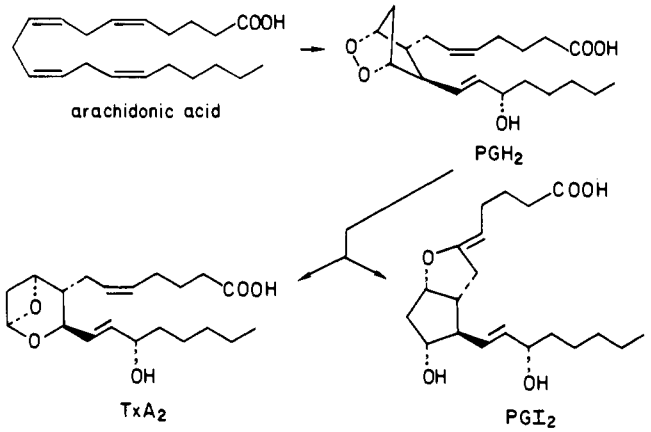
Thromboxane A_2 Synthase Inhibitors. 5-(3-Pyridylmethyl)benzofuran-2-carboxylic Acids

Roy A. Johnson,* Eldon G. Nidy, James W. Aiken, Norman J. Crittenden, and Robert R. Gorman

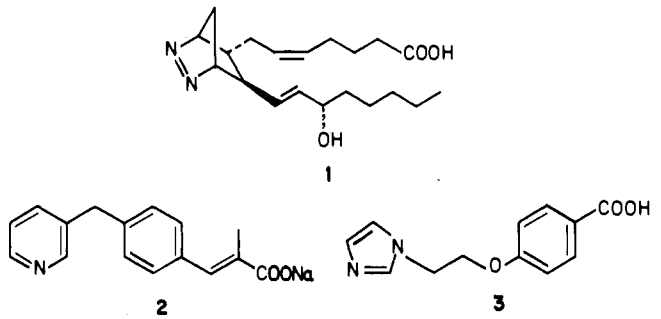
Pharmaceutical Research and Development, The Upjohn Company, Kalamazoo, Michigan 49001. *Received October 29, 1985*

The synthesis and screening of a series of 5-(3-pyridylmethyl)benzofuran-2-carboxylic acids as selective thromboxane A_2 (TxA_2) synthase inhibitors is outlined. The ability of these compounds to inhibit TxA_2 biosynthesis was assayed using microsomal enzyme from human platelets. Substitution of the benzofuran ring caused small changes in potency; modification of the carboxylic acid group caused modest reductions in potency, and substitution of the pyridine ring resulted in large reductions of potency. 5-(3-Pyridylmethyl)benzofuran-2-carboxylic acid sodium salt (**9b**, sodium furegrelate) was chosen for further evaluation as a TxA_2 synthase inhibitor.

Elucidation of the endoperoxide branch of the arachidonic acid cascade during the past decade has placed before scientists a biochemical scheme of exquisite design. At the heart of this scheme is the chemically fascinating endoperoxide molecule, PGH_2.[1] This stable, but reactive molecule serves as the biosynthetic precursor to both thromboxane A_2 (TxA_2)[2] and prostacyclin (PGI_2),[3] as well as to the more "classical" prostaglandins.



The two substances, TxA_2 and PGI_2, have opposing biological activities, the former being a vasoconstrictor and stimulator of platelet aggregation while the latter is a vasodilator and an inhibitor of platelet aggregation. Under normal physiological conditions, these two substances provide a natural homeostatic function. However, if an imbalance of the two substances occurs, the effect upon health may be detrimental. Consequently, immediately after elucidation of this biosynthetic scheme had been completed, the experimental and potential therapeutic value of a thromboxane (TxA_2) synthase inhibitor was recognized. This quickly led to the first reports of the selective inhibitors, imidazole[4] and 9,11-azoprosta-5,13-dienoic acid (1).[5] Following these initial reports, a gradually increasing variety of molecules have been described as selective TxA_2 synthase inhibitors including additional endoperoxide analogues,[6] pyridine and 3-substituted pyridines,[7] 1-substituted imidazoles,[8] and various other related molecules.[9]



To date, two of the most widely studied compounds have been (*E*)-3-[4-(3-pyridylmethyl)phenyl]-2-methyl-2-propenoic acid, sodium salt[7c] (**2**, OKY-1581) and 4-[2-(1-imidazol-1-yl)ethoxy]benzoic acid[8c,e,j] (**3**, UK-37248 or dazoxiben). The prostaglandin endoperoxide analogues also have been extensively tested, but they have not ad-

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(3) (a) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature (London)* **1976**, *263*, 663. (b) Johnson, R. A.; Morton, D. R.; Kinner, J. H.; Gorman, R. R.; McGuire, J. C.; Sun, F. F.; Whittaker, N.; Bunting, S.; Salmon, J.; Moncada, S.; Vane, J. R. *Prostaglandins* **1976**, *12*, 915.

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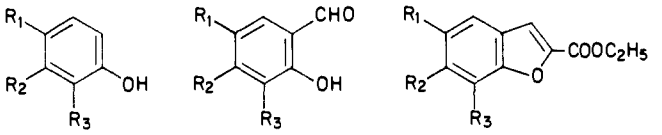
Table I. 5- (and 6-) (3-Pyridylalkyl)benzofuran-2-carboxylic Acid Esters and Synthetic Intermediates



6 7 8

compd no.	R_1	R_2	R_3	method[a]	yield, %	recrystn solvent[b]	mp, °C	formula	anal.[c]
6a	3-Py	H	H	A	65	A-H	202–203	$C_{11}H_9NO$	C,[d] H, N; MS
6b	3-PyCH$_2$	H	H	A	75	A-H	184–186	$C_{12}H_{11}NO$	C, H, N
6c	3-PyCH$_2$	H	CH_3	*e*	70	EA-H	166.5–168.5	$C_{13}H_{13}NO$	C,[f] H, N; MS
6d	3-PyCH$_2$	H	CH_3O	H	51	A	142–143	$C_{13}H_{13}NO_2$	C, H, N; MS
6f	H	3-PyCH$_2$	H	H	42	A-H	117.5–118.5	$C_{12}H_{11}NO$	C, H, N
6g	3-PyCH$_2$CH$_2$	H	H	F	31	A-H	180–181.5	$C_{13}H_{13}NO$	C,[g] N, N; MS
6h	H	3-PyCH$_2$CH$_2$	H	F	69	A-H	107.5–108.5	$C_{13}H_{13}NO$	C, H, N; MS
7a	3-Py	H	H	B	38	A-H	122–123	$C_{12}H_9NO_2$	C, H, N
7b	3-PyCH$_2$	H	H	B	54	A-H	130–132	$C_{13}H_{11}NO_2$	C, H, N
7c	3-PyCH$_2$	H	CH_3	B	45	A-H	49–50	$C_{14}H_{13}NO_2$	C, H, N; MS
7d	3-PyCH$_2$	H	CH_3O	B	43	A-H	114.5–115.5	$C_{14}H_{13}NO_3$	C, H, N; MS
7e	3-PyCH$_2$	H	Br	*e*	45	A-H	115–116	$C_{13}H_{10}BrNO_2$	C, H, Br, N
7f	H	3-PyCH$_2$	H	B	21	A-H	75–76	$C_{13}H_{11}NO_2$	C,[h] H, N
7g	3-PyCH$_2$CH$_2$	H	H	B	53	E-P	63–64.5	$C_{14}H_{13}NO_2$	C, H, N
7h	H	3-PyCH$_2$CH$_2$	H	B	9	E-P	93–94	$C_{14}H_{13}NO_2$	C, H, N[i]; MS
8a	3-Py	H	H	C	61	A-H	91–92	$C_{16}H_{13}NO_3$	C, H, N; MS
8b	3-PyCH$_2$	H	H	C	82	A-H	73–74	$C_{17}H_{15}NO_3$	C, H, N
8c	3-PyCH$_2$	H	CH_3	C	28	H	100–101	$C_{18}H_{17}NO_3$	C, H, N; MS
8d	3-PyCH$_2$	H	CH_3O	C[j]	40	A-H	98.5–99.5	$C_{18}H_{17}NO_4$	C, H, N; MS
8e	3-PyCH$_2$	H	Br	C	55	A-H	109–110	$C_{17}H_{14}NO_3$	C, H, Br, N
8f	H	3-PyCH$_2$	H	C	65	A-H	90–91.5	$C_{17}H_{15}NO_3$	C, H, N
8g	3-PyCH$_2$CH$_2$	H	H	C	19	E-P	91.5–92.5	$C_{18}H_{17}NO_3$	C, H, N; MS
8h	H	3-PyCH$_2$CH$_2$	H	C	30	E-P	81–83	$C_{18}H_{17}NO_3$	C, H, N; MS

[a] See the Experimental Section for descriptions of the methods used. [b] Solvents used: A, acetone; E, ether; EA, ethyl acetate; H, hexane; P, pentane. [c] Elemental analyses for C, H, and N were within ±0.4% of theory except where noted. MS indicates confirmation of the empirical formula by high-resolution mass spectral data. [d] C: calcd, 77.17; found, 76.76. [e] See discussion for method of preparation. [f] C: calcd, 73.22; found, 72.81. [g] C: calcd, 78.36; found, 77.82. [h] C: calcd, 73.22; found, 72.81. [i] N: calcd, 6.16; found 5.28. [j] See discussion for isolation of **10** in this reaction.

vanced as clinical candidates primarily because they have direct agonist effects of their own.[10]

We wished to have available an alternate class of inhibitors in order to further explore the effect of these agents experimentally and, if possible, therapeutically in man. The TxA_2 synthase inhibitory properties of both imidazole and pyridine are greatly enhanced by substitution with alkyl or aryl carboxylic acid groups and some structure–activity relationships have been reported.[7b,c,8a,b,d,e,g-i] We were curious about what effect the use of a benzofuran-2-carboxylic acid group as a substituent would have on the biological properties of these molecules. In this report we describe the synthesis of a series of (3-pyridinylmethyl)benzofuran-2-carboxylic acids and outline the effect of structural variations on the activity of these molecules as thromboxane synthase inhibitors. The detailed biochemical and pharmacological evaluation of one member of this class, **9b**, has been described recently.[11]

Chemistry. The methods used to prepare the desired benzofuran-2-carboxylates are outlined in Scheme I. The examples used to illustrate Scheme I are also the examples for which preparative details are given in the Experimental Section of this report. Analogues of the compounds shown in Scheme I are included in Tables I and II. A brief discussion of these methods and results follows.

The synthesis of **9b** was achieved by the use of methods A–D. The starting material for method A, 3-(4-aminobenzyl)pyridine (**5**) was obtained by reduction of 3-(4-nitrobenzyl)pyridine[12] (**4**) with hydrogen over palladium on carbon. Diazotization of **5** followed by decomposition of the diazonium salt in hot aqueous acid (method A) converted the aniline **5** to phenol **6b** in good yield. Next, we wished to introduce a formyl group ortho to the hydroxyl group of **6b**. This was done in moderate yield by a modification[13] of the Duff reaction[14] in which hexamethylenetetramine in trifluoroacetic acid is used (method B) to convert **6b** into **7b**.

By use of a base-promoted reaction with diethyl bromomalonate,[15] the *o*-hydroxy benzaldehyde **7b** was transformed into a benzofuran-2-carboxylic acid, ethyl ester, **8b**. When this reaction was done with potassium carbonate,[15] **8b** was obtained in 25% yield together with several byproducts. When sodium hydride (solubilization aided by use of dicyclohexyl-18-crown-6) was used as the base (method C), the yield of **8b** was increased to 82%. The ester **8b** was saponified with 1 equiv of sodium hydroxide in aqueous methanol (method D), giving sodium salt **9b**. This sodium salt crystallized beautifully upon dilution of an aqueous solution with acetone. However, other analogues of **9b** prepared by this method (see Table II) were isolated as powders or glasses and were characterized by their spectral and chromatographic properties.

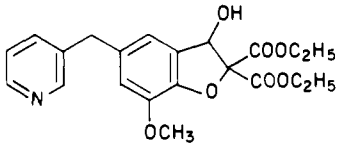
Other methods (Scheme I) were also used to prepare intermediates of type **6**. To prepare analogues having a

(10) Gorman, R. R.; Shebuski, R. J.; Aiken, J. W.; Bundy, G. L. *Fed. Proc.* **1981**, *40*, 19.
(11) Gorman, R. R.; Johnson, R. A.; Spilman, C. H.; Aiken, J. W. *Prostaglandins* **1983**, *26*, 325. The generic name, sodium furegrelate, has been assigned to **9b**.
(12) Bryans, F.; Pyman, F. L. *J. Chem. Soc.* **1929**, 549.
(13) Smith, W. E. *J. Org. Chem.* **1972**, *37*, 3972.
(14) Duff, J. C. *J. Chem. Soc.* **1941**, 547.
(15) (a) Tanaka, S. *J. Am. Chem. Soc.* **1951**, *73*, 872. (b) Witiak, D. T.; Newman, H. A. I.; Poochikian, G. K.; Fogt, S. W.; Baldwin, J. R.; Sober, C. L.; Feller, D. R. *J. Med. Chem.* **1978**, *21*, 833.

two-carbon bridge between the rings (e.g., **6g**), the Wittig reaction of phosphorane **11** with aldehydes such as **12** was used (method E). From this reaction, intermediate **13g** was obtained as a mixture of cis and trans double-bond isomers. These isomers can be separated, but the mixture was reduced catalytically (method F) to the desired **6g**.

Ring-substituted analogues such as **6d** were prepared by use of methods G and H. For example, the addition of 3-lithiopyridine (**14**) to the aldehyde **15** gave intermediate **16d**. Hydrogenolysis of **16d** cleaved the benzyl ether and reduced off the benzylic hydroxyl group to give **6d**. The closely related intermediate **6c** (see Table I) was also prepared by using methods G and H. The precursor produced by these two steps was, however, a methyl ether (**17**, structure not shown) rather than a benzyl ether. This ether was cleaved with 48% HBr, giving the desired **6c** in satisfactory manner.

The benzofuran ring-forming reaction (method C) has been discussed previously in the chemical literature and clearly involves a number of discrete transformations. Several of the intermediate products can be detected by TLC during the course of the reaction, and in the case of cyclization of **7d**, the reaction stopped at the stage of diester **10**. Further treatment of **10**, after isolation and characterization, with sodium hydride led to formation of the desired benzofuran **8d**.



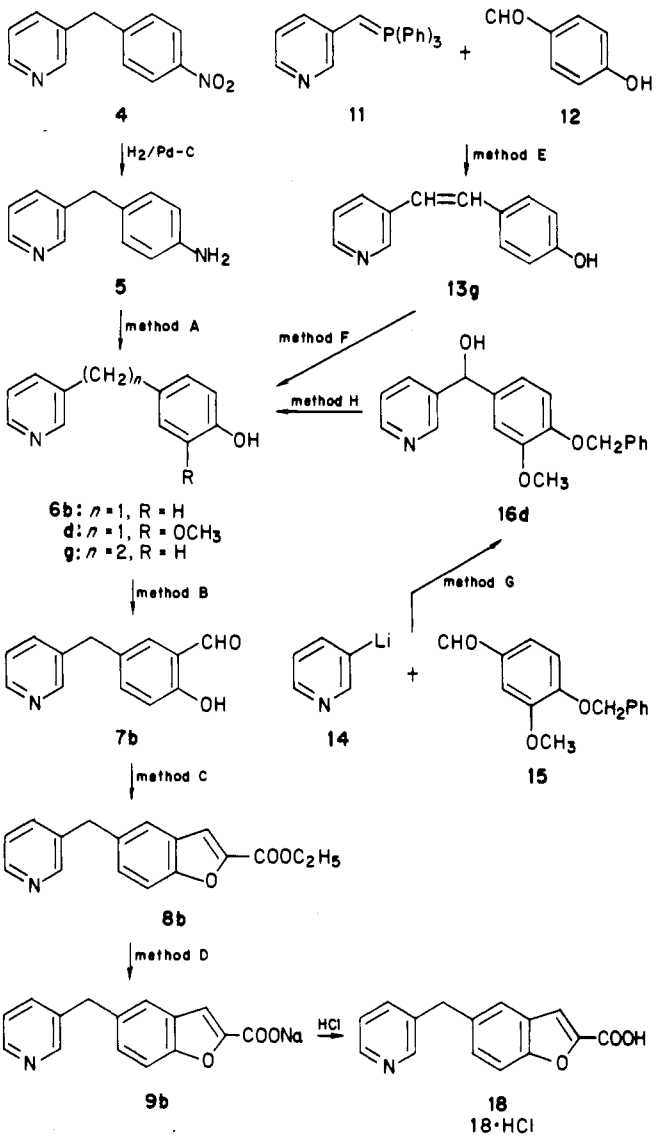
10

3-Bromo-2-hydroxy-5-(3-pyridylmethyl)benzaldehyde (**7e**) was prepared directly from **7b** by bromination with bromine in acetic acid. The details of this reaction are included in the Experimental Section.

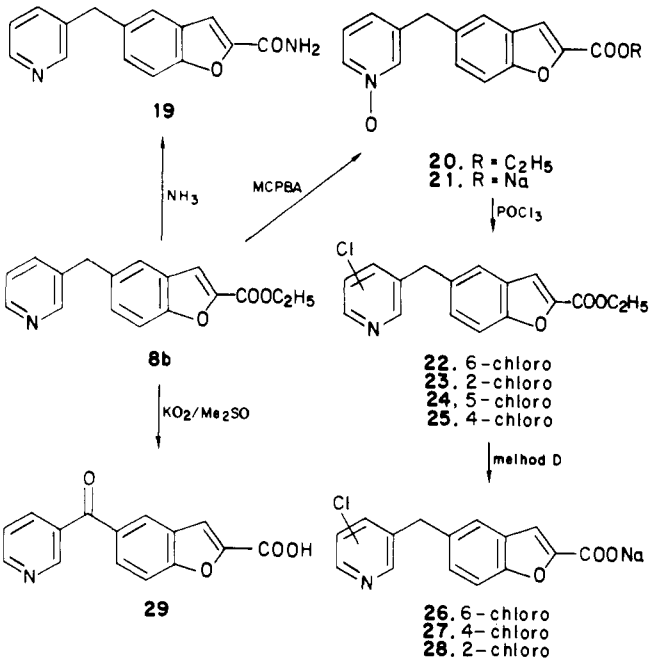
With the choice of **9b** for further pharmacological evaluation, we carried out several modifications of this molecule. First we prepared both the "free acid" **18** and the hydrochloride salt **18**·HCl from the sodium salt **9b** (see Scheme I) in order to compare the physical properties of these different forms. As expected, **18**, while being beautifully crystalline was virtually insoluble in aqueous media (of neutral pH) and therefore less suitable for pharmacological study. The hydrochloride **18**·HCl was soluble in water, but after standing several minutes at room temperature, the solution deposited crystals of **18**, presumably as a result of dissociation of the pyridium hydrochloride in the aqueous medium. The hydrochloride therefore is also a less suitable compound for further evaluation.

Other modifications are outlined in Scheme II. The ethyl ester (**8b**) slowly exchanged with ammonia in alcohol solution to give the amide **19**. The pyridine *N*-oxide (**20**) was readily prepared by oxidation of **8b** with *m*-chloroperbenzoic acid and was converted to the sodium salt **21** by method D. We prepared the *N*-oxide with the possibility in mind that it might be found as a metabolite following administration of **9b** to animals. The *N*-oxide also was useful for the introduction of chlorine substituents into the pyridine ring. As shown, reaction of **8b** with phosphorous oxychloride results in chlorine substitution and at the same time deoxygenates the pyridine-*N*-oxide.[16] All four possible monochloro derivatives, **22–25**, were

Scheme I



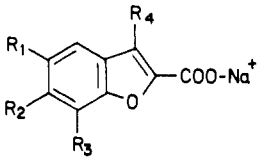
Scheme II



produced in the reaction and could be separated by chromatography. The positions of the chlorine substitu-

(16) Smith, E. M. Ph.D. Dissertation, University of Saskatchewan, 1969. See discussion in Abramovitch, R. A.; Smith, E. M. In *Pyridine and Its Derivatives* Abramovitch, R. A., Ed.; Wiley: New York, 1974; Supplement Part 2, pp 111–114.

Table II. TxA_2 Synthetase Inhibition in Human Platelet Microsomes



compd no.	R_1[a]	R_2	R_3	R_4	ED_{50},[b] ng/mL	potency rel to 9b	anal.[c]
9a	3-Py	H	H	H	30	0.3	C, H, N
9b	3-$PyCH_2$	H	H	H	10	1.0	C, H, N
9c	3-$PyCH_2$	H	CH_3	H	30	0.3	TLC, HPLC
9d	3-$PyCH_2$	H	CH_3O	H	30	0.3	HPLC
9e	3-$PyCH_2$	H	Br	H	30	0.3	TLC, HPLC
9f	H	3-$PyCH_2$	H	H	30	0.3	C, H, N
9g	3-$PyCH_2CH_2$	H	H	H	3000	0.003	TLC,[d] HPLC
9h	H	3-$PyCH_2CH_2$	H	H	3000	0.003	TLC,[d] HPLC[e]
9i[f]	3-$PyCH_2$	H	H	CH_3	30	0.3	
19					300	0.03	C, H, N
21					>10000	<0.001	HPLC
26					>10000	<0.001	TLC, HPLC
27					>10000	<0.001	TLC, HPLC
28					100	0.1	TLC, HPLC
29					3000	0.003	C, H, N
1					30	0.3	
2					30	0.3	
3					30	0.3	

[a] Py, pyridine. [b] ED_{50} is the concentration causing a 50% reduction in the generation of TxA_2 as assayed on the rabbit aortic strip (see Experimental Section). Test accuracy for inhibition of TxA_2 synthesis will reflect statistically significant ($p < 0.05$) differences in potency when compounds differ in potency by $^1/_2$ log unit or more; i.e., 10 ng/mL is different from 30 ng/mL; 30 ng/mL is different from 10 or 100 ng/mL. ED_{50} estimates are rounded to the nearest $^1/_2$ log dose in increment, e.g., 10, 30, 100, 300, 1000, and 3000 ng/mL. Therefore, small differences in potency (less than $^1/_2$ log unit), which may exist, will not be detected in this screen for activity. [c] Elemental analyses for C, H, and N were within ±0.4% of theory. Homogeneity of samples for which elemental analysis are not given was determined by two methods. TLC (thin-layer chromatography) on silica gel was used to determine if hydrolysis of the ester precursors to the sodium salts was complete. Reversed-phase HPLC (high-pressure liquid chromatography) was used to determine homogeneity of the sodium salts. The HPLC method used was adapted from: (a) Wynalda, M. A.; Liggett, W. F.; Fitzpatrick, F. A. *Prostaglandins* **1983**, *26*, 311. (b) Lakings, D. B.; Friis, J. M. *J. Pharm. Sci.* **1985**, *74*, 455. All samples were homogeneous by these methods unless indicated otherwise. [d] A trace of unhydrolyzed ester was detected in the TLC of this compound. [e] The HPLC of this salt contained a second minor component (10% based on integration of the peak size). [f] This compound has been described: Cross, P. E.; Dickinson, R. P.; Thomas, G. N. Eur. Patent Appl. 81 304 930.1, publication no. 050957, May 5, 1982.

tion in each isomers were assigned from the NMR spectra of the compounds (see Experimental Section).

Three of the four monochloropyridines, i.e., the 6-chloro (**22**), the 2-chloro (**23**), and the 4-chloro (**25**), were obtained in sufficient quantity to permit conversion by method D into sodium salts **26**, **27**, and **28**, respectively.

Finally, the derivative of **9b** in which the bridging methylene was oxidized to a ketone was prepared by the reaction of **8b** with potassium superoxide in dimethyl sulfoxide. Under these conditions, the ester is also hydrolyzed and acid **29** is isolated from the reaction. A similar oxidation of a benzylic methylene group with KO_2 has been reported.[17]

Pharmacology. Rabbit aorta contracting substance (RCS) was first described by Piper and Vane[18] as a novel biological activity released from sensitized guinea pig lungs by antigen challenge. Further characterization[19] led to the suggestion that the RCS was one of the unstable endoperoxide intemediates[20] in the prostaglandin biosynthetic pathway. However, quantitative analysis of RCS indicated that there was not enough endoperoxide to account for all of the rabbit aorta contracting activity.[21] Shortly thereafter, Hamberg, Svensson, and Samuelsson identified thromboxane A_2 (TxA_2), demonstrated that it contracted the rabbit aorta, and showed that is half-life was similar to that of RCS.[2] The biological activity of TxA_2 has been estimated to be 10–100 times greater than that of the endoperoxides on the rabbit aorta.[22] Thus, the major component of RCS activity[18] appears to be due to TxA_2.

The ability of our compounds to inhibit TxA_2 biosynthesis was evaluated in vitro by using microsomal enzyme from human platelets. The enzyme was incubated with the potential inhibitor for 5 min before adding the substrate, PGH_2. Thirty seconds after addition of PGH_2, the incubation mixture was passed over rabbit aorta strips (see Experimental Section) and the response of the strip taken as a measure of TxA_2 concentration. In this way, the ED_{50} values for our key compounds were obtained as summarized in Table II. The most potent of these compounds, **9b**, inhibits TxA_2 generation at a concentration of 10 ng/mL.

A number of structure–activity relationships (SARs) for TxA_2 synthase inhibitors have been described previously.[7b,c,8a,b,d,e,g-j] For analogues containing pyridine and imidazole rings, the SARs may be summarized briefly as (a) interaction of the pyridine or imidazole with a cytochrome P-450 of the enzyme,[23] (b) substitution in the 3-position of pyridine or the 1-position of imidazole with a carboxylic acid side chain, and (c) an optimum distance of 8.5–10 Å between heterocyclic nitrogen and the carboxyl group. The results shown in Table II for our series of inhibitors are

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Table III. Inhibition of Thromboxane Synthase Activity in Microsomal Preparations[a] from Human Platelets (HPM), Guinea Pig Lung (GPLM), and Dog Lung (DLM)

substance	ED_{50},[b] ng/mL		
	HPM	GPLM	DLM
9b (furegrelate, U-63557A)	10	800	2
2 (OKY-1581)	30	200	3
3 (dazoxiben, UK-37248)	30	30	15

[a] Microsomal preparations of guinea pig and dog lung were prepared by the same methods used for human platelets.[22] [b] Experiments were run in triplicate.

consistent with these SARs. Our most potent compound, **9b**, is comprised of a pyridine ring attached at the 3-position to a benzofuran-2-carboxylic acid. Models reveal many conformations of **9b** having a distance in the range of 8.5–10 Å between the carboxyl group and the pyridine nitrogen. When the chain connecting the pyridine to the benzofuran system is lengthened to two methylenes (**9g** and **9h**), the distance between carboxyl and nitrogen is increased and is greater than 10 Å in a number of conformations. A significant decrease in activity is observed for these two analogues. Substitution of the benzofuran ring system with groups such as 3-methyl (**9i**), 7-methyl (**9c**), 7-methoxy (**9d**), and 7-bromo (**9e**) affects only slightly the activity of the analogues, whereas 2,4- or 6-chloro substituents on the pyridine ring (**27**, **28**, and **26**, respectively) resulted in significant reductions in potency.

Although **9b** was the most potent agent tested on human platelet microsomal preparations (including the inhibitors **1–3**), it was not uniformly effective on microsomal preparations from other sources. As shown in Table III, an 80-fold higher concentration of **9b** is required to inhibit TxA_2 synthase from guinea pig lung, whereas only $^1/_5$ the concentration that was effective on platelets is needed to inhibit the dog lung synthase. In comparison,[24] the pyridine-containing inhibitor OKY-1581 (**2**) also is varied in effectiveness toward enzymes from different sources, while the imidazole-containing inhibitor dazoxiben (**3**) is nearly equivalent in inhibiting all three enzymes. The reasons for these results are not clear; however, they may reflect differences in the TxA_2 synthase obtained from different tissues and clearly reinforce the importance of evaluating TxA_2 synthase inhibitors on enzyme from a source relevant to the desired therapeutic use of the compounds.

In experiments reported previously,[11] the selectivity of **9b** toward TxA_2 synthase was shown by a lack of any effect on other enzymes of the arachidonic acid cascade, including phospholipase, cyclooxygenase, and prostacyclin synthase.

Experimental Section

Assay of Human Platelet Thromboxane Synthase Activity. Thoracic aorta was removed from rabbits and cut spirally into strips.[25] They were superfused[26] in series[27] with Krebs solution containing flurbiprofen (1 μg/mL), which prevented production of endogenous prostaglandins in the bioassay tissue. Thromboxane A_2 was prepared by mixing PGH_2 with human platelet microsomes for 30 s prior to adding this mixture to the superfusion fluid above the tissues. The human platelet microsomes (HPM) were prepared by the method of Needleman et al.[22] PGH_2 was prepared by the method of Gorman et al.[28]

Potential inhibitors were tested by incubating them with the HPM for 5 min on ice prior to mixing with PGH_2. After the 30-s mixing in a polyethylene vial with the PGH_2, the entire reaction mixture was added to the superfusion fluid above the tissues. Comparison of the ratio of the response of the rabbit aorta to this mixture and to the mixture without any potential inhibitor added gave a percent inhibition at the dose tested. Compounds were initially tested at 10 μg/mL concentration, and if they showed any activity, they were tested at lower doses until an ED_{50} could be approximated by extrapolation from the responses. Radioimmunoassay of TxB_2 (generously done for us by F. A. Fitzpatrick) confirm that a 50% reduction in response of the rabbit aortic strip is equivalent to a 62% reduction in TxB_2. Therefore, the rapid bioassay screen correlates well with, or perhaps slightly underestimates, the potency based on actual amounts of TxA_2 formed.

3-(4-Aminobenzyl)pyridine (5). A solution of 3-(4-nitrobenzyl)pyridine[12] (4, 52.73 g, 0.246 mol) in methanol (400 mL) was shaken with hydrogen over 10% palladium on carbon (2.5 g) in a Parr hydrogenation apparatus until hydrogen uptake was complete (about 1 h). The catalyst was removed by filtration under a flow of N_2 and washed with methanol. The methanol filtrate was reduced in volume to about 150 mL and cooled overnight in a refrigerator. Crystals formed and were collected (32.04 g), and the filtrate was concentrated. The residue is dissolved in methylene chloride and a second crop of crystals was obtained from CH_2Cl_2–hexane. Total yield of colorless to light-yellow-orange crystals of **5** was 43.43 g (0.236 mol, 96%). Recrystallization gave colorless crystals of **5**: mp 121–123 °C; IR (Nujol) 3400, 3300, 3200, 1640, 1610, 1590, 1575, 1515, and 1480 cm^{-1}; UV (EtOH) 241 nm (ε 11 450), 285 sh (1950). Anal. C, H, N.

3-(4-Hydroxybenzyl)pyridine (6b). Method A. An ice-cold solution of sodium nitrite (10.39 g, 0.151 mol) in water (40 mL) was added over a 5-min period to a stirred, ice-cold solution of 3-(4-aminobenzyl)pyridine (24.71 g, 0.134 mol) in water (40 mL) containing concentrated sulfuric acid (27 mL) and ice (67 g). Following addition, the reaction solution was kept on ice and stirred for 1 h. The excess nitrous acid was then quenched by the addition of an aqueous urea solution. The reaction solution was then added dropwise (15 min) to a hot (160 °C oil bath), stirred solution of water (67 mL) and concentrated sulfuric acid (84 mL). The resulting solution was kept at 160 °C for another 10 min and then was cooled to ice-bath temperature. Ice (220 g) was added to the solution, and then the pH was adjusted to 7.0 by careful addition with stirring of aqueous 50% sodium hydroxide. A precipitate formed and was collected by filtration and was washed with water. After the solid was dried, it weighed 190 g (contained much inorganic material) and was boiled with several portions of acetone. These acetone extracts were filtered and then concentrated to yield a solid. Recrystallization of the solid from acetone–hexane gave a first crop (10.59 g) and a second crop (8.07 g, total 18.66g, 0.101 mol, 75%) of crystals, both having mp 178–181.5 °C. Recrystallization from acetone–hexane gave **6b** as colorless crystals: mp 184–186 °C; IR (Nujol) 2900, 2800, 2720, 2680, 2600, 1615, 1590, 1580, 1515, 1485, 1280, 1250, 845, 800, 710, 640 cm^{-1}; UV (EtOH) 226 nm (ε 9100), 258 sh (3850), 264 (4350). Anal. C, H, N (see Table I).

Also prepared by method A from 3-(4-aminophenyl)pyridine[29] was **3-(4-hydroxyphenyl)pyridine (6a)** (65%): mp 202–203 °C after recrystallization from acetone–hexane; IR (Nujol) 2688, 2652, 2624, 2455, 1608, 1595, 1583, 1525, 1292, 1274, 1251, 1180, 842, 830, 812 cm^{-1}; *high-resolution mass spectrum*, 171.0690 (calcd for $C_{11}H_9NO$: 171.0684), *m/e* 154, 142, 115. Anal. C, H, N.

3-(3-Formyl-4-hydroxybenzyl)pyridine (7b). Method B. A solution of **6b** (2.038 g, 0.011 mol) and hexamethylenetetramine (1.61 g, 0.0115 mol) in trifluoroacetic acid (20 mL) was stirred and heated for 4 h in an 80 °C oil bath. Excess trifluoroacetic acid was removed under reduced pressure, and water (35 mL) was added to the residue. The resulting solution was left at room temperature for 20 min before the pH of the solution was adjusted to about 7 by the addition of solid sodium bicarbonate. A gummy oil precipitated. The mixture was extracted with ethyl acetate

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(3×), and the extract solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was chromatographed over silica gel (130 g) packed as a slurry in 1:1 acetone–hexane. Elution was with the same solvent and 150-mL fractions were collected. The desired product **7b** (1.263 g, 0.005 93 mol, 54%) was obtained in fractions 5–7 as a colorless, crystalline solid. Two recrystallizations from acetone–hexane gave colorless crystals of **7b**: mp 130–132 °C; ^1H NMR ($CDCl_3$) δ 9.89 (s, 1 H, CHO), 8.55 (m, 2 H, pyridine C_2H and C_6H), 6.70–7.66 (m, 5 H, aromatic H), 3.98 (s, 2 H, Py–CH_2–Ar); IR (mull) 2560 (OH), 1670 (C=O), 1610, 1595, 1580, 1505, 1480 (C=C/C=N) cm^{-1}; Anal. (see Table I). Compounds **7a,c,d,f–h** also were prepared by method B.

5-(3-Pyridylmethyl)benzofuran-2-carboxylic Acid, Ethyl Ester (8b). Method C. A mixture of sodium hydride (1.25 g, 0.052 mol) in dry toluene (20 mL) was stirred under N_2. To this was added slowly a solution of **7b** (4.26 g, 0.020 mol) in toluene (300 mL). Then a solution of diethyl bromomalonate (5.26 g, 0.022 mol) in toluene (20 mL) was added dropwise. Dicyclohexyl-18-crown-6 (36 drops) was added, and the resulting mixture was stirred at room temperature until TLC (1:1 acetone–hexane) indicated the reaction was complete (usually 48 h, but the time for this reaction may vary from 12 h to several days). The reaction was poured into a mixture of ice (100 mL), saturated sodium bicarbonate solution (100 mL), and brine (200 mL). The mixture was extracted with EtOAc (3×), and the combined extracts were washed with brine (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated to a yellow oil (6.57 g). The oil was chromatographed over silica gel (200 g, 30% acetone–hexane, 200-mL fractions), and the desired product **8b** was eluted in fractions 7–12. Recrystallization from acetone–hexane gave **8b** (4.62 g, 0.0165 mol, 82%) as a crystalline solid: mp 73–74 °C; ^1H NMR ($CDCl_3$) δ 8.54 (m, 2 H, pyridine C_2H and C_6H), 7.67–7.00 (m, 6 H, aromatic H), 4.45 (q, 2 H, J = 7 Hz, OCH_2CH_3), 4.10 (s, 2 H, Py–CH_2–Ar), 1.41 (t, 3 H, J = 7 Hz, OCH_2CH_3). Anal. (see Table I). Compounds **8a,c–h** were also prepared by method C. In the case of **8d**, the intermediate **10** was isolated and then converted to **8d** (see below).

5-(3-Pyridylmethyl)benzofuran-2-carboxylic Acid, Sodium Salt (9b, Sodium Furegrelate, U-63557A). Method D. A solution of **8b** (16.83 g, 0.060 mol) in methanol (50 mL), aqueous 1.0 N sodium hydroxide (60.0 mL, 0.060 mol), and distilled water (125 mL) was stirred at room temperature for 1.5 h. TLC (1:1 acetone–hexane) showed no remaining **8b**. The solution was concentrated to dryness, leaving a crystalline residue. The crystalline mass was dissolved in water (100 mL) and crystallization caused by the addition of acetone (2.5 L). The glistening white crystals are collected by filtration, and after drying, 15.68 g (0.057 mol, 95%) of **9b** is obtained: mp >300 °C; IR (Nujol) 3355, 3277, 1600, 1590, 1572, 1402, 1333, 1192, 1140, 939, 834, 805, 790, 718 cm^{-1}; UV (EtOH) λ 2.11 (ε 28 250), 261 sh (19 750), 268 (22 150), 276 sh (14 550), 288 (sh (7850), 299 (5800). Anal. ($C_{15}H_{10}NO_3Na·H_2O$) C, H, N, Na, H_2O.

Also prepared by this method were compounds **9a,c–i**, and **21**, **26–28**; in most cases these salts were obtained either as amorphous powders or as glasses. ^1H NMR spectra of the salts in D_2O were obtained and were consistent with the assigned structures.

Isolation of 3-Hydroxy-7-methoxy-5-(3-pyridylmethyl)-2,3-dihydrobenzofuran-2,2-dicarboxylic Acid, Diethyl Ester (10). During the attempted preparation of **8d** from **7d** (2.43 g, 0.010 mol) by method C, the reaction was observed by TLC to stop with approximately equal amounts of starting material and a new product remaining. The reaction was quenched, and following chromatography (two Merck size B Lobar silica gel columns, ethyl acetate) there was obtained first 1.21 g (0.003 02 mol, 30%) of **10** followed by mixed fractions (0.184 g) and starting material (0.410 g). Recrystallization from acetone–hexane gave **10** as colorless crystals: mp 140.5–142 °C; IR (Nujol) 3004–3093 (sh), 2728, 1744, 1722, 1622, 1608, 1596, 1582, 1503, 1309, 1303, 1260, 1222, 1144, 1092, 1068, 1043, 1032, 1023, 856, 728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.42 (m, 2 H, pyridine C_2 and C_6 protons), 7.50 (m, 1 H, pyridine C_4 or C_5 proton), 7.24 (m, 1 H, pyridine C_4 or C_5 proton), 6.85 (s, 1 H, aromatic), 6.70 (s, 1 H, aromatic), 5.98 (s, 1 H, Ar–CH(OH)), 4.30 (m, 4 H, OCH_2CH_3), 3.90 (s, 2 H, Py–CH_2–Ar), 3.83 (s, 3 H, OCH_3); mass spectrum, 401.1483 (34%) ($C_{21}H_{23}NO_7$ requires 401.1474), m/e 311 (100), 282 (20), 266 (14), 254 (18), 241 (27). Anal. C, H, N.

This compound (**10**) was treated with sodium hydride in dry THF under N_2 and was slowly converted (after 10-fold dilution with THF) to the desired **8d** (see Table I).

(*E,Z*)-1-(4-Hydroxyphenyl)-2-(3-pyridyl)ethylene[30,31] (13g). Method E. Under an N_2 atmosphere a dry flask was charged with dry THF (170 mL) and *n*-butyllithium in hexane (80 mL of 1.6 M solution, 0.128 mol) and then cooled in an ice bath. 3-Pyridylmethyltriphenylphosphonium chloride hydrochloride (17.04 g, 0.04 mol) was added in portions as the solid. A dark reddish brown slurry resulted. The ice bath was removed, and the mixture was stirred for 30 min at room temperature. Then a solution of 4-hydroxybenzaldehyde (**12**, 4.0 g, 0.033 mol) in THF was added dropwise over a 15-min period. The reaction mixture was stirred overnight at room temperature and then was poured into ice-cold 2 N HCl (final pH ~2) and extracted with ether (2×). The aqueous phase was then made alkaline with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (4×). The combined EtOAc extracts were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated, giving a red oil. The oil was chromatographed over silica gel (500 g, 1:1 EtOAc–hexane) to give **13g** as an oil (1.50 g, 0.0076 mol, 23%); ^1H NMR ($CDCl_3$) δ 8.53 (d, 1 H, pyridine C_2H), 8.40 (d of d, 1 H, pyridine C_6H), 7.01 (d, 2 H, J = 8 Hz, aromatic H). The compounds were used without further analyses in the next step.

(*E,Z*)-1-(3-Hydroxyphenyl)-2-(3-pyridyl)ethylene[31] (13h) also was prepared by method E. From the reaction of 3-hydroxybenzaldehyde (2.0 g, 0.016 mol) with 3-pyridylmethylenetriphenylphosphorane (**11**, 0.020 mol), there was obtained after chromatography (400 of g silica gel, 25% acetone–hexane) the less polar, minor isomer of **13h** (0.41 g), a mixture of isomers (0.59 g), and the more polar, major isomer of **13h** (1.27 g, total 2.27 g, 0.0115 mol, 72%). NMR spectrum of the minor isomer: ($CDCl_3$) δ 8.34 (d, 1 H, J = 2 Hz, pyridine C_2 or C_6 proton), 8.17 (d of d, 1 H, J = 5.5 Hz, pyridine C_2 or C_6 proton). NMR spectrum of the major isomer: (acetone-d_6) δ 8.80 (d, 1 H, pyridine C_2 or C_6 proton), 8.48 (d or d, 1 H, pyridine C_2 or C_6 proton). The compounds were used without further purification in the preparation of **6h**.

1-(4-Hydroxyphenyl)-2-(3-pyridyl)ethane (6g). Method F. The ethylene **13g** (1.96 g, 0.0099 mol) was dissolved in absolute EtOH (50 mL) and reduced over 10% Pd–C (120 mg) in a Parr hydrogenation apparatus. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The crude product was chromatographed over silica gel (two Merck Lobar size B columns) using 50–75% EtOAc in hexane. The product **6g** (0.613 g, 0.003 08 mol, 31%) was a solid. Two recrystallizations from acetone–hexane gave **6g**: mp 180–181.5 °C; IR (mull) 2749, 2684, 2614 (OH), 1611, 1593, 1579, 1515 (C=C/C=N) cm^{-1}; Anal. (see Table I). Compound **6h** also was prepared by method F.

3-[[3-Methoxy-4-(benzyloxy)phenyl]hydroxymethyl]-pyridine (16d). Method G. A solution of *n*-BuLi (75.5 mL of a 1.6 M solution in THF, 0.12 mol) in ether (125 mL) under N_2 was cooled to −78 °C. To this was added dropwise over a period of 45 min a solution of 3-bromopyridine (15.8 g, 0.10 mol) in ether (125 mL). A light-yellow solid precipitated from solution. The mixture was stirred at −78 °C while adding dropwise over a period of 20 min a solution of 3-methoxy-4-benzyloxybenzaldehyde (**15**, 24.2 g, 0.10 mol) in ether (250 mL) plus THF (25 mL). The reaction mixture was allowed to warm to room temperature and was stirred overnight. The mixture was poured into water and extracted with ether (2 × 500 mL). The ether extracts were pooled, washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue (34 g) was combined with the crude product (33 g) from a second identical preparation and was chromatographed over silica gel (1.2 kg, 40% acetone in hexane), using acetone–hexane to elute the column. The product was eluted with 50–60% acetone–hexane and was a crystalline solid (42.14 g, 0.131 mol, 65%). Two recrystallizations

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from acetone–hexane gave **16d** as white crystals: mp 107.5–108.5 °C; IR (Nujol) 3150, 1607, 1592, 1583, 1514, 1261, 1222, 1132, 1077, 1030, 1008, 921, 862, 806, 752, 710, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (d, 1 H, py C_1 proton), 8.25 (d of d, 1 H, py C_6 proton), 7.66 (m, 1 H), 6.70–7.53 (m, 9 H, aromatic), 5.68 (s, 1 H, Py–*CH*–(OH)–Ar), 5.08 (s, 2 H, OCH_2Ph), 3.73 (s, 3 H, OCH_3); mass spectrum, *m/e* 321 (M^+), 230, 106, 91. Anal. C, H, N.

3-[[3-(Benzyloxy)phenyl]hydroxymethyl]pyridine (16e) also was prepared by method G and was obtained in 73% yield as white crystals: mp 86–88 °C; IR (Nujol) 3167, 1600, 1593, 1579, 1258, 1082, 1070, 1023, 799, 740, 718, 699, 694 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.53 (d, 1 H, py C_1 or C_6 proton), 8.37 (d of d, 1 H, py C_1 or C_6 proton), 6.8–7.9 (m, 11 H, aromatic), 5.78 (s, 1 H, carbinol proton), 5.03 (s, 2 H, benzylic methylene). Anal. H, N, C: calcd, 78.53; found, 77.89.

2-Methoxy-4-(3-pyridylmethyl)phenol (6d). Method H. A solution of 16 (18.12 g, 0.056 mol) in absolute ethanol (200 mL) and 70% perchloric acid (5.2 mL, 0.056 mol) was shaken with 10% palladium on carbon (7.0 g) and hydrogen in a Parr hydrogenation apparatus. Hydrogen uptake was complete after 4 h, and the catalyst was removed by filtration. The ethanol was removed under reduced pressure, and the residue was taken up in the ethyl acetate and washed with aqueous sodium bicarbonate. The aqueous layer was reextracted with ethyl acetate, and the combined ethyl acetate layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue (dark red) partially crystallized. From this and from a second identical reduction there was obtained after crystallization from ethanol 7.52 g of crystals, mp 138–141 °C. Recrystallization from acetone gave 6.36 g of **6d**, mp 141–142 °C. The filtrates were pooled and chromatographed over silica gel (1 kg) packed in 35% acetone–hexane and gave an additional 6.35 g (total 12.71 g, 0.059 mol, 51%) of **6d**: mp 142–143 °C; IR (Nujol) 2785–2431, 1607, 1592, 1579, 1510, 1427, 1277, 1244, 1193, 1155, 1129, 1047, 1039, 870, 819, 805, 742, 709 cm^{-1}; ^1H NMR (acetone-d_6) 8.57 δ (d, 1 H, pyridine C_2 proton), 8.47 (d of d, 1 H, pyridine C_6 proton), 7.65 (m, 1 H, pyridine C_5 or C_4 proton), 7.30 (four-line pattern, 1 H, pyridine C_5 or C_4 proton), 6.63–7.00 (m, 3 H, aromatic proton), 3.92 (s, 2 H, Py–CH_2–Ar), 3.80 (s, 3 H, OCH_3); mass spectrum, 215.0937 (100%) (calcd for $C_{13}H_{13}NO_2$: 215.0946), *m/e* 200 (14), 184 (15), 172 (32), 153 (26). Anal. C, H, N. Compound **6f** was also prepared by this method.

2-Methyl-4-(3-pyridylmethyl)phenyl (6c). A solution of 2-methyl-4-(3-pyridylmethyl)anisole (17) (18.83 g, 0.088 mol) in aqueous 48% hydrobromic acid (50 mL) was heated for 18 h in an 110 °C oil bath. The reaction solution was poured into ice water, and the pH of the resulting solution was adjusted to about 7 by careful addition of 50% aqueous sodium hydroxide. The mixture was extracted with ethyl acetate (4×), and the pooled extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue (15.62 g) was a solid that was crystallized from acetone–hexane, giving 12.29 g (0.0618 mol, 70%) of **6c**, mp 165–168 °C capillary. Two recrystallizations from ethyl acetate–hexane gave **6c** having mp 166.5–168.5 °C capillary; IR (Nujol) 2713, 2676, 2579, 1740 (m), 1611, 1592, 1588, 1512, 1480, 1435, 1425, 1273, 1262, 1245, 1213, 1186, 1143, 1120, 1046, 1033, 810, 739, 710, 645, 630 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.52 (d, 1 H, J = 2.5 Hz, pyridine C_2 proton), 8.42 (d of d, 1 H, J = 2, 5 Hz, pyridine C_6 proton), 7.60 (d of t, 1 H, J = 2.5, 8 Hz, pyridine C_4 proton), 7.27 (m, 1 H pyridine C_5 proton), 6.67–7.05 (m, 3 H, aromatic protons), 3.88 (s, 2 H, Py–*CH*$_2$–Ar), 2.16 (s, 3 H, CH_3); mass spectrum, 199.0995 (100%) (calcd for $C_{13}H_{13}NO$: 199.0997), *m/e* 184 (74), 170 (11), 154 (10), 121 (31). Anal. H, N, C: calcd, 78.36; found, 76,51, 76.42.

3-Bromo-2-hydroxy-5-(3-pyridylmethyl)benzaldehyde (7e). Bromine (0.54 mL, 1.59 g, 0.010 mol) was added dropwise to a stirred solution of **7b** (2.13 g, 0.010 mol) in glacial HOAc (50 mL). Orange crystals formed in the reaction. These were collected by filtration after 30 min and weighed 1.86 g. The filtrate contained unreacted **7b** (TLC, 75% EtOAc in hexane), and additional bromine (0.2 mL) was added. After 30 min, additional orange crystals (2.18 g) were collected. Recrystallization was attempted from acetone–hexane but was unsuccessful, so the solvent was removed under reduced pressure leaving a greenish residue. The residue was dissolved in H_2O; the pH was adjusted to 7 by the addition of Na_2CO_3; and the resulting mixture was extracted with ether (1×) and EtOAc (4×). The pooled extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was taken up in acetone (incompletely dissolved) and chromatographed over silica gel (65 g) packed as a slurry in 1:1 acetone–hexane. Fractions of 25 mL were collected, and **7e** was obtained in fractions 12–22. Recrystallization from acetone–hexane gave a first crop (0.919 g) of yellow crystals, mp 87–88 °C, and a second crop (0.212 g, 1.131 g total, 0.0045 mol, 45%) of pale-yellow crystals, mp 114–115 °C. Recrystallization of the first crop from acetone–hexane gave the isomorphic crystalline form of **7e**: mp 115–116 °C; IR (mull) 3055 (weak) (OH), 1650 (C=O) cm^{-1}; UV (EtOH) at 0.018 56 g/L 221 nm (ϵ 21 600), 258.5 (11 500), 263 (12 350), 342 (3000), 400 (1750), at 0.007 42 g/L 220 (29 500), 263 (12 250), 268 sh (10 600), 345 (2750), 399 (3250); mass spectrum, *m/e* 293 (97%), 291 (100). Anal. (see Table I).

5-(3-Pyridylmethyl)-2-benzofurancarboxylic Acid (18). Aqueous 1.0 N HCl (2.0 mL, 0.0020 mol) was added to a solution of **9b** (0.550 g, 0.0020 mol) in water (1 mL). A white solid separated immediately and was collected by filtration using an extra milliliter of water to aid in transfer of the mixture. The solid was washed with water (4 mL) and dried, giving 0.458 g (0.001 81 mol, 91%) of crystalline 18, mp 227–228 °C. The solid can be recrystallized from hot water or from acetone. From acetone, colorless crystals, mp 229–230 °C, were obtained with the following properties: IR (mull) 1722 (CO_2^-), 1567, 1332, 1199, 1143, 1060, 804, 775, 764, 712 cm^{-1}. Anal. C, H, N.

5-(3-Pyridylmethyl)-2-benzofurancarboxylic Acid Hydrochloride (18·HCl). Ether (20 mL) saturated with HCl(g) was added to a solution of 18 (0.210 g, 0.000 83 mol) in acetone (375 mL). The volume of the resulting solution was reduced to about 250 mL by evaporation on a steam bath. At this point crystals were seen, and so the solution was cooled on ice for 2 h. The crystals (18·HCl) were collected (0.165 g, 0.000 571 mol, 69%) and had mp 216–248 °C. Treatment with additional HCl did not change the melting point: IR (mull) 2575 (NH^+), 1716 (C=O), 1633, 1611, 1566, 1555, 1278, 1265, 1193, 1137, 938, 833, 819, 807, 791, 767, 761, 745, 715, 690 cm^{-1}. Anal. C, H, N. Crystalline 18·HCl dissolved readily in water at 25 °C, but after standing several minutes, a crystalline precipitate formed. These crystals were collected and had mp 227–229 °C.

5-(3-Pyridylmethyl)-2-benzofurancarboxylic Acid Amide (19). A solution of **8b** (2.0 g, 0.0071 mol) in absolute EtOH saturated with anhydrous NH_3 was stirred intermittenly at 25 °C (total 100 h) and at reflux temperature (2× for a total of 12 h). The solution was resaturated with NH_3 following each reflux period. Although some unreacted **8b** was still detected by TLC, the ethanol was removed under reduced pressure and the residue was recrystallized from acetonitrile to give **19** (1.396 g, 0.005 53 mol, 78%) as a colorless crystals, mp 151.5–152.5 °C, which contained no **8b** as determined by TLC. A second recrystallization from CH_3CN gave colorless crystals of **19**: mp 151–5.152.5 °C; IR (mull) 3249, 3085 (NH_2), 1695 (C=O), 1635 (amide II) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55 (m, 2 H, pyridine C_2 and C_6 H), 7.16–7.64 (m, 6 H, aromatic H), 4.10 (s, 2 H, Py–CH_2–Ar); mass spectrum, $C_{15}H_{12}N_2O_2$ requires 252.0899 (found: 252.0891 (100%)), *m/e* 236 (8), 208 (10), 180 (21). Anal. C, H, N.

5-(3-Pyridylmethyl)-2-benzofurancarboxylic Acid, Ethyl Ester, *N*-Oxide (20). A solution of **8b** (0.563 g, 0.0020 mol) and *m*-chloroperbenzoic acid (0.447 g of an 85% purity reagent, 0.0022 mol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 2 h. More CH_2Cl_2 was added, and the solution was extracted with saturated aqueous $NaHCO_3$ (2×), with H_2O, and then was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product (0.621 g) was recrystallized 2 times from acetone–hexane, giving **20** (0.445 g, 0.0015 mol, 75%) as colorless crystals: mp 120–121 °C; IR (mull) 1739 (C=O), 1602, 1568, 1490 (C=C/C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.09 (m, 2 H), 7.27 (m, 6 H, aromatic H), 4.45 (q, 2 H, J = 9 Hz, OCH_2CH_3), 4.03 (s, 2 H, Py–CH_2–Ar), 1.42 (t, 3 H, J = 9 Hz, OCH_2CH_3); mass spectrum, calcd for $C_{17}H_{15}NO_4$, 297.1001; found, 297.0994. Anal. C, H, N.

5-[3-(2-Chloropyridyl)methyl]-2-benzofurancarboxylic Acid, Ethyl Ester (23); 5-[3-(4-Chloropyridyl)methyl]-2-benzofurancarboxylic Acid, Ethyl Ester (25); 5-[3-(5-Chloropyridyl)methyl]-2-benzofurancarboxylic Acid, Ethyl Ester (24); and 5-[3-(6-Chloropyridyl)methyl]-2-benzofurancarboxylic Acid, Ethyl Ester (22). A solution of **20** (0.878

g, 0.00295 mol) in phosphorus oxychloride (10 mL) was stirred and heated to the reflux temperature of $POCl_3$ for 30 min. At this time TLC indicated that the desired reaction was complete. The reaction solution was poured into ice water and ether, and the pH of the aqueous phase was adjusted to ~8 by the addition of solid potassium carbonate. The ether layer was separated, and the aqueous phase was extracted 3 more times with ether. The ether extracts were pooled, washed with water, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residual oil was chromatographed over two Merck size B Lobar silica gel columns, using 20% ethyl acetate–hexane followed at fraction 113 by 40% ethyl acetate–hexane to elute the column. Fractions of 25 mL were collected, and the products eluted in the following order of increasing polarity: isomer **22** (0.295 g, 0.00093 mol, 31%) was obtained in fractions 31–43; isomer **23** (0.271 g, 0.00086 mol, 29%) was obtained in fractions 47–56; isomer **24** (0.017 g, 0.000054 mol, 2%) was obtained in fractions 78–87; and isomer **25** (0.114 g, 0.00036 mol, 12%) was obtained in fractions 145–154. After pooling the appropriate fractions and removing solvent, all the products crystallized. The individual isomers were recrystallized and analyzed as follows.

Recrystallization of fractions 31–43 from acetone–hexane gave 0.272 g of **22** as colorless crystals: mp 112–112.5 °C; IR (Nujol) 1734, 1723, 1563, 1439, 1320, 1304, 1288, 1214, 1194, 1145, 1140, 1097, 1025, 836, 812, 767, 746 cm^{-1}, ^1H NMR ($CDCl_3$) δ 8.36 (d, 1 H, J = 2.5 Hz, pyridine C_2 proton), 7.18–7.65 (m, 6 H, aromatic protons), 4.45 (q, 2 H, J = 7 Hz, OCH_2CH_3), 4.07 (s, 2 H, Py-CH_2-Ar), 1.42 (t, 3 H, J = 7 Hz, OCH_2CH_3); mass spectrum, 315.0649 (100%) (calcd for $C_{17}H_{14}{}^{35}ClNO_3$: 315.0662), m/e 270 (28), 242 (30), 214 (21). Anal. ($C_{17}H_{14}ClNO_3$) C, H, N.

Recrystallization of fractions 47–56 from hexane gave 0.243 g of **23** as colorless crystals: mp 82–83 °C; IR (Nujol) 1727, 1568, 1564, 1409, 1322, 1311, 1298, 1214, 1200, 1193, 1146, 1098, 1063, 1020, 843, 811, 786, 767 cm^{-1}; ^1H NMR δ 8.32 (d of d, 1 H, J_{H_5} = 5 Hz, J_{H_4} = 2 Hz, pyridine C_6 proton), 7.10–7.68 (m, 6 H, aromatic protons), 4.43 (q, 2 H, J = 7 Hz, OCH_2CH_3), 4.17 (s, 2 H, Py-CH_2-Ar), 1.40 (t, 3 H, J = 7 Hz, OCH_2CH_3); mass spectrum, 315.0658 (100%) (calcd for $C_{17}H_{14}{}^{35}ClNO_3$: 315.0662), m/e 270 (30), 242 (13), 214 (14). Anal. C, H, N.

The crystalline fractions 79–87 of **24** were used for analysis without recrystallization: mp 90–91 °C; ^1H NMR ($CDCl_3$) δ 8.48 (m, 2 H, pyridine C_2 and C_6 protons), 7.23–7.73 (m, 5 H, aromatic protons), 4.48 (q, 2 H, J = 7 Hz, OCH_2CH_3), 4.09 (s, 2 H, Py-CH_2-Ar), 1.43 (t, 3 H, J = 7 Hz, OCH_2CH_3); mass spectrum, 315.0661 (100%) (calcd for $C_{17}H_{14}{}^{35}ClNO_3$: 315.0662), m/e 271 (37), 242 (13), 214 (25).

Recrystallization of fractions 145–154 from hexane gave 0.098 g of **25** as colorless crystals: mp 103–104 °C; IR (Nujol) 1714, 1578, 1556, 1436, 1408, 1347, 1318, 1302, 1267, 1221, 1199, 1134, 1125, 1084, 1012, 943, 882, 833, 825, 820, 804, 782, 762, 741, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.52 (s, 1 H, pyridine C_2 proton), 8.47 (d, 1 H, J = 5.5 Hz, pyridine C_6 proton), 7.23–7.73 (m, 5 H, aromatic protons), 4.48 (q, 2 H, J = 7 Hz, OCH_2CH_3), 4.09 (s, 2 H, Py-CH_2-Ar), 1.43 (t, 3 H, J = 5 Hz, OCH_2CH_3); mass spectrum, 315.0655 (100%) (calcd for $C_{17}H_{14}{}^{35}ClNO_3$: 315.0662), m/e 270 (25), 242 (15), 214 (22). Anal. C, H, N.

5-(3-Pyridinylcarbonyl)-2-benzofurancarboxylic Acid (29). A mixture of potassium superoxide (0.20 g, 0.00282 mol) in dimethylsulfoxide (10 mL) containing dicyclohexyl-18-crown-6 (0.10 g) was stirred for 30 min at room temperature. The ester **8b** (0.140 g, 0.00050 mol) was added directly to the mixture and stirring was continued. The reaction was followed by TLC (1:1 acetone–hexane or 75% ethyl acetate–hexane) until no starting material remained in the reaction mixture. After 4 h the reaction was poured into water (75 mL), and the pH of the resulting solution was adjusted to 4–5 by addition of 1 N aqueous hydrochloric acid. A white precipitate gradually began to form. The solution was cooled on ice, and after several hours the solid was collected by filtration. The dry solid weighed 0.049 g (0.000183 mol, 36%) and did not melt below 300 °C. The solid could be crystallized from either acetic acid–water or methanol. A sample dissolved in hot glacial acetic acid and crystallized by the addition of water was submitted for analysis: IR (Nujol) 1731 (br), 1655, 1613, 1598, 1584, 1572, 1321, 1254, 1180, 1143, 1125, 1116, 1097, 1053, 754 cm^{-1}; mass spectrum, m/e 267 (74%), 222 (31), 189 (100), 151 (25). Anal. C, H, N.

Peptidyl Carbamates Incorporating Amino Acid Isosteres as Novel Elastase Inhibitors

George A. Digenis,* Bushra J. Agha, Kiyoshi Tsuji,† Masayuki Kato,† and Masaki Shinogi‡

College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536-0053. *Received August 12, 1985*

The design and synthesis of 13 novel peptidyl carbamates are described. When tested for inhibitory activity toward porcine pancreatic elastase, trypsin, and chymotrypsin, six compounds were found to specifically inhibit elastase without affecting the other two serine proteases. All the active inhibitors had an amino acid isostere at the P_1 position. Kinetic studies indicated that the inhibition was competitive with K_i values ranging from 4.23×10^{-5} to 2.4×10^{-6} M. The degree of inhibition was found to be dependent on the specificity of the peptide chain for the extended subsites on the enzyme as well as on the nature of P_1'. Preliminary work on one inhibitor indicates that the inhibition is reversible and proceeds via the rapid formation of a strong enzyme–inhibitor complex, followed by slow acylation of the serine residue on the active site of the enzyme. Peptidyl carbamates represent a novel class of elastase inhibitors.

Proteinases from polymorphonuclear leukocytes and macrophages, especially elastases (human leukocyte (HL) elastase and cathepsin G), appear to be responsible for the chronic tissue destruction associated with inflammation, arthritis, and emphysema.[1-4] During infection or inflammation, the normal lung is protected from proteolytic digestion by the protease inhibitor, α_1-antitrypsin. This protective mechanism appears to be nonoperative in individuals with an α_1-antitrypsin deficiency due to genetic or other causes. Synthetic elastase inhibitors capable of replacing α_1-antitrypsin would therefore be expected to be potentially useful in the treatment of pulmonary emphysema and related diseases.[5]

†Present address: Fujisawa Pharmaceutical Co., Ltd., 1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532, Japan.

‡Present address: Kobe Women's College of Pharmacy, 4-19-1, Motoyamakita-Machi, Higashinadada-KU, Kobe Shi 658, Japan.

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