to yield plasma concentrations of the antiinflammatory agent 2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide (2), similar to those obtained following an equivalent dose of the established prodrug of 5-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide (1).

Over the past several decades many pharmaceutical agents have been devised using the concept of prodrugs. This strategy has been useful in overcoming a variety of problems which would have precluded the development of many parent compounds as medicinal agents. Some virtues ascribed in the literature to prodrugs include improved solubility and bioavailability, tissue-specific delivery, diminution of side effects, sustained release of metabolically unstable agents, and lengthened shelf life.[2] Although less abundant than rationally devised prodrugs, there are many examples of compounds shown to exhibit their particular biological effect only after some metabolic transformation[3] or where a metabolite has efficacy similar to that of the parent compound.

(1) Contribution No. 783 from the Syntex Institute of Organic Chemistry.

(2) Reviews: (a) Armstrong, R. N. Mechanistic Aspects of Xenobiotic Metabolism as Related to Drug Design. *Annu. Rep. Med. Chem.* 1988, *23*, 315-324. (b) Bodor, N.; Kaminski, J. J. Prodrugs and Site-Specific Chemical Delivery Systems. *Annu. Rep. Med. Chem.* 1987, *22*, 303-313.

(3) Recent examples: Borgna, J.-L.; Rochefort, H. Hydroxylated Metabolites of Tamoxifen are Formed in vitro and Bound to Estrogen Receptor in Target Tissues. *J. Biol. Chem.* 1981, *256*, 859-868 (tamoxifen). Machin, P. J.; Hurst, D. N.; Osbond, J. M. β-Adrenoceptor Activity of the Stereoisomers of the Bufuralol Alcohol and Ketone Metabolites. *J. Med. Chem.* 1981, *28*, 1648-1651 (bufuralol). Biollaz, J.; Schelling, J. L.; Des-Combes, B. J.; Brunner, D. B.; Desponds, G.; Brunner, H. R.; Ulm, E. H.; Hichens, H.; Gomez, H. J. Enalapril Maleate and a Lysine Analogue in Normal Volunteers; Relationship Between Plasma Drug Levels and the Renin Angiotensin System. *Br. J. Clin. Pharmacol.* 1982, *14*, 363-368 (enalapril).

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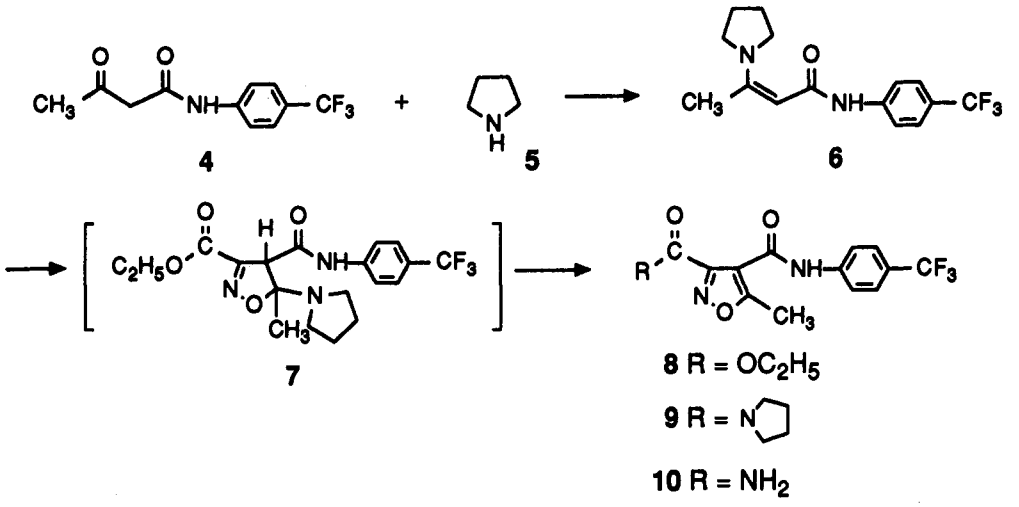
Scheme I



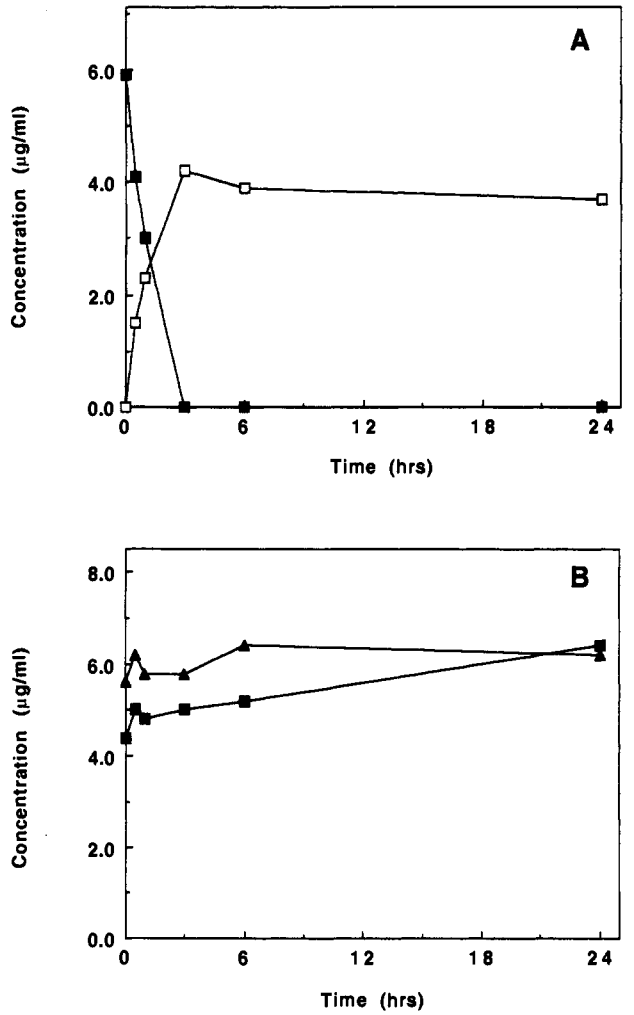
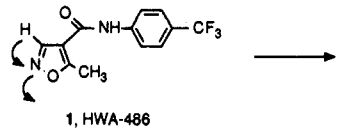


Figure 1. Plasma stability of compounds 1, 3, and 10 in vitro: panel A, conversion of compound 1 (■) to compound 2 (□) in rat plasma at 37 °C; panel B, stability of compound 3 (■) and compound 10 (△) in rat plasma at 37 °C (n = 2).

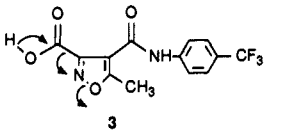
The metabolic processes reported for activation of prodrugs include decarboxylations, oxidations, reductions, hydrolysis of esters and carbamates, and various demethylations of ethers and amines. However, the number of reaction types available for metabolic activation of prodrugs remains rather limited and the revelation of a new reaction type is of considerable interest to medicinal chemistry. The discovery that the antiarthritic agent 5-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide (HWA-486, 1) was most likely a prodrug for 2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide (A771726, 2) resulting from fragmentation of the isoxazole ring[4] revealed a new type of metabolic transformation shown in eq 1. Our interest in modifying the



(1)

solubility, the duration of action, and other pharmacokinetic properties of compounds 1 and 2 led to the consideration of alternate prodrugs of 2.

The subject of this report is the discovery of a more extended in vivo fragmentation of a 3-carboxyisoxazole, 3, to produce the same active cyano enol 2 and carbon dioxide as illustrated in eq 2.



(2)

There is adequate precedent for this type of fragmentation in the chemical literature[5] and in particular the

(4) Bartlett, R. R.; Schleyerbach, R.; Kaemmerer, F. J. Drugs for the Treatment of Graft-versus-host and Autoimmune disease. Ger. Offen. DE 3534440 A1, 1987; *Chem. Abstr.* 1987,*107*, 861*j*. Although there are few reports on the pharmacological effects of cyanobuteneamide 2, its known prodrug isoxazole 1 has a variety of useful antiarthritic and immunological properties: Pasternak, R. D.; Wadopian, N. S.; Wright, R. N.; Simonoff, P.; Gylys, J. A.; Buyniski, J. P. Disease Modifying Activity of HWA 486 in Rat Adjuvant-induced Arthritis. *Agents Actions* 1987, *21*, 241-3. Popovic, S.; Bartlett, R. R. Disease-modifying Activity of HWA 486 on the Development of SLE in MRL/1 Mice. *Agents Actions* 1986, *19*, 313-14. Bartlett, R. R. Immunopharmacological Profile of HWA 486, A Novel Isoxazole Derivative II. In Vivo Immunomodulating Effects Differ from those of Cyclophosphamide, Prednisolone or Cyclosporin A. *Int. J. Immunopharmacol.* 1986, *8*, 199–204. Bartlett, R. R.; Dimitrijevic, M.; Mattar, T.; Zielinski, T.; Germann, T.; Ruede, E.; Thoenes, G. H.; Kuechle, C. C. A.; Schorlemmer, H. U. Leflunomide (HWA 486), A Novel Immunomodulating Compound for the Treatment of Autoimmune Disorders and Reactions Leading to Transplantation Rejection. *Agents Actions* 1991, *32*, 10–21.

(5) Barnes, R. A. *Heterocyclic Compounds*; Elderfield, R. C., Ed.; J. Wiley & Sons, Inc.: New York, 1957; p 471.

kinetics of the fragmentation of 3-carboxybenzisoxazoles has been investigated.[6]

Chemistry

The requisite 3-carboxyisoxazole **3**, necessary to test this putative biotransformation, was prepared as shown in Scheme I, using a nitrile oxide–enamine cycloaddition reaction to assemble the isoxazole ring. Thus, acetoacetic anilide **4**[7] was converted into enamine **6** by reaction with pyrrolidine **5** in refluxing benzene. Generation of carbethoxyformonitrile oxide by treating ethyl chlorooximidoacetate with triethylamine (the usual reaction conditions[8]) in the presence of enamine **6** resulted in cycloaddition and concomitant elimination of pyrrolidine.[9] Unfortunately, the reaction product consisted of a mixture of about equal amounts of ester **8** and amide **9** resulting from amination of **8** by the pyrrolidine byproduct. This side reaction was completely suppressed by conducting the cycloaddition reaction in methylene chloride without triethylamine, under which circumstances the nucleophilic character of pyrrolidine was diminished by formation of the hydrochloride salt. 3-Carboxamido isoxazole **10** was prepared by amination of **8** with ammonia. Finally, hydrolysis of the ethyl ester moiety of **8** with lithium hydroxide in aqueous methanol gave isoxazole-3-carboxylic acid **3**.

Biological Results

The fragmentation of isoxazole **1** and 3-carboxyisoxazole **3** was investigated in vitro in plasma and in vivo after oral administration to rats. For comparison, the 3-carboxamide derivative **10**, which is not expected to undergo the reaction illustrated in eq 2, was also examined under these conditions. The fragmentation reaction was monitored by reverse-phase HPLC methods capable of resolving all three compounds and cyano enol product **2**. At 37 °C in fresh rat plasma, **1** was rapidly converted to **2** within 3 h (Figure 1A). In aqueous solutions below pH 8, **1** was very stable ($t_{1/2}$ > 48 h), suggesting that enzymes, plasma proteins, or other constituents in rat plasma accelerate opening of the isoxazole ring. Substitution at C3 of the ring blocked this reaction with both the 3-carboxy and 3-carboxamide derivatives resistant to fragmentation during incubation in rat plasma through 24 h (Figure 1B).

Oral administration of 50 mg/kg **1** to rats efficiently delivered high and sustained levels of cyano enol **2** to the systemic circulation (Figure 2A). As early as 1 h after treatment, more than 80% of the absorbed dose had already been converted to the cyano enol. It is not known if tissue metabolism of **1** contributes to the fragmentation reaction, although the data presented above suggest that significant conversion could take place in plasma after absorption. In contrast to the findings with **1**, substantial systemic levels of 3-carboxyisoxazole **3** were found during the first 6 h after oral administration to rats (Figure 2B). Eventually, the 3-carboxyisoxazole was converted to **2**, so

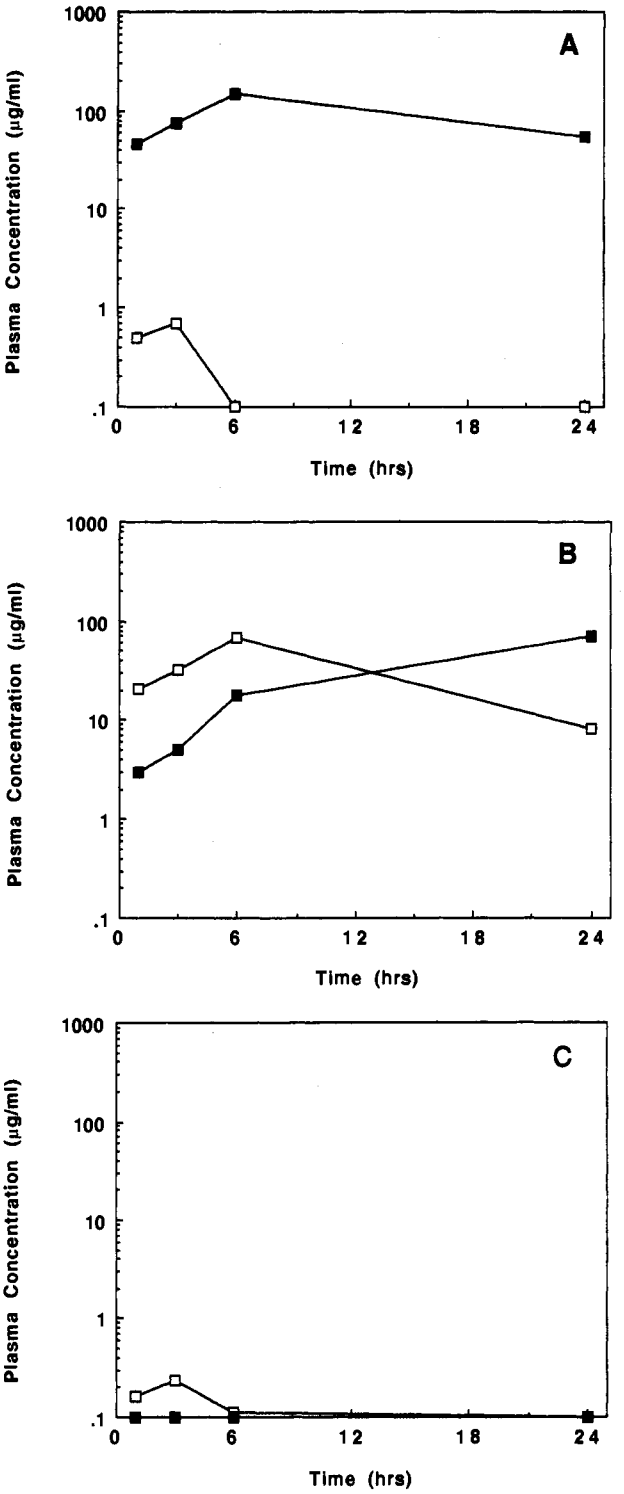


Figure 2. Pharmacokinetics of compounds **1**, **3**, and **10** in rats. Plasma levels of the parent compounds and the common metabolite **2** (■) were determined at various times after a 50 mg/kg oral dose in rats (n = 2): panel A, compound **1** (□); panel B, compound **3** (□); panel C, compound **10** (□).

that by 24 h postdose, plasma levels of the cyano enol arising from a 50 mg/kg dose of either **1** or carboxyisoxazole **3** were similar (56 and 70 μg/mL, respectively). Since the 3-carboxyisoxazole **3** was stable in vitro in plasma, tissue metabolism is presumably required to generate the cyano enol **2** in vivo in rats. Finally, oral administration of the 3-carboxamide derivative to rats led to the transient appearance of low circulating levels of the parent compound (Figure 2C). There was no evidence for the formation of enol **2** at concentrations above the limit

(6) Kemp, D. S.; Paul, K. Decarboxylation of Benzisoxazole-3-carboxylic Acids. Catalysis by Extraction of Possible Relevance to the Problem of Enzymatic Mechanism. *J. Am. Chem. Soc.* **1970**, *92*, 2553–2554.
(7) Compound **5** is readily available by acylation of 4-aminobenzotrifluoride with diketene: Kaemmerer, J. F.; Schleyerback, R. Isoxazole derivatives. U. S. Patent 4,351,841, 1982; *Chem. Abstr.* **1980**, *93*, 239392*m*.
(8) Kozikowski, A. P.; Adamczyk, M. Methods for the Stereoselective Cis Cyanohydroxylation and Carboxylation of Olefins. *J. Org. Chem.* **1983**, *48*, 366–372.
(9) Stork, G.; McMurry, J. E. A General Synthesis of 4-Isoxazolecarboxylic Acids. *J. Am. Chem. Soc.* **1967**, *89*, 5461–5462.

of detection of the HPLC assay (~10 ng/mL). However, three unidentified peaks were noted in the HPLC profile. None of the peaks had a UV absorption spectrum characteristic of the cyano enol product 2, suggesting that these putative metabolites retain the intact isoxazole ring structure.

Conclusions

This report illustrates a new fragmentation reaction for the design of prodrugs. 3-Carboxyisoxazole 3 can be metabolized by rats to yield cyano enol product 2, and hence function as a prodrug for this antiinflammatory agent. The cyano ketone moiety is present in other classes of medicinal agents[10] and therefore this fragmentation reaction may be useful as well in preparing prodrugs of these compounds.

Experimental Section

Chemistry. Infrared spectra were recorded on a Pye Unicam 3-200 spectrometer as KBr pellets. The 90-, 300-, and 500-MHz ^{1}H NMR spectra were obtained on Varian EM 390 and Bruker WM 300 and AM 500 spectrometers in $CDCl_3$ solution using TMS as internal standard. Melting points are uncorrected and were observed on a Hoover-Thomas apparatus. A Control Equipment Corporation Model 240 xA analyzer was used to obtain combustion analyses.

***N*-[4-(Trifluoromethyl)phenyl]-3-pyrrolidino-2-butenamide (6).** A mixture of acetoacetic anilide 4 (27.06 g, 0.11 mol) and pyrrolidine (11 mL, 0.132 mol) in 250 mL of benzene was refluxed using a Dean–Stark trap to separate water for 1 h. On cooling to room temperature the precipitate was collected by filtration and washed with cold benzene to yield enamine 6 (29.32 g, 74%): mp 198–200 °C; IR 1640, 1590, 1520, 1320 cm^{-1}; ^{1}H NMR (90 MHz) δ 7.5 (m, 4 H), 6.95 (br s, 1 H, NH), 4.40 (s, 1 H), 3.2 (m, 4 H), 2.5 (s, 3 H) 1.9 ppm (m, 4 H). Anal. ($C_{15}H_{17}F_3N_2O$) C, H, N.

3-Carbethoxy-5-methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide (8). A slurry of enamine 6 (11.64 g, 0.039 mol) in 100 mL of CH_2Cl_2 was cooled in an ice bath and treated with ethyl chlorooximidoacetate (7.55 g, 0.05 mol) in one portion. The reaction mixture was stirred at 0 °C for 3 h and then poured into water. The aqueous layer was extracted with CH_2Cl_2 (200 mL), and the combined organic layers were washed with 5% HCl and saturated aqueous $NaHCO_3$ and then dried over $MgSO_4$. Evaporation of the solvent and recrystallization of the residue from EtOH gave isoxazole 8 (10.91 g, 78%): mp 91–92 °C; IR 1710, 1680, 1610 cm^{-1}; ^{1}H NMR (300 MHz) δ 11.35 (br, NH), 7.83 (d, J = 8.4 Hz, 2 H), 7.42 (d, J = 8.4 Hz, 2 H), 4.59 (d, J = 7.2 Hz, 2 H), 2.88 (s, 3 H), 1.51 ppm (t, J = 7.2 Hz, 3 H). Anal. ($C_{15}H_{13}F_3N_2O_4$) C, H, N.

3-Carboxy-5-methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide (3). A solution of lithium hydroxide (3.21 g, 0.134 mol) in 25 mL of water was diluted with 150 mL of MeOH and cooled to −15 °C. Ethyl ester 8 (20.675 g, 0.0604 mmol) in 250 mL of MeOH was added to the lithium hydroxide solution over 25 min and the reaction mixture was then stirred at −15 °C for 1 h. The reaction mixture was acidified with aqueous HCl, diluted with 400 mL of water, and then stirred for 30 min at 0 °C. The product was collected by filtration and recrystallized from *tert*-butyl methyl ether, to yield after drying in vacuo (56 °C), acid 3 (16.25 g, 86%): mp 226–227 °C; IR 1691, 1649, 1622, 1574 cm^{-1}; ^{1}H NMR (500 MHz) δ 10.99 (s, NH), 7.85 (d, J = 8.5 Hz, 2 H), 7.72 (d, J = 8.5 Hz, 2 H), 2.63 ppm (s, 3 H). Anal. ($C_{13}H_9F_3N_2O_4$) C, H, N.

3-Carbamoyl-5-methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide (10). A partially saturated solution of ammonia was prepared by bubbling anhydrous ammonia into 20 mL of EtOH for 5 min. Ethyl ester 8 (2.00 g, 5.84 mmol) was added to the reaction mixture which was stirred in a closed flask for 30 min. Evaporation of the ethanol and excess ammonia in vacuo and recrystallization of the residue from CH_2Cl_2/MeOH gave carboxamide 10 (1.67 g, 91%): mp 205–206 °C; IR 3482, 3327, 1653, 1624 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) 12.4 (br s, NH), 8.78 (br, s, NH), 8.5 (br s, NH), 7.80 (d, J = 8.5 Hz, 2 H), 7.72 (d, J = 8.5 Hz, 2 H), 2.76 ppm (s, 3 H). Anal. ($C_{13}H_{10}F_3N_3O_3$) C, H, N.

HPLC Analysis. After addition of an internal standard, plasma samples were acidified with HCl and extracted three times with three volumes of redistilled diethyl ether. The pooled ether extracts were dried in a stream of nitrogen and redissolved in methanol. Samples were injected onto a Perkin-Elmer Pecosphere 3-μm C18 cartridge column (3.3 × 0.46 cm). The mobile phase was methanol–35 mM KH_2PO_4 (pH 4.5) 55:45 containing 4 mM triethylamine. The flow rate was 1.0 mL/min. The mobile phase strength was increased to 65% methanol for resolution of the 3-carboxamide isoxazole 10. The compounds were monitored by UV absorption using a Perkin-Elmer LC-235 diode-array detector. Plasma concentrations were determined from standard curves using peak height for quantitation. The results were corrected for recovery of the internal standard. The internal standard that was used for the analysis of compound 10 was compound 1. The internal standard for compounds 1 and 3 was 5-methyl-*N*-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide.

Plasma Stability. Heparinized plasma was prepared from 180–200-g male Lewis rats (Charles River). Ten-microgram samples of the isoxazoles and A771726 were added to 2 mL of plasma and incubated at 37 °C. At 0, 0.5, 1, 3 and 6 h, 50–100-μL samples were moved and analyzed by HPLC.

Pharmacokinetics. Male Lewis rats (two per group) were treated by oral gavage with a 50 mg/kg dose of each compound formulated as a suspension in 0.5% sodium carboxymethylcellulose. Heparinized blood samples were collected from the retrorbital plexus at 1, 3, 6 and 24 h postdose and analyzed by HPLC. The results reported are the average of the individual plasma concentrations for each of the two rats, which rarely differed by more than 10%.

Registry No. 2, 108605-62-5; 3, 134888-93-0; 4, 351-87-1; 5, 123-75-1; 6, 134888-97-4; 8, 134889-01-3; 10, 137895-22-8; ethyl chlorooximidoacetate, 14337-43-0.

(10) (a) Other 3-cyano-2-hydroxy-2-propenamide antiinflammatories: Gallagher, P. T.; Hicks, T. A. Preparation of N-heteroarylacrylamides and their use as pharmaceuticals useful for treatment of immune disease. European Patent 259,972, 1987; *Chem. Abstr.* 1988, *109*, 92763*w*. Walker, G. N. α-Carbamoylpyrrolepropionitriles. U.S. Patent 4,256,759, 1981; *Chem. Abstr.* 1981, *95*, 7046*q*. (b) 3β-hydroxysteroid dehydrogenase inhibitors: trilostane and epostane, Christiansen, R. G.; Neumann, H. C.; Salvador, U. J.; Bell, M. R.; Schane, H. P.; Creange, J. E.; Potts, G. O.; Anzalone, A. J. Steroidogenesis Inhibitors. 1. Adrenal Inhibitory and Interceptive Activity of Trilostane and Related Compounds. *J. Med. Chem.* 1984, *27*, 928–931.