aortic blood pressure, and left ventricular blood pressure were measured directly; left ventricular contractility was assessed by deriving dP/dt_{max} from left ventricular pulse pressure. The study consisted of fifteen 30-min periods. The first two periods were control periods prior to dosing, while the remaining thirteen periods were postdose experimental periods. Compound **15a**, 10 mg/kg, was administered as a dry powder in a gelatin capsule.

PDE Assays. Three isoenzymes of PDE were isolated from dog heart and dog kidney by chromatography on DEAE-cellulose.[30] These were labeled type I (calmodulin sensitive), type II (cGMP sensitive), and type IV (high-affinity PDE, PDE III) phosphodiesterase as recommended by the Committee on Nomenclature.[31] Assays for PDE activity were done by the two-step method of Thompson et al.,[30] using [^{3}H]cAMP as the substrate. Enzyme activity was initiated by addition of substrate to tubes containing buffer (pH 7.4) and sufficient enzyme to hydrolyze less than 20% of the substrate in 10 min at 30 °C. Dimethyl sulfoxide was used to dissolve compounds, and control assays containing identical concentrations of the solvent (1%) were run.

Acknowledgment. We thank Dr. Claude I. Judd for helpful discussions and encouragement. In addition, we thank Lee W. Emmert for technical support and Linda Austin for typing the manuscript.

Registry No. 1, 492-98-8; **2**, 101226-39-5; **3**, 112513-85-6; **4**, 111852-05-2; **5**, 111852-03-0; **6**, 111852-08-5; **7**, 101226-46-4; **8**, 101226-47-5; **9**, 101226-50-0; **10**, 101226-51-1; **11**, 101226-52-2; **12**, 123124-76-5; **13a**, 10111-08-7; **13b**, 13750-81-7; **13c**, 113825-16-4; **13d**, 37050-18-3; **13e**, 111851-98-0; **13f**, 69767-96-0; **13g**, 6002-15-9; **13h**, 10045-65-5; **13i**, 56248-10-3; **13j**, 123125-08-6; **13k**, 123125-09-7; **13l**, 102808-02-6; **14a**, 102807-85-2; **14b**, 102807-87-4; **14c**, 123125-01-9; **14d**, 111851-99-1; **14e**, 123125-02-0; **14f**, 123125-03-1; **14g**, 123125-04-2; 14h, 102807-88-5; 14i, 123125-05-3; **14j**, 123125-06-4; **14k**, 123125-07-5; **14l**, 102807-86-3; **15a**, 102807-93-2; **15b**, 102807-95-4; **15c**, 111851-87-7; **15d**, 111851-93-5; **15e**, 111851-88-8; **15f**, 111851-91-3; **15g**, 111851-90-2; **15h**, 102807-96-5; **15i**, 111851-92-4; **15j**, 111851-79-7; **15k**, 111851-80-0; **15l**, 111851-78-6; **15m**, 102807-94-3; **15n**, 111851-86-6; **15o**, 111852-14-3; **15p**, 111851-81-1; **16**, 111852-11-0; **16** (4′-Br), 111851-12-1; **17**, 123124-77-6; **18**, 111852-13-2; **19**, 123124-78-7; **20**, 123124-79-8; **21**, 123124-80-1; **22**, 111851-82-2; **23**, 111851-83-3; **24**, 123124-81-2; **25**, 123124-82-3; **26**, 111928-57-5; **27**, 123125-12-2; **27**·2HCl, 123124-83-4; **28**, 123124-84-5; **29**, 46323-27-7; **30**, 111851-95-7; **31**, 111851-97-9; **32a**, 500-22-1; **32b**, 98-03-3; **32c**, 498-62-4; **32d**, 4701-17-1; **32e**, 98-01-1; **32f**, 3920-50-1; **32g**, 35344-95-7; **33a**, 33468-84-7; **33b**, 33468-72-3; **33c**, 123125-10-0; **33d**, 81654-10-6; **33e**, 33468-88-1; **33f**, 102807-89-6; **33g**, 102807-90-9; **34a**, 123124-97-0; **34b**, 123124-98-1; **34c**, 123124-99-2; **34d**, 123124-00-8; 34e, 102807-99-8; **34f**, 102807-97-6; **34g**, 102807-98-7; **35**, 123124-85-6; **35**·2Ts-OH, 123124-86-7; **36**, 81371-82-6; **37**, 123124-87-8; **38**, 123124-88-9; **39**, 123124-89-0; **39**·HCl, 123125-11-1; **40**, 1122-28-7; **41**, 123124-90-3; **42**, 123124-91-4; **43**, 123124-92-5; 44, 123124-93-6; **45**, 33016-47-6; **46**, 123124-94-7; **47**, 123124-95-8; **48**, 123124-96-9; **49**, 102807-91-0; **50**, 102807-92-1; 1,1-dibromo-3,3,3-trifluoroacetone, 431-67-4.

N-Phenyl-2-pyridinecarbothioamides as Gastric Mucosal Protectants

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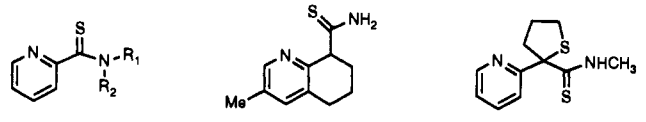
A series of substituted 2-pyridinecarbothioamides was synthesized and evaluated for gastric mucosal protectant activity in the rat. Out of this investigation *N*-(3,5-difluorophenyl)-2-pyridinecarbothioamide (**23**, AY-31,574) was identified. This compound was much more potent than sucralfate and ranitidine against ethanol-induced lesions. Compound **23** was equipotent with ranitidine against gastric injury caused by stress. Unlike ranitidine, **23** was found to be devoid of antisecretory activity in the pylorus-ligated rat model, making it a selective mucosal protectant. Such a potent selective mucosal protectant may provide a novel clinical approach in treating ulcers.

In recent years interest has grown in discovering therapeutic agents which prevent ulcers by increasing defensive forces present in the gut. The property of a drug protecting the integrity of the mucosa against aggressors such as acid, ethanol, and NSAID's without affecting the aggressor has been coined cytoprotection.[1] The primary approach toward curing ulcers clinically has been to decrease the aggressive forces injuring the ulcer. For instance, the H_2-antagonists cimetidine[2] and ranitidine[3] and the proton pump inhibitor omeprazole[4] have been used to reduce acid secretion, which successfully accelerates healing. However, omeprazole has an excessive duration of antisecretory action (>24 h) and like long-acting H_2 antagonists, it causes dysplasia when administered long-term in rats.[5] Therefore, the total elimination of gastric acid secretion (aggressive factors) appears to cause deleterious side effects. The potential advantage of purely cytoprotective agents such as sucralfate, which is an aluminum complex, has been demonstrated in the clinic by lower ulcer recurrence rates.[6] In the course of efforts to discover a novel type of antiulcer agent, thioamides of general structure **1** (Scheme I) were identified, which were potent cytoprotective agents with no antisecretory activity. In contrast, thioamides such as tiquinamide (**2**)[7] and picartamide (**3**),[8] on which **1** was based, are potent antisecretory agents. Because it was expected that a selective

Scheme I

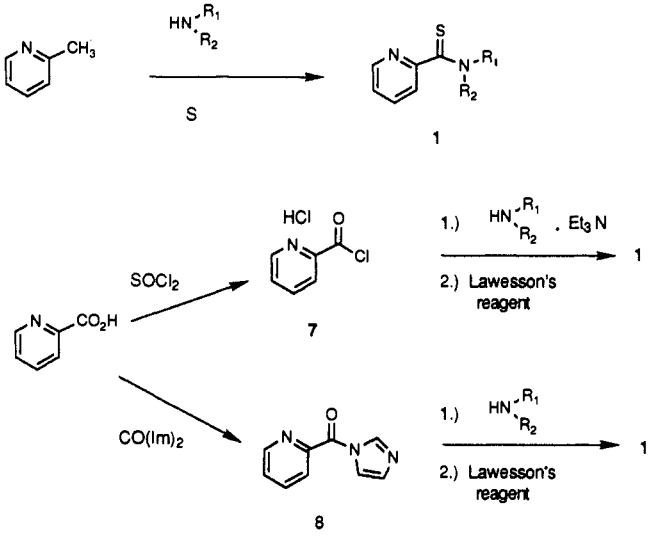


1 **2** **3**

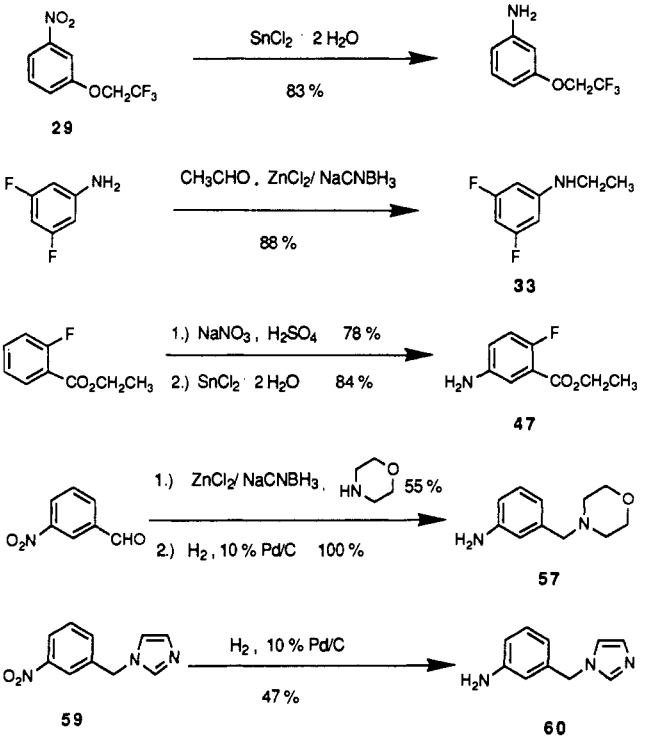
(1) Meeroff, J. C. *Dig. Dis. Sci.* **1985**, *30*, 697.
(2) Brimblecombe, R. W.; Duncan, W. A. M.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Leslie, G. B.; Parsons, M. E. *Gastroenterology* **1978**, *74*, 339.
(3) Brogden, R. N.; Carmine, A. A.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1982**, *24*, 267.
(4) Clissold, S. P.; Campoli-Richards, D. M. *Drugs* **1986**, *32*, 15.
(5) Ekman, L.; Hansson, E.; Havu, N.; Carlsson, E.; Lundberg, C. *Scand. J. Gastroenterol.* **1985**, *20*, Suppl. 108, 53.
(6) (a) Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1984**, *27*, 194. (b) Marks, I. N.; Wright, J. P.; Lucke, W.; Girdwood, A. H. *Scand. J. Gastroenterol.* **1983**, *18*, Suppl. 83, 53-56.
(7) Beatti, D. E.; Dixon, G. T.; Shriver, D. A.; Alps, B. J. *Drug. Res.* **1979**, *29(II)*, Nr. 9.
(8) Minaire, Y.; Forichon, J.; Woehrle, R. *Lancet* **1982**, *i*, 1179.

0022-2623/90/1833-0327$02.50/0

Scheme II



Scheme III



mucosal protectant would have good therapeutic potential, a program was initiated to identify a candidate in this series worthy of selection for development.

Chemistry

The thioamides of general structure **1** were prepared by one of three processes (Scheme II). Picoline can be reacted with some amines in the presence of sulfur at reflux to yield the thioamide directly.[9] This procedure was used for the synthesis of **4–6** (Table I). In most cases one obtains better results by heating picolinic acid with thionyl chloride to give acid chloride **7**,[10] which reacts cleanly with most amines to give the amide. Lawesson's reagent[11] was then used for thiation to the thioamide. The only drawback of this protocol is that the acid chloride **7** is fairly unstable. It was sometimes more convenient to form the amide by activating picolinic acid with 1,1′-carbonyldiimidazole to give imidazolide **8**, which was then treated shortly thereafter with the amine in the same pot. However, the imidazolide reacts very slowly with less nucleophilic amines.

The aniline required for the synthesis of **28** was prepared from the known compound **29**[12] (Scheme III) by reduction with stannous chloride dihydrate in refluxing ethyl acetate.[13] Introduction of an *N*-methyl group as in **30** and **31** was achieved by alkylation of the amide with sodium hydride and iodomethane in dimethylformamide (52% and 56% yield). Whereas, *N*-ethyl derivative **32** was made from substituted aniline **33**, which was available by reductive amination of acetaldehyde with 3,5-difluoroaniline and zinc-modified cyanoborohydride.[14] Nicotinic and isonicotinic analogues **35** and **36** were synthesized through the corresponding acid chlorides. Oxidation of sulfide **14** with *m*-chloroperbenzoic acid at 0 °C in chloroform yielded sulfoxide **43** in low yield (36%). The anilines required for the preparation of thioamides **44–46** were prepared by nitration[15] of the disubstituted aromatic compound followed by reduction as illustrated in the synthesis of **47**.

Acid **45** was obtainable (31% yield) from **44** by saponification with potassium carbonate in wet methanol and tetrahydrofuran. Introduction of the cyanoamidine functionality (**50**) was achieved (23% yield) by refluxing thioamide **5** with lead cyanamide in acetonitrile.[16]

Compounds **52–56**, which contain various basic heterocycles, were synthesized from substituted anilines such as **57**, which was prepared by the method shown in Scheme III. Reductive amination of 3-nitrobenzaldehyde using morpholine and zinc-modified cyanoborohydride, followed by reduction with hydrogen, afforded **57**. Imidazole-containing analogue **58** was prepared by catalytic reduction of known **59**[17] to yield **60**, which was converted to the amide in the usual manner. However thiation with Lawesson's reagent was not effective in giving **52–56** and **58**. Instead, phosphorus pentasulfide in refluxing pyridine was utilized successfully.

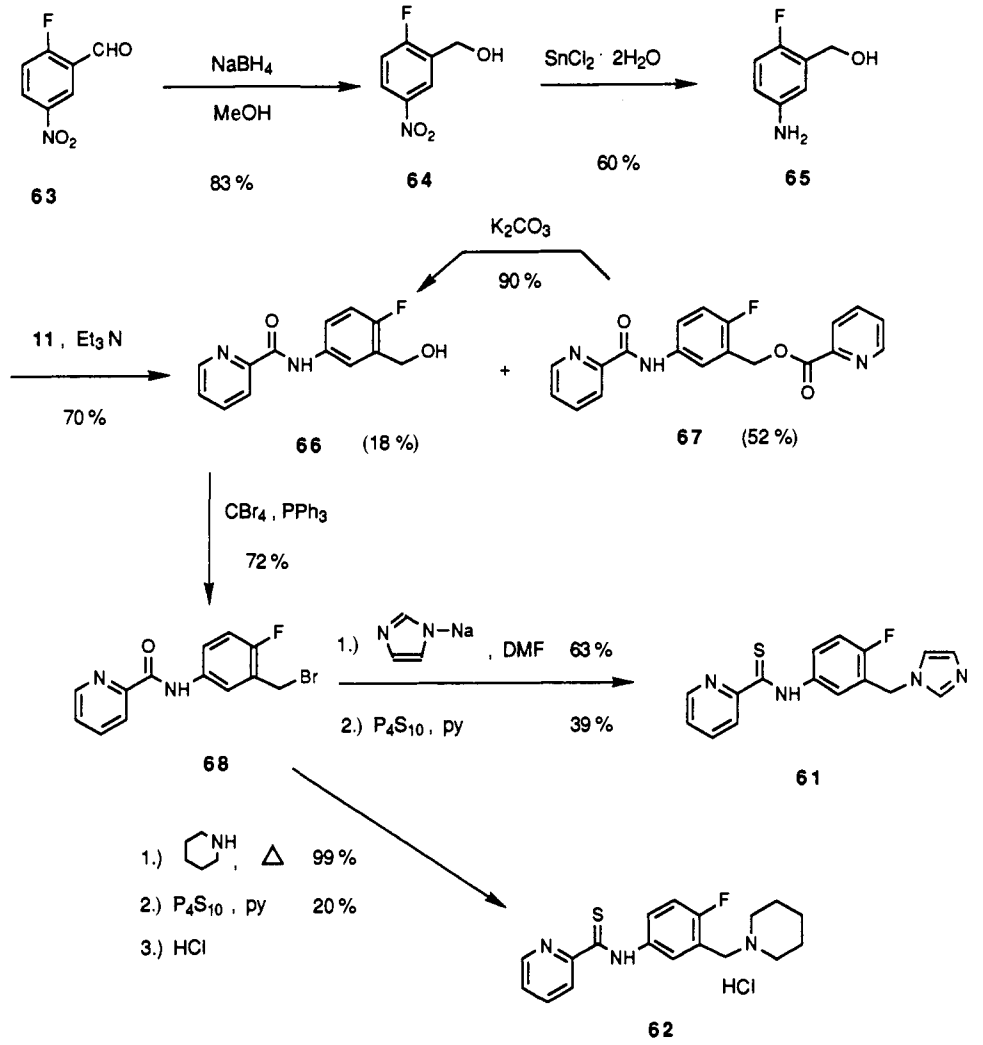
The total synthesis of the related fluoro compounds **61** and **62** is illustrated in Scheme IV. Reduction of nitrobenzaldehyde **63**[15] with sodium borohydride afforded nitro alcohol **64**, which could be further reduced with stannous chloride to amino alcohol **65**. Treatment of **65** with 2 equiv of acid chloride **7** gave a mixture of **66** and **67**; saponification of **67** affords additional **66**. Alcohol **66** was converted to bromide **68** with carbon tetrabromide and triphenylphosphine.[18] Reaction of **68** with the sodium salt of imidazole at room temperature in dimethylformamide, followed by phosphorus pentasulfide treatment in pyridine afforded imidazolyl thioamide **61**. Piperidinyl analogue **62** was prepared by refluxing piperidine with **68** in toluene, followed by thiation.

Biological Results and Discussion

Thioamides **4–6** (Table I), which contain a combination

(9) (a) Emmert, B. *Chem. Ber.* **1958**, *91*, 1388. (b) Hisano, T.; Ichikawa, M. *Chem. Pharm. Bull.* **1976**, *24*, 1451.
(10) Späth, E.; Spitzer, H. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1477.
(11) Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S. O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 223.
(12) Idoux, J. P.; Gupton, J. T.; McCurry, C. K.; Crews, A. D.; Jurss, C. D.; Colon, C.; Rampi, R. C. *J. Org. Chem.* **1983**, *48*, 3771.
(13) Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, 839.
(14) Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927.
(15) Gale, D. J.; Wilshire, J. F. K. *Aust. J. Chem.* **1970**, *23*, 1063.
(16) Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. *J. Med. Chem.* **1977**, *20*, 901.
(17) Baggaley, K. H.; Heald, M.; Hindley, R. M.; Morgan, B.; Tee, J. L.; Green, J. *J. Med. Chem.* **1975**, *18*, 833.
(18) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86.

Scheme IV



of pyridyl, phenyl, and pyrazinyl rings, were the first compounds found to be active against gastric lesions caused by ethanol. Unlike ranitidine, these compounds had little to no antisecretory effect in the pylorus-ligated rat model at a screening dose of 100 mg/kg (e.g. **5**, Table II). However, they were cytoprotective in the ethanol induced lesion model, with compound **5** possessing an ED_{50} of 1.1 mg/kg. Structures such as **9–11**, which do not have an aryl ring directly bonded to nitrogen, were found to be inactive. Thioamides **4–6** were evaluated for acute toxicity at 1000 mg/kg (po), and it was determined that **5** was superior in being nonlethal (Table II); therefore it was considered the lead structure.

A structure–activity relationship study was undertaken to identify the type of substituent in the aniline ring which would improve activity relative to **5**. Introduction of electron-donating lipophilic groups, such as benzyloxy, methoxy, or methylthio, which are contained in **12–14** at the 4-position on the aniline, decreased the cytoprotective effect. Encouraging results were seen when fluorines, which are slightly electron withdrawing and slightly lipophilic relative to hydrogen, were introduced (**15–17**). 3-Fluoro analogue **16** was equipotent to **5**, while the 2-fluoro substituent was not constructive. The difluoro derivatives (**18–23**) were of even greater interest in being equal or superior to **5** in this primary cytoprotective screen. In the stress induced ulcer model, which will be discussed later (Table II), a clear superiority of **23** was seen relative to **5**. 2,4,6-Trifluoro thioamide **24** was also prepared and it was found to be equipotent to **21**, which was the most potent of the difluoro analogues. 3,5-Dichloro derivative **25** was less effective than 3,5-difluoro analogue **23**. Like **25**, other congeners with strongly withdrawing lipophilic substituents, such as **26–28**, were found to be less potent than **23**.

Attention was then turned to other sections of the parent structure **5**. Incorporation of *N*-methyl or *N*-ethyl substituents on the otherwise potent thioamides **16** and **23** yielded compounds **30–32**, which had greatly diminished cytoprotective activity. A similar derivative, **34**, containing an *N*-propyl group tied back on the aniline ring, was also inactive. The 3-pyridyl (**35**) and 4-pyridyl (**36**) analogues of **23**, which were prepared to test the importance of the pyridine's orientation, were found to be less attractive pharmacologically.

Further investigation of aniline substitution was undertaken. Disubstituted analogues **37–40** containing at least one halogen were prepared, because of the previous success of incorporating halogens. Only the 3-fluoro-4-methyl compound (**39**) was considerably active. Substituents which are electron withdrawing and less lipophilic than fluorine were also studied. For instance, the 3-cyano (**41**) and 4-cyano (**42**) analogues were prepared and found to be equivalent to **23** in the primary screen. Methylsulfinyl thioamide **43** was less active. When a carboethoxy substituent, which is electron withdrawing like a cyano group but less hydrophilic, was combined with a fluorine in **44**, activity was lost; the corresponding acid (**45**) was also inactive. Compound **46**, which incorporates the advantageous fluoro and cyano substituents, was found to have insignificant activity. Therefore, it appears that the com-

Table I. Cytoprotection Screening Data for 2-Pyridinecarbothioamides

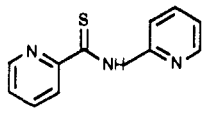
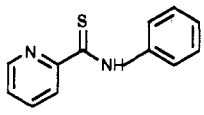
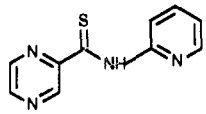
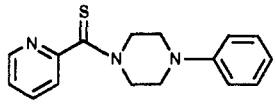
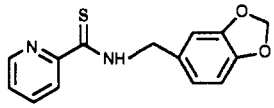
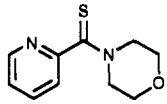
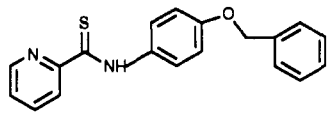
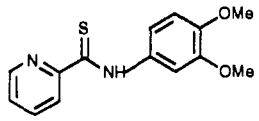
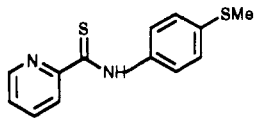
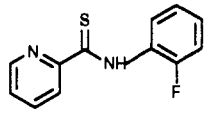
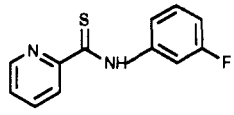
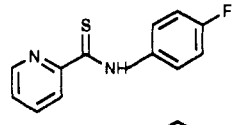
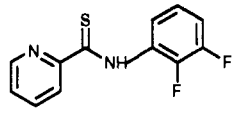
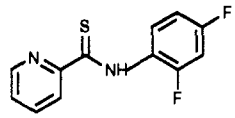
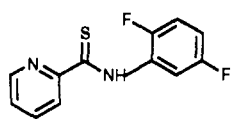
no.	structure	mp, °C	formula[a]	% yield[b]	purificn[c]	ethanol-induced lesions[d] dose, mg/kg po	% inhibn
4		81–83	$C_{11}H_9N_3S$	66	A, G	ED_{50} = 2.6 (1.2–5.9)	
5		51–53	$C_{12}H_{10}N_2S$	79	A	ED_{50} = 1.1 (0.2–4.7)	
6		136–138	$C_{10}H_8N_4S$	20	A, G	ED_{50} = 5.2 (2.4–11)	
9		99–101	$C_{16}H_{17}N_3S$	16	A, H	50	+6
10		75–77	$C_{14}H_{12}N_2O_2S$	17	A, H	50	11
11		105.5–107	$C_{10}H_{12}N_2OS$	22	A, G	50	44
12		90–91	$C_{19}H_{16}N_2OS$	23	A, I	5	47
13		100–101	$C_{14}H_{14}N_2O_2S$	42	A, J	5	0
14		81.5–92	$C_{13}H_{12}N_2S_2$	43	A	5	31
15		94–97	$C_{12}H_9FN_2S$	39	A	100	69[e]
16		73–76	$C_{12}H_9FN_2S$	36	A, G	ED_{50} = 1.0 (0.6–1.7)	
17		82–85	$C_{12}H_9FN_2S$	36	A	ED_{50} = 3.8 (2.3–6.1)	
18		89–90	$C_{12}H_8F_2N_2S$	29	A	ED_{50} = 1.0 (0.5–2.2)	
19		124–129	$C_{12}H_8F_2N_2S$	71	A	ED_{50} = 4.8 (3.0–7.6)	
20		141–143	$C_{12}H_8F_2N_2S$	27	A	ED_{50} = 6.2 (1.8–20)	

Table I (Continued)

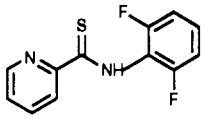
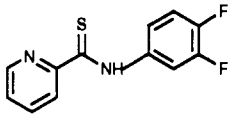
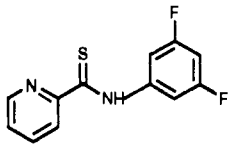
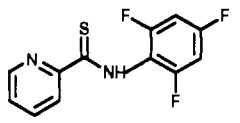
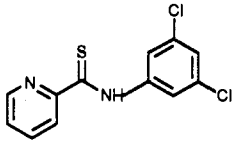
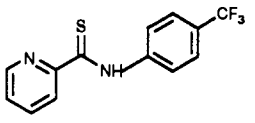
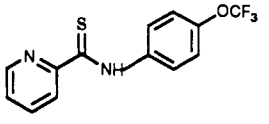
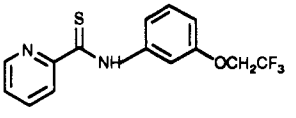
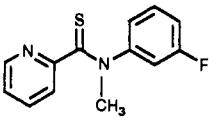
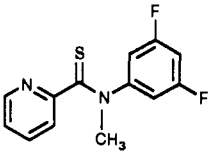
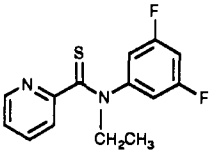
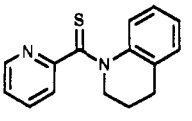
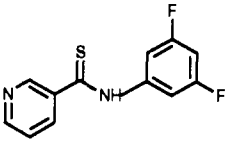
no.	structure	mp, °C	formula[a]	% yield[b]	purificn[c]	ethanol-induced lesions[d] dose, mg/kg po	% inhibn
21		138–140	$C_{12}H_8F_2N_2S$	37	A	ED_{50} = 0.38 (0.2–0.6)	
22		98–100	$C_{12}H_8F_2N_2S$	50	A, I	ED_{50} = 1.6 (1.0–2.4)	
23		140–142	$C_{12}H_8F_2N_2S$	39	A, K	ED_{50} = 2.9 (1.5–5.5)	
24		160–163	$C_{12}H_7F_3N_2S$	20	A, K	ED_{50} = 0.38 (0.2–0.6)	
25		144–145	$C_{12}H_8Cl_2N_2S$	15	A	25	89[e,f]
26		114.5–116.5	$C_{13}H_9F_3N_2S$	22	A, J	ED_{50} = 12 (7.9–19)	
27		63–64	$C_{13}H_9F_3N_2OS$	60	A, H	5	35
28		71–73	$C_{14}H_{11}F_3N_2OS$	48	A, I	10	7
30		100–103	$C_{13}H_{11}FN_2S$	17	A	10	31
31		131–134	$C_{13}H_{10}F_2N_2S$	16	A, I	25	40
32		111–113	$C_{14}H_{12}F_2N_2S$	18	A, I	10	13
34		92–94	$C_{15}H_{14}N_2S$	26	A, K	10	17
35		170–172	$C_{12}H_8F_2N_2S$	42	A, K	25	55

Table I (Continued)

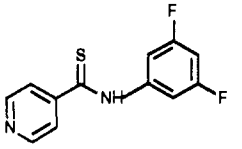
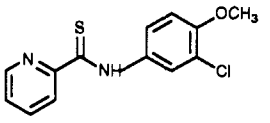
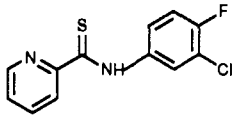
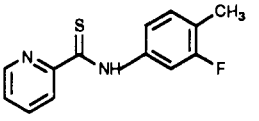
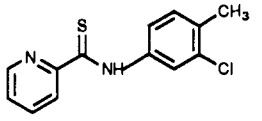
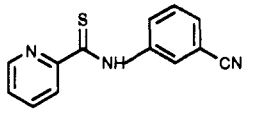
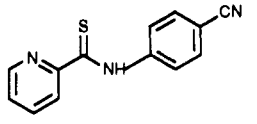
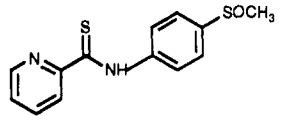
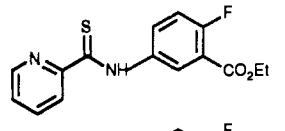
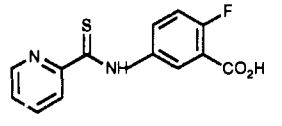
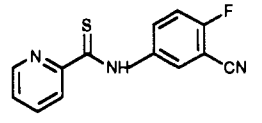
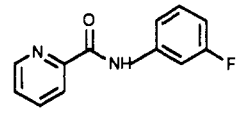
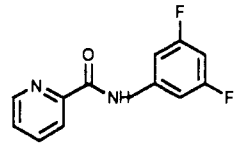
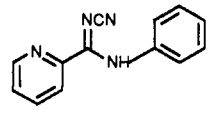
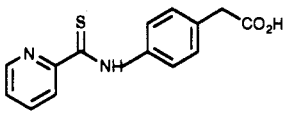
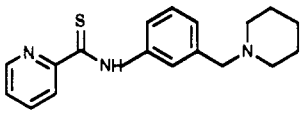
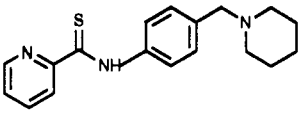
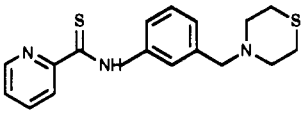
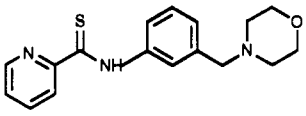
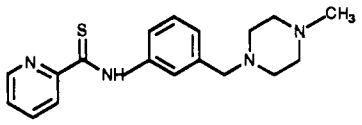
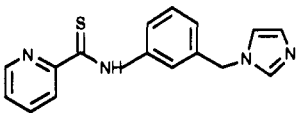
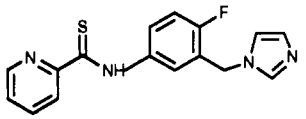
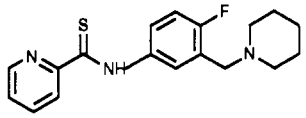
no.	structure	mp, °C	formula[a]	% yield[b]	purificn[c]	ethanol-induced lesions[d] dose, mg/kg po	% inhibn
36		194–196	$C_{12}H_8F_2N_2S$[g]	23	A, K	25	85[e,f]
37		150–152	$C_{13}H_{11}ClN_2OS$	8	A, J	25	44
38		140–142	$C_{12}H_8ClFN_2S$	28	A	25	56[e]
39		84–87	$C_{13}H_{11}FN_2S$	65	A	ED_{50} = 5.4 (3.0–10)	
40		132–134	$C_{13}H_{11}ClN_2S$	14	A	25	22
41		159–160	$C_{13}H_9N_3S$	39	A, K	ED_{50} = 1.6 (1.1–2.4)	
42		142–144	$C_{13}H_9N_3S$	22	A	ED_{50} = 1.1 (0.5–2.0)	
43		153–164	$C_{13}H_{12}N_2OS_2$	15	A, I	5	54
44		66–68	$C_{15}H_{13}FN_2O_2S$	24	A, K	10	10
45		231–236	$C_{13}H_9FN_2O_2S$	7	L	10	42
46		159–162	$C_{13}H_8FN_3S$	16	A, K	10	29
48		65–82	$C_{12}H_9FN_2O$	74	J	10	24
49		108–110	$C_{12}H_8F_2N_2O$	67	J	25	11
50		88–92	$C_{13}H_{10}N_4$	9	A, I	5	37

Table I (Continued)

no.	structure	mp, °C	formula[a]	% yield[b]	purific[n][c]	ethanol-induced lesions[d] dose, mg/kg po	% inhibn
51		133–136	$C_{14}H_{12}N_2O_2S$	27	K	25	21
52		225–230	$C_{18}H_{21}N_3S{\cdot}2HCl{\cdot}H_2O$	5	B, M	ED_{50} = 5.3 (3.8–7.4)	
53		123–125	$C_{18}H_{21}N_3S$	5	C	ED_{50} = 6.2 (4.8–8.1)	
54		130–132	$C_{17}H_{19}N_3S_2$	8	C	10	32
55		90–92	$C_{17}H_{19}N_3OS$	11	C	10	64[e]
56		250 dec	$C_{18}H_{22}N_4S{\cdot}2HCl{\cdot}^1/_2H_2O$	2	D, N	10	10
58		148–150	$C_{16}H_{14}N_4S{\cdot}2HCl{\cdot}H_2O$	6	E, O	ED_{50} = 2.2 (1.4–3.4)	
61		154–156	$C_{16}H_{13}FN_4S$	6	E	ED_{50} = 0.9 (0.5–1.5)	
62		254–256	$C_{18}H_{20}FN_3S{\cdot}HCl$	4	F, J	ED_{50} = 2.6 (1.5–4.6)	

[a] All compounds exhibited satisfactory (±0.4%) elemental analyses except where noted. [b] Unoptimized yields from commercially available starting materials. [c] Chromatography used (A) ethyl acetate/petroleum ether mixtures, (B) dried 97/2/1 CH_2Cl_2/MeOH/NH_4OH, (C) 2% MeOH/CH_2Cl_2, (D) dried 97/3/1.5 CH_2Cl_2/MeOH/NH_4OH, (E) dried 97.75/1.5/0.75 CH_2Cl_2/MeOH/NH_4OH, (F) 5% MeOH/CH_2Cl_2. Recrystallization used (G) CH_2Cl_2/hexane, (H) hexane, (I) EtOAc/petroleum ether, (J) ether/petroleum ether, (K) EtOAc/hexane, (L) acetone, (M) wet MeOH/acetone/EtOAc, (N) wet MeOH/acetone, (O) wet MeOH/EtOAc, (P) MeOH/acetone. [d] 95% confidence interval is in parentheses. [e] $p < 0.05$. [f] $ED_{50} > 10$ mg/kg. [g] H: calcd, 3.22; found, 2.80.

Table II. Further Evaluation of 5 and 23 Including Comparison to Sucralfate and Ranitidine

structure	ethanol-induced lesions: ED_{50},[a] mg/kg po	antisecretory (PLR): ED_{50},[a] mg/kg po	stress-induced ulcers: ED_{50},[a] mg/kg po	acute lethality: LD_{50}, mg/kg po
sucralfate	60 (36–98)	NS[b]	194 (75–501)	NL[c]
ranitidine	NS[d]	2.4 (2.1–2.7)	3.6 (2.2–5.9)	NL[e]
5	1.1 (0.3–4.7)	NS[b]	10 (7.1–15)	>1000
23	2.9 (1.5–5.5)	NS[f]	0.9 (0.1–5.5)	>1500

[a] 95% confidence interval is in parentheses. [b] No significant activity at 100 mg/kg. [c] Not lethal, see ref 6a. [d] No significant activity at 25 mg/kg. [e] Not lethal, see ref 3. [f] No significant activity at 200 mg/kg.

bination of a fluorine and an electron-withdrawing hydrophilic substituent causes inactivity.

A limited effort was made to determine the necessity of the carbothioamide subunit. Amides **48** and **49**, corresponding to the potent thioamides **16** and **23**, were found to be inactive. Cyanoamidine **50** exhibited insignificant cytoprotection.

Because of **23**'s lack of water solubility, it was of interest to test the antiulcer activity of derivatives containing acidic or basic functionality, which would form water-soluble salts. The acidic functionality incorporated into **51** was not a productive change as was the case with **45**. Since basic groups are present more often than not in antiulcer agents, it was of special interest to investigate the effect of such substituents. Some of the basic analogues (**52–56**) had good cytoprotective activity. The piperidinylmethyl substituent joined at the 3-position (**52**) or the 4-position (**53**) gave compounds of fairly good potency. Of the other three 3-substituted thioamides **54–56**, only the morpholinyl analogue (**55**) was of similar cytoprotective activity. Im-

idazole-containing compound **58** was as potent as **23**. Fluorine-containing derivatives **61** and **62** corresponding to **58** and **52** were slightly more potent against ethanol-induced lesions.

The thioamides which demonstrated potency (ED_{50} < 5 mg/kg) in the ethanol-necrosis model were then evaluated for their antisecretory action in the pylorus-ligated rat model. In addition, these compounds were differentiated by evaluation in one or more of the secondary ulcer models involving assault by stress, aspirin, indomethacin, or cysteamine. In Table II, the data for **5** and **23** are shown for three additional models, and two references are included for comparison. Compounds **5**, **23**, and sucralfate lacked activity in the antisecretory model, in contrast to the H_2-receptor antagonist ranitidine. The superiority of **23** over **5** is demonstrated in the stress-induced ulcer model, in which **5** is less potent. Ranitidine and sucralfate are also active in this model, although sucralfate is much less potent. In the ethanol induced lesion model ranitidine was ineffective and again sucralfate was weakly active. Like the lead structure of **5**, **23** was found to be nonlethal in the acute lethality model.

In conclusion, the 3,5-difluoro derivative **23** (AY-31,574) demonstrated a profile superior to the other analogues in having potent activity (1–10 mg/kg) in all the secondary ulcer models mentioned above. The protection provided by **23** against lesions caused by ethanol and stress is equal or superior to the therapeutic agents ranitidine and sucralfate. Compound **23** lacks antisecretory activity, making it a selective mucosal protectant. It is expected that such a potent cytoprotective agent would provide a novel clinical approach in treating peptic ulcer related diseases, including gastritis and esophagitis, and in preventing and counteracting damage to the gastrointestinal mucosa caused by frequent oral use of NSAID's and alcohol.

Experimental Section

Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. infrared spectra were recorded with a Perkin-Elmer 781 spectrophotometer. ^1H NMR spectra were obtained at either 200 or 400 MHz on a Varian XL-200 or Bruker AM-400 spectrometer, respectively. Mass spectra were measured on either a Finnigan 8230 or a Hewlett-Packard 5995A mass spectrometer. Elemental analyses were obtained on a Control Equipment 240-XA elemental analyzer. Flash chromatography refers to the technique described by Still.[19] The diameter of the column used is noted, but the height of the silica gel (400–230 mesh) was 6 inches in all cases. Magnesium sulfate was used as the drying agent, and all reactions requiring anhydrous conditions were performed under nitrogen.

***N*-(3,5-Difluorophenyl)-2-pyridinecarbothioamide (23).** Picolinic acid (38.3 g, 0.311 mol) and 1,1′-carbonyldiimidazole (50.3 g, 0.310 mol) were combined in dry dimethylformamide (300 mL) for 1 h, and then 3,5-difluoroaniline (40.0 g, 0.310 mmol) was introduced. After 3 days, the reaction mixture was poured into a 0.5 N HCl solution (1.5 L) to give a solid, which was filtered and washed with water. The solid was then suspended in a 0.5 N sodium hydroxide solution (1 L) and again filtered and washed with water. The vacuum-dried solid was recrystallized from ether in petroleum ether to give pure **49** (48.6 g, 67%, mp 111–113 °C). Some **49** (47.4 g, 0.203 mol) was treated with Lawesson's reagent[11] (64.6 g, 0.160 mol) in anhydrous toluene (450 mL) at reflux temperature for 4 h. The solvent was removed, and the residue was dissolved in dichloromethane and preadsorbed onto silica gel. Flash chromatography (6 in., elution with 5% ethyl acetate in petroleum ether) and recrystallization from ethyl acetate in hexane afforded a bright yellow solid, **23** (29.6 g, 58%, mp 140–142 °C): ^1H NMR δ ($CDCl_3$, 400 MHz) 10.86 (br s, NH), 8.75 (d, J = 8 Hz, 1 H), 8.55 (br d, J = 4.5 Hz, 1 H), 7.90 (td, J = 8 and 1.5 Hz, 1 H), 7.83 (dd, J = 8.5 and 2 Hz, 2 H), 7.52–7.49 (m, 1 H), 6.73 (tt, J = 8.5 and 2 Hz, 1 H); UV λ_{max} (CH_3OH) 338 mm (ϵ 9380), 284.5 (14 500), 235 (15 400); MS m/z 250 (M^+, 81), 249 (68), 217 (100), 79 (35), 78 (94). Anal. ($C_{12}H_8F_2N_2S$) C, H, N.

***N*-(3,5-Difluorophenyl)-*N*-methyl-2-pyridinecarbothioamide (31).** To a suspension of 80% sodium hydride (1.22 g, 41 mmol) in anhydrous dimethylformamide (90 mL) was added dropwise a solution of **49** (8.71 g, 37 mmol) in more dimethylformamide (30 mL). The reaction mixture was warmed to 65 °C for 1 h and then treated with iodomethane (2.60 mL, 42 mmol) at room temperature. After 2.5 h the reaction mixture was poured into water (500 mL) and extracted with ethyl acetate (2 × 300 mL). The combined organic layers were washed with brine, dried, preadsorbed onto silica gel, and purified by flash chromatography (300 g, elution with 40% ethyl acetate in petroleum ether) to yield pure *N*-methyl amide (5.16 g, 21 mmol, 56%, mp 102–104 °C), which was treated with Lawesson's reagent (4.36 g, 11 mmol) in anhydrous toluene (65 mL) at reflux temperature for 1.5 h. The toluene was evaporated and the residue was dissolved in dichloromethane, preadsorbed onto silica gel, and purified by flash chromatography (300 g, elution with 10% ethyl acetate in petroleum ether) and recrystallization (ethyl acetate in petroleum ether) to afford **31** as a yellow solid (2.36 g, 43%, mp 131–134 °C): ^1H NMR δ ($CDCl_3$, 400 MHz) 8.25 (br s, 1 H), 7.60 (br s, 2 H), 7.09 (br s, 1 H), 6.63 (br s, 3 H), 3.89 (br s, 3 H); IR (KBr, cm^{-1}) 1600, 1110; MS m/z 264 (M^+, 19), 123 (100), 119 (41), 78 (26). Anal. ($C_{13}H_{10}F_2N_2S$) C, H, N.

***N*-[4-(Methylsulfinyl)phenyl]-2-pyridinecarbothioamide (43).** A cold (5 °C) solution of 14 (1.45 g, 5.57 mmol) in chloroform (75 mL) was treated with 85% *m*-chloroperbenzoic acid (1.24 g, 6.11 mmol) over a 10-min period. After 3.5 h the reaction mixture was extracted with sodium bicarbonate solution and the organic layer was dried and concentrated to afford a brown oil, which was purified by flash chromatography (3 cm, elution with 90% ethyl acetate in petroleum ether) and recrystallization (ethyl acetate in petroleum ether) to give **43** as gold crystals (0.56 g, 36%, mp 153–164 °C): ^1H NMR δ ($CDCl_3$, 400 MHz) 10.78 (br s, NH), 8.78 (d, J = 8 Hz, 1 H), 8.57 (br d, J = 4 Hz, 1 H), 8.34 (d, J = 9 Hz, 2 H), 7.92 (td, J = 8 and 1.5 Hz, 1 H), 7.75 (d, J = 9 Hz, 2 H), 7.53–7.50 (m, 1 H), 2.77 (s, 3 H); IR (KBr, cm^{-1}) 3440, 1040. Anal. ($C_{13}H_{12}N_2OS_2$) C, H, N.

***N*-Fluoro-5-[(2-pyridinylthioxomethyl)amino]benzoic Acid (45).** Ester 44 (16.3 g, 56 mmol), potassium carbonate (19.6 g, 85.5 mmol), methanol (175 mL), water (175 mL), and tetrahydrofuran (350 mL) were stirred at ambient temperature under nitrogen for 48 h. The volatiles were removed in vacuo and the remaining liquid was diluted with additional water (1200 mL) and extracted with ethyl acetate (1400 mL). The aqueous layer was acidified with 1 N hydrochloric acid solution and extracted with ethyl acetate (1400 mL), which was dried and evaporated to give a solid. Recrystallization from acetone yielded **45** as a yellow solid (4.79 g, 31%, mp 231–236 °C): ^1H NMR δ (DMSO, 400 MHz) 12.4 (s, OH), 11.7 (s, NH), 8.68 (br d, J = 4 Hz, 1 H), 8.52 (d, J = 8 Hz, 1 H), 8.46 (dd, J = 6 and 3 Hz, 1 H), 8.09 (m, 1 H), 8.04 (td, J = 8 and 2 Hz, 1 H), 7.66 (m, 1 H), 7.40 (t, J = 9 Hz, 1 H); IR (KBr, cm^{-1}) 3250, 1710, 1500, 1300; MS m/z 276 (M^+, 68), 275 (100), 243 (93), 79 (37), 78 (87). Anal. ($C_{13}H_9FN_2O_2S$) C, H, N.

***N*′-Cyano-*N*-phenyl-2-pyridinemethanimidamide (50).** A refluxing solution of **5** (6.36 g, 30 mmol) in acetonitrile (130 mL) and dimethylformamide (12 mL) was treated with 3 equiv of lead cyanamide (3 × 7.34 g, 89.1 mmol) at 0 and 20 min and 3 days. After a total of 5 days the reaction mixture was cooled, filtered through Celite, and diluted with dichloromethane (250 mL). The organic layer was washed with brine (4 × 250 mL), adsorbed onto silica gel, and purified by flash chromatography (300 g, gradient elution with 30–40% ethyl acetate in petroleum ether). Recrystallization from ethyl acetate in petroleum ether yielded analytically pure **50** (1.53 g, 23%, mp 88–92 °C): ^1H NMR δ ($CDCl_3$, 400 MHz) 10.1 (br s, NH), 8.85 (br d, 1 H), 8.70 (br d, J = 5 Hz, 1 H), 7.98 (td, J = 8 and 2 Hz, 1 H), 7.67 (br d, J = 8 Hz, 2 H), 7.59–7.55 (m, 1 H), 7.44 (t, J = 8 Hz, 2 H), 7.29 (t, J = 8 Hz, 1 H); IR ($CHCl_3$, cm^{-1}) 2180; MS m/z 222 (M^+, 62), 221 (39), 181 (100), 105 (22). Anal. ($C_{13}H_{10}N_4$) C, H, N.

3-(4-Morpholinylmethyl)aniline (57). A 0.5 M solution of zinc-modified cyanoborohydride was prepared by dissolving zinc chloride (15.6 g, 0.114 mol), which had been flame dried under

(19) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

vacuum to a melt, in anhydrous methanol (250 mL). Sodium cyanoborohydride (14.4 g, 0.228 mol) was introduced and the solution was diluted to 456 mL. A reaction mixture containing 3-nitrobenzaldehyde (30 g, 200 mmol) and morpholine (34.9 mL, 400 mmol) in anhydrous methanol (150 mL) was treated at 0 °C with the 0.5 M stock solution (400 mL, 200 mmol). The ice bath was removed and the reaction was stirred overnight. A white precipitate was removed by filtration and the filtrate was concentrated to give a yellow residue, which was partitioned between methylene chloride (500 mL) and pH 7 buffer solution (500 mL). The organic layer was dried, and the volatiles were removed to give crude material, which was dissolved in ether and treated with ethereal hydrogen chloride to make the salt. The solid was collected, dissolved in water, treated with 1 N sodium hydroxide solution, extracted with ethyl acetate, and concentrated to give pure yellow crystals (25.16 g, 113 mmol, 55%, mp 45–48 °C). The nitro aromatic compound was reduced to the aniline by combining it with 10% palladium on carbon (0.7 g) in ethanol (300 mL) and treating it with 50 psi of hydrogen overnight. The reaction mixture was filtered through Celite and concentrated to give **57** as a yellow oil (21.8 g, 100%): ^{1}H NMR δ ($CDCl_3$, 400 MHz) 7.08 (t, J = 8 Hz, 1 H), 6.60 (m, 2 H), 6.57 (d, J = 8 Hz, 1 H), 3.70 (m, 6 H), 3.40 (s, 2 H), 2.43 (m, 4 H); MS m/z 192 (M^+, 16), 107 (100), 106 (60).

4-Fluoro-3-(hydroxymethyl)aniline (65). Sodium borohydride (15.0 g, 0.397 mol) was added portionwise to a 0 °C solution of 2-fluoro-5-nitrobenzaldehyde (**23**,[15] 82.4 g, 0.487 mol) in anhydrous methanol (1 L). The reaction mixture was stirred at 0 °C for 0.5 h and was quenched by adding 1 N hydrochloric acid solution (500 mL), which resulted in a pH range of between 4 and 5. The product, which precipitated out when methanol was removed, was collected by filtration. To obtain additional product, the filtrate was extracted with ether (4 × 500 mL). The ether layer was washed with brine, dried, and concentrated to give crude material, which was combined with the above solid and recrystallized from ethyl acetate in petroleum ether to give pure alcohol **64** (69.4 g, 0.406 mol, 83%, mp 50–53 °C). A solution of **64** in ethyl acetate (1 L) was treated with stannous chloride dihydrate (451 g, 1.99 mol) at reflux temperature for 24 h. The reaction mixture was poured into ice water (1.5 L), basified with 2.5 N sodium hydroxide solution to pH 9, and extracted with ethyl acetate (4 × 1 L), which was washed with brine and dried. Removal of ethyl acetate gave aniline **65** [34.2 g, 60%, mp 80–84 °C (lit.[20] mp 95–98 °C)], which was used for the next step without further purification: ^{1}H NMR δ (DMSO, 400 MHz) 6.78 (dd, J = 10 and 9 Hz, 1 H), 6.67 (dd, J = 6 and 3 Hz, 1 H), 6.41 (m, 1 H), 5.11 (t, J = 6 Hz, OH), 4.97 (br s, NH_2), 4.43 (d, J = 6 Hz, 2 H); IR (KBr, cm^{-1}) 3360, 1250, 1040; MS m/z 141 (M^+, 100), 124 (43), 120 (47), 112 (57), 92 (45), 83 (32), 65 (29).

***N*-[4-Fluoro-3-(hydroxymethyl)phenyl]-2-pyridinecarboxamide (66).** Aniline **65** (34.2 g, 0.243 mol) was dissolved in dichloromethane (600 mL) under anhydrous conditions and cooled to 0 °C. Triethylamine (120 mL, 0.861 mol) was added, followed by a solution of picolinic acid chloride hydrochloride[10] (86.0 g, 0.483 mol) in dichloromethane (300 mL). The reaction mixture was stirred at 0 °C for 0.5 h and at room temperature for 1 h, and poured into ice water (2 L). The resulting mixture was basified with 1 N sodium hydroxide solution, extracted with dichloromethane (3 × 800 mL), washed with brine, and dried to give a brown oil, which was purified by flash chromatography (6 in., elution with 25% ethyl acetate in petroleum ether) to give the title compound **66** (10.0 g, 18%, mp 99–102 °C) as the first compound off the column, followed by **67** (46.5 g, 52%, mp 116–118 °C). Additional **66** can be obtained by treating **67** (25.0 g, 71 mmol) with potassium carbonate (19.8 g, 143 mmol) in water (20 mL), methanol (200 mL), and tetrahydrofuran (200 mL). The mixture was left for 2 days and then concentrated in vacuo. The residue was partitioned between ethyl acetate (500 mL) and water (500 mL), and the aqueous layer was extracted again with ethyl acetate (2 × 200 mL). The organic layer was washed with 2.5 N sodium hydroxide solution (500 mL) and brine (500 mL), dried, and concentrated to afford **66** (15 g, 90%).

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66: ^{1}H NMR δ ($CDCl_3$, 400 MHz) 10.02 (br s, NH), 8.57 (d, J = 5 Hz, 1 H), 8.25 (d, J = 8 Hz, 1 H), 7.88 (td, J = 8 and 2 Hz, 1 H), 7.84–7.80 (m, 1 H), 7.75 (dd, J = 6 and 3 Hz, 1 H), 7.48–7.45 (m, 1 H), 7.08 (t, J = 9 Hz, 1 H), 5.11 (s, 2 H); IR (KBr, cm^{-1}) 3400, 1200, 1000; MS m/z 246 (M^+, 46), 106 (36), 79 (100), 78 (92), 69 (33).

67: ^{1}H NMR δ ($CDCl_3$, 400 MHz) 10.04 (br s, NH), 8.78 (d, J = 5 Hz, 1 H), 8.59 (d, J = 4 Hz, 1 H), 8.27 (d, J = 8 Hz, 1 H), 8.16 (d, J = 8 Hz, 1 H), 7.90–7.84 (m, 4 H), 7.48 (m, 2 H), 7.12 (t, J = 9 Hz, 1 H), 5.53 (s, 2 H); IR (KBr, cm^{-1}) 3280, 1730; MS m/z 351 (M^+, 5), 245 (63), 150 (44), 106 (66), 79 (100).

***N*-[3-(Bromomethyl)-4-fluorophenyl]-2-pyridinecarboxamide (68).** Carbon tetrabromide (39.4 g, 118 mmol) and triphenylphosphine (33.0 g, 118 mmol) were added to a 0 °C solution of alcohol **66** (22.9 g, 98 mmol) in anhydrous tetrahydrofuran (350 mL). The reaction mixture was stirred for 2 h, warmed to room temperature, and left overnight. The white precipitate was removed by filtration, and the filtrate was preadsorbed onto silica gel and submitted to flash chromatography (6 in., elution with 10% ethyl acetate in petroleum ether) to give the pure bromide **68** (20.6 g, 72%, mp 137–138 °C): ^{1}H NMR δ ($CDCl_3$, 400 MHz) 10.03 (br s, NH), 8.61 (d, J = 5 Hz, 1 H), 8.28 (d, J = 8 Hz, 1 H), 7.92 (m, 2 H), 7.68 (m, 1 H), 7.49 (m, 1 H), 7.08 (t, J = 9 Hz, 1 H), 4.53 (s, 2 H); IR (KBr, cm^{-1}) 3280, 1680, 1230; MS m/z 310 (M^+, 8), 308 (M^+, 8), 230 (29), 229 (100), 106 (39), 96 (26), 79 (78), 78 (99).

***N*-[4-Fluoro-3-(1*H*-imidazol-1-ylmethyl)phenyl]-2-pyridinecarbothioamide (61).** The sodium salt of imidazole (4.0 g, 44.8 mmol) was introduced to a solution of bromide **68** (10.11 g, 34.5 mmol) in anhydrous dimethylformamide (200 mL). The reaction mixture was stirred at room temperature for 2 h, poured into water (1.2 L), extracted with ethyl acetate (3 × 500 mL), washed with brine, dried over magnesium sulfate, and evaporated. The crude amide was recrystallized from methanol in ethyl acetate to give a pure, off-white solid (6.4 g, 22 mmol, 63%, mp 115–117 °C). The thioamide was prepared by treating the amide in anhydrous pyridine (200 mL) with phosphorus pentasulfide (4.8 g, 11 mmol) at reflux temperature for 4 h. The reaction mixture was poured into ice water (1 L), basified with 1 N sodium hydroxide solution, extracted with ethyl acetate (3 × 700 mL), washed with brine, and dried. The ethyl acetate layer was preadsorbed onto silica gel, and the residue was purified by flash chromatography [4 in., elution with 30% (90/5/2.5 dichloromethane/methanol/ammonium hydroxide) in dichloromethane] to yield **61** (2.7 g, 39%, mp 154–156 °C): ^{1}H NMR ($CDCl_3$, 400 MHz) 11.97 (br s, NH), 8.75 (d, J = 8 Hz, 1 H), 8.54 (d, J = 5 Hz, 1 H), 8.01 (m, 1 H), 7.89 (td, J = 8 and 2 Hz, 1 H), 7.81 (dd, J = 7 and 2 Hz, 1 H), 7.62 (s, 1 H), 7.48 (m, 1 H), 7.19 (t, J = 9 Hz, 1 H), 7.10 (s, 1 H), 7.00 (s, 1 H), 5.22 (s, 2 H); IR (KBr, cm^{-1}) 3400, 1500; MS m/z 312 (M^+, 8), 244 (60), 122 (36), 108 (46), 107 (39), 78 (79), 69 (100). Anal. ($C_{16}H_{13}FN_4S$) C, H, N.

***N*-[4-Fluoro-3-(piperidinylmethyl)phenyl]-2-pyridinecarbothioamide Hydrochloride (62).** To a solution of bromide **68** (4.9 g, 17 mmol) in anhydrous toluene (150 mL) was introduced piperidine (3.4 mL, 33 mmol). The reaction mixture was refluxed for 1 h, cooled, and diluted with ethyl acetate (400 mL). The organic layer was washed with 1 N sodium hydroxide solution (2 × 500 mL) and brine, dried, and concentrated to give a light brown oil (5.2 g, 17 mmol, 99%). The crude oil was dissolved in anhydrous pyridine (150 mL) and treated with phosphorus pentasulfide (3.7 g, 8.3 mmol) at reflux temperature for 3 h. The reaction mixture was poured into ice water (1 L), basified with 1 N sodium hydroxide solution, and extracted with ethyl acetate (3 × 700 mL). The organic layers were washed with brine, dried, and preadsorbed onto silica gel. Flash chromatography (4 in., elution with 5% methanol in dichloromethane) was performed on the crude residue to give the pure thioamide as an oil. The hydrochloride salt was prepared by dissolving the thioamide in ether and adding hydrogen chloride saturated ether. Salt **62** was collected and recrystallized from methanol in acetone to afford a yellow solid (1.23 g, 20%, mp 254–256 °C): ^{1}H NMR δ (DMSO, 400 MHz) 12.39 (br s, NH), 10.50 (br s, NH), 8.68 (d, J = 5 Hz, 1 H), 8.53 (d, J = 8 Hz, 1 H), 8.15 (dd, J = 7 and 2 Hz, 1 H), 8.05 (td, J = 8 and 2 Hz, 1 H), 8.01 (m, 1 H), 7.68 (m, 1 H), 7.42 (t, J = 9 Hz, 1 H), 4.32 (d, J = 4 Hz, 2 H), 3.40–3.33 (m, 2 H),

2.94-2.90 (m, 2 H), 1.77-1.68 (m, 5 H), 1.35 (m, 1 H); IR (KBr, cm^{-1}) 3450, 2500, 1500; MS m/z 329 (M^+, 39), 246 (16), 244 (15), 212 (61), 207 (100), 122 (22), 108 (34), 84 (38), 78 (20). Anal. ($C_{18}H_{21}ClFN_3S$) C, H, N.

Ethanol-Induced Gastric Lesions.[21] Charles River male albino rats (190-219 g) were deprived of food but not water for 18-24 h prior to use. Rats were dosed orally with drug or vehicle 1 h before ethanol administration (1 mL per rat po). One hour after ethanol administration, the rats were sacrificed by CO_2 asphyxiation. The stomachs were removed and kept moist with saline until the lesions were scored by an investigator unaware of the treatment groups (single blind). The grading of gastric ulcers took into account the size of the ulcers in mm^2 and the number of ulcers in each size category. The mean ulcer score for each treatment group was compared to the mean score of the vehicle-treated group, and the percent inhibition of ulcer formation was calculated.

Gastric Acid Secretion in the Pylorus-Ligated Rat.[22] One hour after oral administration of test compounds or vehicle, each rat was anesthetized with methohexital (30 mg/kg ip). A midline incision was made and the pylorus was ligated. The incision was closed with wound clips and the rat was allowed to recovery from anesthesia. Four hours after surgery, the rats were sacrificed by CO_2 asphyxiation. Gastric contents were collected, volumes were measured, and acid concentrations were determined by titration to pH 7.0 with 0.1 N NaOH with an automatic titrator. Total acid output (in microequivalents per 4 h) was determined by multiplying volume (in milliliters) by acid concentration (in microequivalents per milliliter).

Stress-Induced Gastric Ulcers.[23] Rats were immobilized in plastic restrainers and placed in a cold room at 4-5 °C for 3 h. Then, the rats were euthanized, and the gastric ulcers were graded as above. Test drugs or vehicle were administered po 30 min prior to immobilization.

Acute Lethality. The rats were orally dosed with drug and observed for lethality over a 7-day period.

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Statistics. The mean score of each treatment group (±SEM) was compared with that of the control group and expressed as a percentage of inhibition. Statistical significance was determined by Dunnett's multiple comparison technique. Values for ED_{50} with 95% confidence limits were calculated by standard regression analysis of the dose-response data.

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Growth Inhibition and Induction of Cellular Differentiation of Human Myeloid Leukemia Cells in Culture by Carbamoyl Congeners of Ribavirin[1]

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A series of 1,2,3-triazole (**2**), pyrazole (**3** and **5**), and pyrrole (**4**) ribonucleosides with two adjacent carbamoyl groups have been synthesized and evaluated for cell growth inhibition and induction of cellular differentiation of HL-60 cells in culture. Glycosylation of the TMS derivatives of dimethyl 1,2,3-triazole-4,5-dicarboxylate (**6**) and diethyl pyrazole-3,4-dicarboxylate (**7**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**8**) in the presence of TMS triflate gave predominantly the β-nucleosides **9** and **14**, respectively. Ammonolysis of **9** and **14** furnished 2-β-D-ribofuranosyl-1,2,3-triazole-4,5-dicarboxamide (**2**) and 1-β-D-ribofuranosylpyrazole-3,4-dicarboxamide (**3**), respectively. Stereoselective ring annulation of 1-deoxy-1-hydrazinyl-2,3-*O*-isopropylidene-D-ribose (**16**) with tetracyanoethylene (**15**) gave 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-3,4-dicarbonitrile (**17**). Deisopropylidenation of **17**, followed by oxidative hydrolysis of the reaction product (**18**), gave the 5-amino derivative of **3** (**5**). Stereospecific glycosylation of the sodium salt of preformed diethyl pyrrole-3,4-dicarboxylate (**22**) with 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose (**23**) was accomplished to furnish blocked nucleoside **24**, which on ammonolysis and deisopropylidenation gave 1-β-D-ribofuranosylpyrrole-3,4-dicarboxamide (**4**). The structures of **2** and **3** were assigned by single-crystal X-ray diffraction studies, which showed extensive inter- and intramolecular hydrogen bonding. Nucleosides **2**-**5** are devoid of significant cytotoxic properties against L1210 and WI-L2 leukemia cells in culture. However, these compounds were found to be inducers of cellular differentiation of HL-60 cells in the range of 30-60 μM and were comparable to ribavirin in this regard.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, **1**),[2] a synthetic azole nucleoside analogue of guanosine[3,4] synthesized and reported from our laboratory,[5] is singular in its broad-spectrum antiviral activity.[6,7]

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