and 100 mL of MeOH was shaken in an atmosphere of hydrogen at about 50 psi and at room temperature for 20 h. The catalyst was filtered and the filtrate concentrated to give 1.0 g (95%) of the title compound: NMR ($CDCl_3$) δ 3.0 (t, 2 H, CH_2NH_2), 2.5 (m, 7 H, pyrrol and amine protons), 1.5 (m, 2 H), 1.05 (s, 3 H, CH_3).

General Method B. Synthesis of 7-[3-(Aminomethyl)-3-phenyl-1-pyrrolidinyl]-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (19a). To 1.13 g (4.00 mmol) of 1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[11] in 40 mL of CH_3CN were added 0.88 g (5.00 mmol) of **15a** and 1.03 g (10.0 mmol) of triethylamine. The mixture was refluxed for 3 h and then stirred at room temperature for 18 h. The solids were filtered and washed with CH_3CN and ether to give 1.55 g (89%) of the title compound: mp 233-235 °C; IR (KBr) 1723, 1626 cm^{-1}; NMR (TFA) δ 9.3 (s, 1 H, C_2H), 8.1 (d, J = 13 Hz, 1 H, C_5H), 7.5 (m, 5 H, phenyl), 4.5 (m, 4 H, pyrrol), 4.1 (m, 1 H, cyclopr), 3.8 (s, 2 H, CH_2NH_2), 2.65 (m, 2 H, pyrrol), 1.5 (m, 4 H, cyclopr). Anal. Calcd for $C_{24}H_{23}F_2N_3O_3 \cdot 0.15H_2O$: C, 65.19; H, 5.31; N, 9.50. Found: C, 65.13; H, 5.19; N, 9.65.

Compound **20a** was prepared in a similar fashion, using **18a**,[13] the trifluoroquinolone, and triethylamine to give 7-[3-(acetylamino)-3-methyl-1-pyrrolidinyl]-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid. A suspension of 1.05 g (2.59 mmol) of this product in 35 mL of 6 N HCl and 20 mL of AcOH was refluxed for 18 h and then cooled and concentrated. The residue was triturated with *i*-PrOH, and the solids were filtered and recrystallized from *i*-PrOH to give 0.85 g (53% over two steps) of **20a**.

N-Phenyl-*N'*-pyridinylureas as Anticonvulsant Agents[1]

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A series of *N*-phenyl-*N'*-pyridinylureas was examined for anticonvulsant activity. Extensive structure/activity investigations revealed optimal activity in the *N*-(2,6-disubstituted-phenyl)-*N'*-(4-pyridinyl)urea series, with **37** exhibiting the best overall anticonvulsant profile. Compound **37** was effective against seizures induced by maximal electroshock but did not protect mice from clonic seizures produced by the convulsant pentylenetetrazol. The overall pharmacological profile suggests that **37** would be of therapeutic use in the treatment of generalized tonic-clonic and partial seizures. Compound **37** was selected for Phase 1 clinical trials.

The search for new anticonvulsant drugs remains an active area of investigation since available antiepileptic drugs are effective in only 60%-80% of patients.[2] While absence (petit mal) seizures are well treated in most instances, significant therapeutic improvement is still needed for the treatment of partial-complex (focal) and generalized tonic-clonic (grand mal) seizures.[3] In addition, most marketed anticonvulsants suffer from a broad range of undesirable side effects[4] such as sedation, teratogenicity, cognitive dulling, blood dyscrasia, and hepatotoxicity. Failure to achieve control of seizures is frequently due to use-limiting side effects seen with increasing doses of the drugs before a satisfactory therapeutic dose is reached.

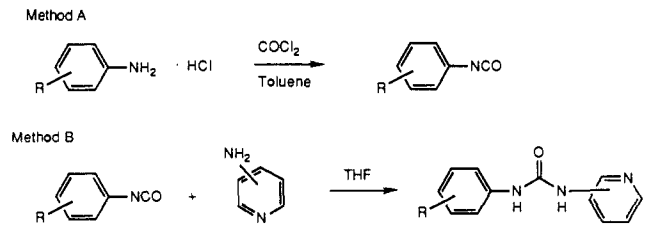
In collaboration with the NIH-NINCDS Antiepileptic Drug Discovery Program,[5] we discovered the potent anticonvulsant effects of a series of *N*-phenyl-*N'*-(4-pyridinyl)ureas.[6] While this class of compounds has been described to promote cell growth and differentiation in plants,[7-10] we are unaware of reports of these compounds displaying anticonvulsant activity.

Initially, anticonvulsant activity was observed with *N*-(2,6-dimethylphenyl)-*N'*-(4-pyridinyl)urea (**1**). While this compound was effective in blocking seizures in mice induced by maximal electroshock (an accepted model for generalized tonic-clonic seizures), we sought both an improvement in potency as well as greater separation between the doses affording protection from seizures and those exhibiting undesirable behavioral side effects. To achieve these goals, we systematically examined structural modifications using **1** as a starting point.

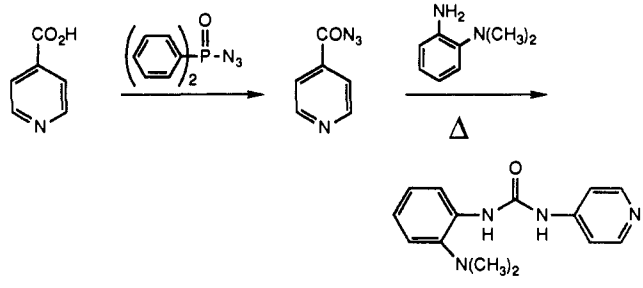
Chemistry

The ureas were prepared by reaction of an isocyanate with an amine (method B, Scheme I). In most cases the isocyanates were commercially available or were prepared by reaction of an aniline hydrochloride with excess phosgene[11] (method A, Scheme I). The two exceptions were compounds **17** and **44**. In the former case, attempts to

Scheme I

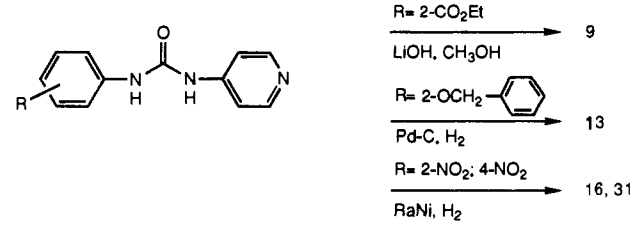


Scheme II



17

Scheme III



† Department of Chemistry.
‡ Department of Pharmacology.

(1) Presented in part at 194th American Chemical Society Meeting, New Orleans, LA, 1987.

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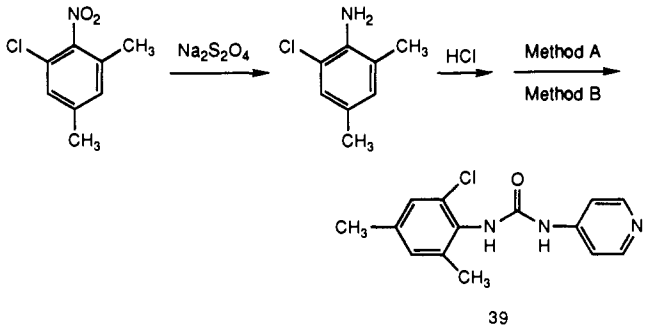
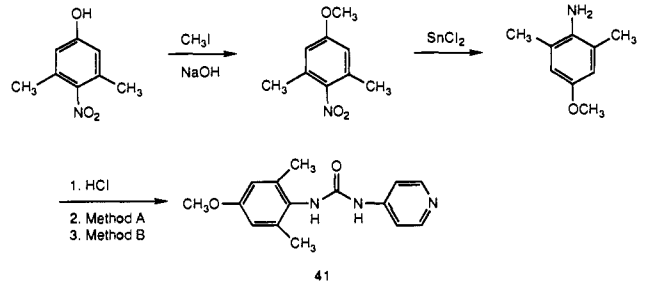
Scheme IV



Scheme V



convert *N,N*-dimethyl-1,2-benzenediamine to the corresponding isocyanate were unsuccessful under a variety of conditions. Treatment of isonicotinic acid with diphenyl phosphorazidate[12] afforded the corresponding acyl azide (Scheme II). Heating this azide in the presence of *N,N*-dimethyl-1,2-benzenediamine gave **17** through in situ generation of 4-pyridinyl isocyanate. In the case of **44**, the isocyanate was prepared by reaction of 2-chloro-6-(trifluoromethyl)benzeneamine with (trichloromethyl) chloroformate.[13]

Compounds **9**, **13**, **16**, and **31** were prepared as shown in Scheme III by standard methods.

The required aniline for the preparation of **39** was obtained by the sodium dithionite reduction of 1-chloro-3,5-dimethyl-2-nitrobenzene[14] as shown in Scheme IV. For compound **41** the aniline was prepared by methylation of 4-nitro-3,5-dimethylphenol[15] followed by reduction of the nitro group with stannous chloride (Scheme V).

Compound **43** was synthesized from 3-chloro-2-nitrobenzenemethanol (Scheme VI) prepared by the method of Ishizumi[16] from 3-chloro-2-nitrobenzoic acid. The alcohol was protected as the *tert*-butyldimethylsilyl ether before the aniline was generated by reduction of the nitro group with hydrogen in the presence of Raney Ni. The urea was obtained by application of methods A and B followed by removal of the silyl protecting group with tetrabutylammonium fluoride.

The synthesis of **44** (Scheme VII) required 2-chloro-6-(trifluoromethyl)benzenamine.[17] 2-Chloro-6-methylbenzenamine was reacted with phthalic acid to generate the corresponding phthalimide. Conversion of the methyl group to the trichloromethyl group was carried out by treating a melt of the phthalimide with chlorine gas. Subsequent reaction with anhydrous hydrogen fluoride gave the trifluoromethyl derivative, which was converted to the aniline by treatment with hydrazine.

Compound **51** was prepared as shown in Scheme VIII from 2-methyl-4-aminopyridine.[18]

Discussion

Testing in the NIH–NINCDS Antiepileptic Drug Discovery Program showed that **1** possessed anticonvulsant activity. Further examination by us confirmed this finding. Compound **1** is a potent anticonvulsant in mice with an ED_{50} (intraperitoneal administration) of 21 mg/kg with ataxia not seen until doses 7 times higher (TD_{50} = 138 mg/kg); the confidence limits of these values are shown in Table V, and the raw data and probit fit are shown in Figure 2. After single doses of 30 mg/kg **1**, the time of peak anticonvulsant effect was approximately 1.5 h postdosing with no activity seen after 4 h. Anticonvulsant effects were also observed in mice by the oral route of administration with an ED_{50} of 22 mg/kg. The overall activity profile suggested that structural modifications to **1** should be examined to increase the potency, duration of action, and separation between the doses exhibiting anticonvulsant and ataxic effects.

We began the investigation by varying the substituents on the phenyl ring. Table I lists 44 *N*-phenyl-*N'*-(4-pyridinyl)ureas prepared. The unsubstituted derivative, **2**, showed no activity at 30 mg/kg but a robust anticonvulsant effect at 100 mg/kg, indicating a less potent compound than **1**. Series of 2-, 3-, and 4-substituted phenyl analogues (**3–31**) were prepared with the substituents chosen to examine the effects of electron-donating and -withdrawing groups as well as changes in bulk and lipophilicity. Substitution in the 2-position of the phenyl ring with electron-donating groups is generally beneficial to activity although none is as potent as **1**. The exception appears to be those groups that are capable of hydrogen-bonding interactions such as the OH and NH_2 derivatives (**13** and **16**) where activity is decreased. Electron-withdrawing substituents in the 2-position show diminished anticonvulsant activity. Any substitution in the 3- or

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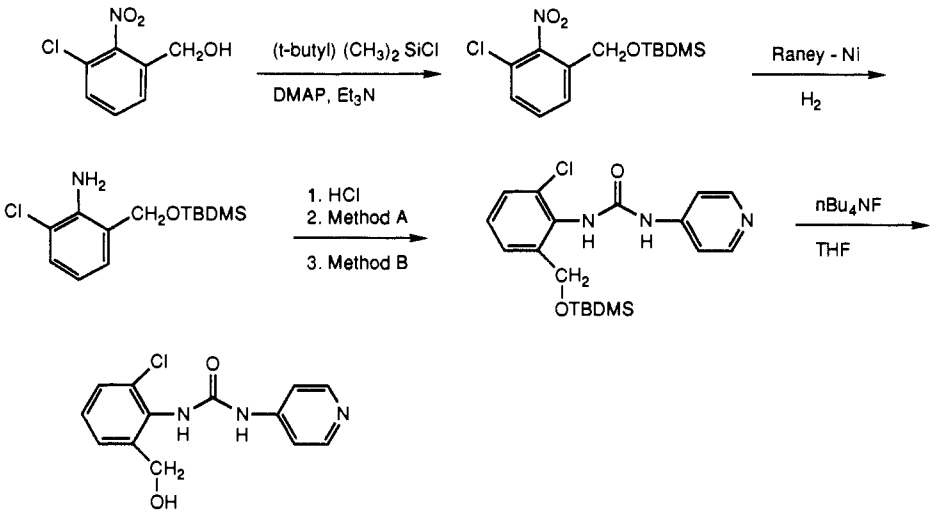
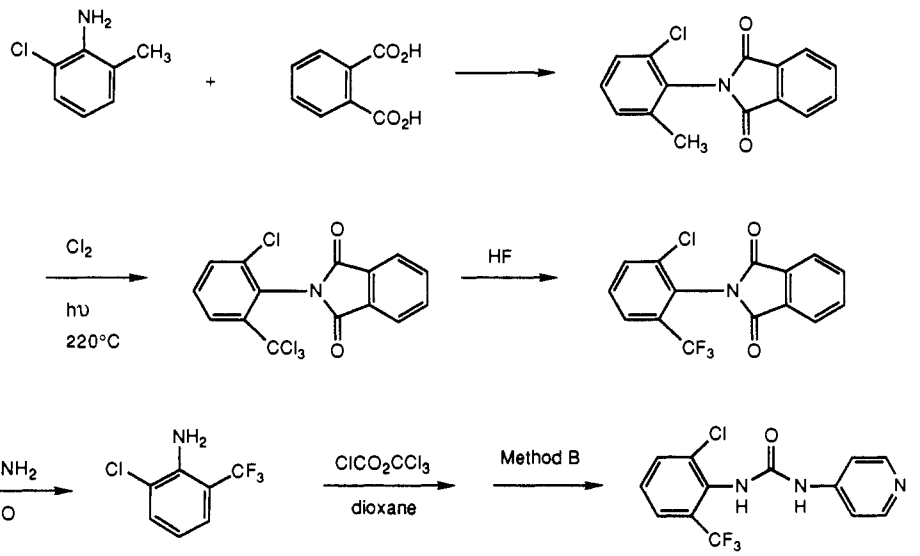
Scheme VI



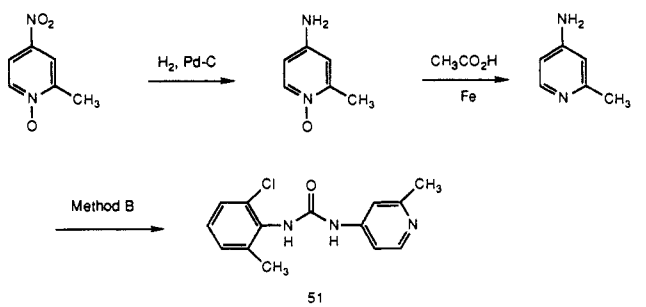
43

Scheme VII



44

Scheme VIII



51

4-position of the phenyl ring leads to a dramatic decrease in anticonvulsant activity.

Multiple substituents on the phenyl ring were examined next. A combination of groups at the 2- and 4-positions of the phenyl ring resulted in complete loss of anticonvulsant activity (**32** and **33**). When double substitution was made at positions 2 and 6, an increase in the desired activity was seen. The dichloro (**34**) and diethyl (**35**) analogues had profiles similar to that seen with the chemical lead, **1**. Further increases in the bulk and lipophilicity of these groups as in the diisopropyl compound (**36**) resulted in a loss of anticonvulsant activity. This result may be due to the isopropyl groups distorting the conformation of the two aromatic rings.

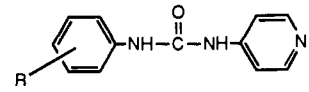
The 2-chloro-6-methyl derivative **37** showed anticonvulsant activity at 30 mg/kg in the initial tests with no signs of ataxia until 300 mg/kg. This was the best overall profile yet observed. In addition, the activity seen with 30 mg/kg **37** was still present 4 h postdose, suggesting a longer duration of action than for **1**.

Additional derivatives were prepared with substituents in the 2-, 4-, and 6-positions of the phenyl ring. Good activity was seen with the 2,4,6-trimethyl analogue **38**, but it was slightly less potent than **37** and also appeared to have a shorter duration of anticonvulsant activity. Interestingly, we expected the 2-chloro-4,6-dimethyl analogue **39** to be a potent anticonvulsant compound on the basis of the results with **37** and **38**, but it was found to be considerably less potent.

Overall, it appeared that small, lipophilic, non-hydrogen-bonding groups at the 2- and 6-positions of the phenyl ring represented the optimal substitution pattern for this series of 4-pyridinylureas.

Considering the duration of action for the most potent compounds, **1** and **37**, it is possible that metabolic hy-

Table I. Structure/Anticonvulsant Activity of *N*-Phenyl-*N'*-(4-pyridinyl)ureas



compd no.	R	mp, °C	ref and expl method[a]	yield, %[b]	molecular formula[c]	minimum effective dose (MED), mg/kg[d]	minimum ataxic dose (MAD), mg/kg[e]
1	2,6-$(CH_3)_2$	168–170	B	75	$C_{14}H_{15}N_3O{\cdot}0.33H_2O$	30	100
2	H	166–167	B	72	$C_{12}H_{11}N_3O$	100	300
3	2-Cl	174–176	B	57	$C_{12}H_{10}ClN_3O{\cdot}0.5H_2O$	100	>300
4	2-Br	177–178	B	77	$C_{12}H_{10}BrN_3O{\cdot}0.5H_2O$	100	>300
5	2-F	161–162	B	82	$C_{12}H_{10}FN_3O$	100	100
6	2-CF_3	194–196	B	70	$C_{13}H_{10}F_3N_3O$	100	300
7	2-CN	190–191	A, B	20	$C_{13}H_{10}N_4O$	>300	100
8	2-NO_2	211–212	B	88	$C_{12}H_{10}N_4O_3$	300	>300
9	2-CO_2H	>300		48	$C_{13}H_{11}N_3O_3{\cdot}HCl{\cdot}0.5H_2O$	>300	>300
10	2-$CO_2C_2H_5$	>280	B	85	$C_{15}H_{15}N_3O_3$	300	>300
11	2-CH_3	189–190	B	78	$C_{13}H_{13}N_3O$	100	>300
12	2-$CH(Ch_3)_2$	112–114	B	56	$C_{15}H_{17}N_3O{\cdot}0.3H_2O$	100	300
13	2-OH	155		76	$C_{12}H_{11}N_3O_2$	>300	>300
14	2-OCH_3	196–197	B	55	$C_{13}H_{13}N_3O_2$	100	>300
15	2-$OCH_2C_6H_5$	151	A, B	43	$C_{19}H_{17}N_3O_2$	>300	30
16	2-NH_2	188–189		72	$C_{12}H_{12}N_4O$	>300	>300
17	2-$N(CH_3)_2$	136–140		39	$C_{14}H_{16}N_4O{\cdot}0.25H_2O$	100	100
18	3-CF_3	238–239	B	63	$C_{13}H_{10}F_3N_3O$	>300	>300
19	3-NO_2	267–268	B	79	$C_{12}H_{10}N_4O_3$	>300	>300
20	3-CH_3	203–204	B	80	$C_{13}H_{13}N_3O$	>300	>300
21	4-Cl	242–243	B	65	$C_{12}H_{10}ClN_3O$	>300	>300
22	4-F	188–190	B	90	$C_{12}H_{10}FN_3O$	300	300
23	4-I	271–272	B	78	$C_{12}H_{10}IN_3O$	>o00	>300
24	4-NO_2	284–285	B	91	$C_{12}H_{10}N_4O_3$	>300	>300
25	4-$COCH_3$	210–211	B	82	$C_{14}H_{13}N_3O_2$	>300	>300
26	4-$CO_2C_2H_5$	184–185	B	58	$C_{15}H_{15}N_3O_3$	>300	>300
27	4-CH_3	188–189	B	82	$C_{13}H_{13}N_3O$	>300	100
28	4-$CH(CH_3)_2$	151–152	B	62	$C_{15}H_{17}N_3O$	>300	>300
29	4-OCH_3	175–176	B	73	$C_{13}H_{13}N_3O_2$	100	300
30	4-OC_6H_5	164–166	B	66	$C_{18}H_{15}N_3O_2$	>300	>300
31	4-NH_2	>270		72	$C_{12}H_{12}N_4O{\cdot}2HCl{\cdot}H_2O$	>300	300
32	2,4-$(CH_3)_2$	191–192	B	24	$C_{14}H_{15}N_3O$	>300	>300
33	2,4-Cl_2	242–243	B	79	$C_{12}H_9Cl_2N_3O$	>300	>300
34	2,6-Cl_2	217–219	B	70	$C_{12}H_9Cl_2N_3O$	100	>300
35	2,6-$(C_2H_5)_2$	173–175	B	65	$C_{16}H_{19}N_3O$	30	100
36	2,6-$[CH(CH_3)_2]_2$	174–176	A, B	19	$C_{18}H_{23}N_3O{\cdot}0.3H_2O$	>300	>300
37	2-Cl,6-CH_3	210–212	B	84	$C_{13}H_{12}ClN_3O{\cdot}H_2O$	30	300
38	2,4,6-$(CH_3)_3$	181–182	B	71	$C_{15}H_{17}N_3O$	30	300
39	2-Cl,4,6-$(CH_3)_2$	>190		30	$C_{14}H_{14}ClN_3O$	100	>300
40	2,4,6-Cl_3	231–232	B	64	$C_{12}H_8Cl_3N_3O$	300	300
41	2,6-$(CH_3)_2$,4-OCH_3	196–197		62	$C_{15}H_{17}N_3O_2$	>300	>300
42	2-CH_3,3-Cl	159–161	B	14	$C_{13}H_{12}ClN_3O$	>300	>300
43	2-CH_2OH,6-Cl	165–166		52	$C_{13}H_{12}ClN_3O_2$	>300	300
44	2-CF_3,6-Cl	140–142		28	$C_{13}H_9ClF_3N_3O$	100	300

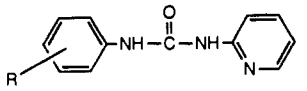
[a] The original reference is footnoted under Experimental Section. The general methods are those described under Experimental Section and are designated by letters. [b] Isolated yield. [c] Satisfactory analytical data ± 0.4% were obtained for all compounds. [d] Dose level (10, 30, 100, 300, or >300 mg/kg) at which two or more mice (N = 5) were protected against tonic extension produced by maximal electroshock (MES) at 30 min postadministration. [e] Lowest dose (10, 30, 100, 300, or >300 mg/kg) at which two or more mice (N = 5) fell off inverted screen at 30 min postadministration.

droxylation of the methyl group on the aromatic ring could occur. The hydroxymethyl analogue **43** was synthesized and found to have no activity in accordance with the above hypothesis. The trifluoromethyl derivative **44** was designed to inhibit the possible metabolism of the methyl group. This compound was slightly less active, and the anticonvulsant effect appeared to be approximately equal in duration to that seen with **37**. This result suggests that the relatively short durations, of **1** and **37** are not due to metabolic hydroxylation of the 6-methyl group.

With an extensive study of the phenyl substitution pattern of the *N*-phenyl-*N'*-(4-pyridinyl)ureas complete, an examination of the pyridinyl group was undertaken. Series of 2- and 3-pyridinyl derivatives were prepared with the optimized phenyl substitution patterns. In the 2-pyridinyl series (**45–47**; see Table II) the compounds were much less active than the corresponding 4-pyridinyl derivatives. The 3-pyridinylureas (**48–50**; see Table III) showed intermediate potency between the 2- and 4-pyridinyl analogues.

Further studies were carried out to examine the differences among the 2-, 3-, and 4-pyridinylureas. An examination of the pK_a' (50% aqueous methanol) and log P values for the 2,6-dichloro and 2-chloro-6-methyl analogues (**46**, **47**, **49**, and **50**) revealed pK_a' values increasing from approximately 3 for the 2-pyridinyl compounds to 4 for the 3-pyridinyl series and further to 5.5–6.0 for the 4-pyridinyl series. No obvious trends were detected in the log P values to explain the changes in activities observed. The 2-methyl-4-pyridinyl analogue **51** (see Table IV) was prepared with the expectation that the methyl group would cause an increase in the pK_a' value due to the inductive effect. The measured pK_a' value was slightly higher than that of **37** at 6.2; however, while potent anticonvulsant

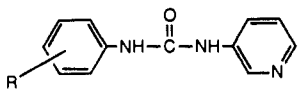
Table II. Structure/Anticonvulsant Activity of *N*-Phenyl-*N'*-(2-pyridinyl)ureas[a]



compd no.	R	mp, °C	ref and expl method	yield, %	molecular formula	minimum effective dose (MED), mg/kg	minimum ataxic dose (MAD), mg/kg
45	2,6-$(CH_3)_2$	197–198	B	60	$C_{14}H_{15}N_3O$	300	>300
46	2,6-Cl_2	199–200	B	86	$C_{12}H_9Cl_2N_3O$	>300	100
47	2-Cl,6-CH_3	210–212	B	71	$C_{13}H_{12}ClN_3O \cdot HCl$	100	>300

[a] For explanations of the column headings, see footnotes to Table I.

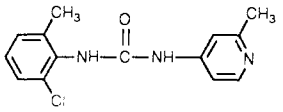
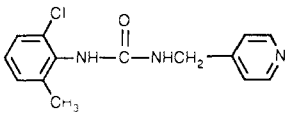
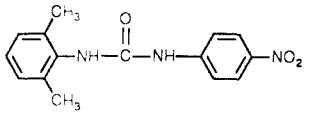
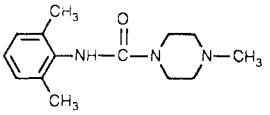
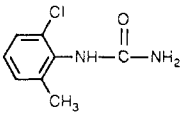
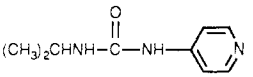
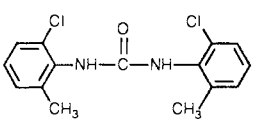
Table III. Structure/Anticonvulsant Activity of *N*-Phenyl-*N'*-(3-pyridinyl)ureas[a]



compd no.	R	mp, °C	ref and expl method	yield, %	molecular formula	minimum effective dose (MED), mg/kg	minimum ataxic dose (MAD), mg/kg
48	2,6-$(CH_3)_2$	190–192	B	79	$C_{14}H_{15}N_3O$	100	300
49	2,6-Cl_2	225–227	B	72	$C_{12}H_9Cl_2N_3O$	>300	300
50	2-Cl,6-CH_3	246–247	B	72	$C_{13}H_{12}ClN_3O$	100	300

[a] For explanations of the column headings, see footnotes to Table I.

Table IV. Structure/Anticonvulsant Activity of *N*-Phenyl-*N'*-(4-pyridinyl)ureas[a]

compd no.	compd	mp, °C	ref and expl method	yield, %	molecular formula	minimum effective dose (MED), mg/kg	minimum ataxic dose (MAD), mg/kg
51		237–240	B	27	$C_{14}H_{14}ClN_3O$	30	100
52		209–210	B	51	$C_{14}H_{14}ClN_3O$	>300	>300
53		237–238	B	68	$C_{15}H_{15}N_3O_3$	>300	>300
54		197–198	B	81	$C_{14}H_{21}N_3O$	100	300
55		234–235	B	85	$C_8H_9ClN_2O$	100	300
56		110–111	B	57	$C_9H_{13}N_3O$	300	300
57		>260	B	72	$C_{15}H_{14}Cl_2N_2O$	>300	>300

[a] For explanations of the column headings, see footnotes to Table I.

effects were seen at 30 mg/kg, the effect was gone by 2 h postdosing. In addition, severe ataxia was seen at 100 mg/kg in contrast with the near absence of side effects with **37** at the same dose. The overall profile of **51** was less desirable than that seen with **37**.

Several additional changes were made in the pyridinyl region of the molecule (**52–55**). The pyridinyl moiety was separated from the urea by addition of a methylene group, which resulted in total loss of activity. Next, the pyridinyl group was replaced by an isosteric 4-nitrophenyl group (**53**). The total lack of activity suggests that the basic nitrogen is important for activity. This is further supported by the activity of **54**, which contains a basic center. Interestingly, the unsubstituted urea **55** was active at 100

Table V. ED_{50} Values (mg/kg) for Protection from Maximal Electroshock Seizures (MES) in Mice and for Ataxia (ATAX) by the Inverted Screen Test after Intraperitoneal Administration

compd	time of MES test (max effect), min	MES ED_{50}	time of ATAX test (max effect), min	ATAX ED_{50}
1	90	21 (18–24)[a]	120	138 (119–160)
34	60	30 (25–36)	120	293 (182–472)
37	60	46 (35–60)	120	251 (197–320)

[a] Numbers in parentheses are 95% confidence intervals.

Table VI. ED_{50} Values (mg/kg) for Compound 37·HCl and Phenytoin in Mice and Rats after Oral Administration

compd	species	time of MES test, min	MES ED_{50}	time of ATAX test, min	ATAX ED_{50}
37·HCl	mouse	30	14 (12–16)[a]	60	210 (170–260)
37·HCl	rat	60	9.2 (4.4–19)	240	408 (310–530)
phenytoin	mouse	120	8.3 (6.9–10)	120	78 (68–90)
phenytoin	rat	30	30 (22–39)	240	>300

[a] Numbers in parentheses are 95% confidence intervals.

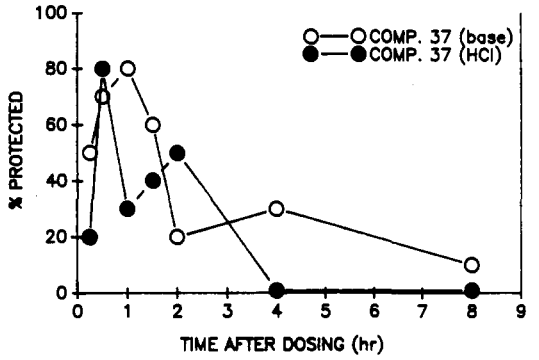


Figure 1. Anticonvulsant activity of compound **37** as the free base and hydrochloride salt at various times after oral administration to mice. A 30 mg/kg concentration of the free base (open circles) caused the same maximum effect as a 15 mg/kg concentration of the HCl salt. Note that the maximum effect occurred with a shorter latency from administration with the HCl salt than with the base. Percent protected refers to the percent of 10 mice that failed to have tonic hind-limb extensor seizures following maximal electroshock.

mg/kg for at least 2 h with no signs of ataxia at that dose. Finally, replacement of the phenyl ring with an alkyl group (**56**) or replacement of the pyridinyl group with the 2-chloro-6-methylphenyl group (**57**) resulted in a total loss of activity.

These studies identified **1**, **34**, and **37** as having the most desirable in vivo profiles for a potential anticonvulsant drug. Results with these three compounds are summarized in Table V. On the basis of duration of anticonvulsant action and results in an animal model for partial seizures (which will be reported elsewhere), compound **37** was selected for additional study.

Compound **37** had a favorable ratio of anticonvulsant dose (ED_{50} = 46 mg/kg ip) to ataxic dose (ED_{50} = 251 mg/kg ip). Preparation of the hydrochloride salt of **37** increased aqueous solubility and allowed more rapid drug absorption, as evidenced by earlier time of peak anticonvulsant effect with oral dosing in mice (Figure 1). The dose–response relationship of **37** (hydrochloride) for protection from maximal electroshock seizures in mice is shown in Figure 2. Ataxia using the inverted screen

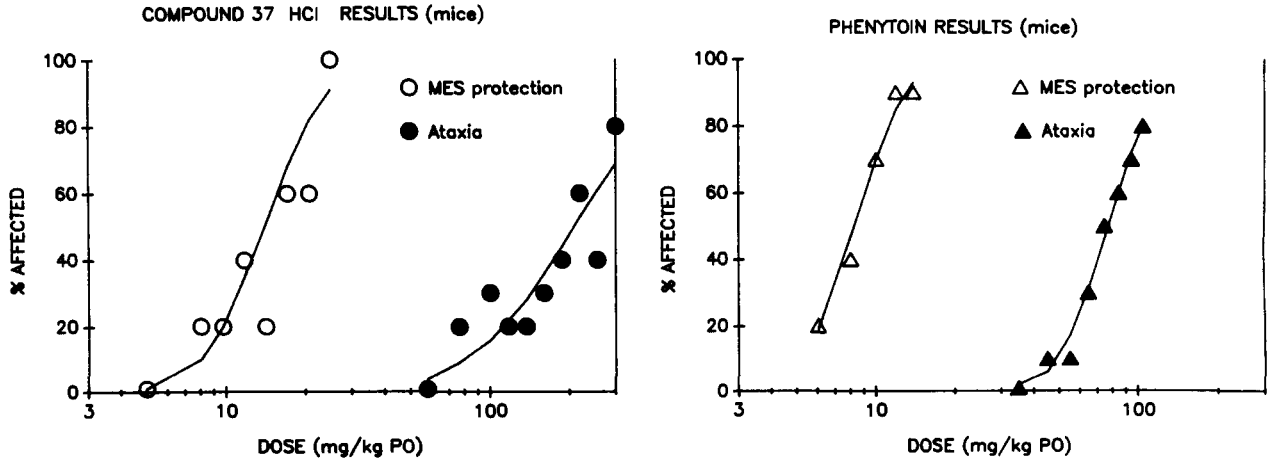


Figure 2. Anticonvulsant activity and ataxia produced by oral administration of compound **37**·HCl and phenytoin in mice. The left panel shows the percent of 10 mice that were protected from tonic extensor seizures from maximal electroshock as a function of compound **37**·HCl dose. Observations were made 0.5 h after dosing (time of maximum effect). Similarly, ataxia denotes percent of mice falling from an inverted square of wire mesh at the time of peak ataxic effect (1 h after dosing). The right panel shows similar results with phenytoin (all data taken 2 h after oral dosing).

procedure was seen only at significantly higher doses. Dose–response relationships are compared with those for phenytoin (5,5-diphenyl-2,4-imidazolidinedione) in Figure 2 and Table VI. Similarly, **37** (hydrochloride) prevented maximal electroshock seizures in rats at doses that did not cause ataxia (Table VI).

These results are similar to those observed with phenytoin but are unlike those with anticonvulsant benzodiazepines, ethosuximide (3-ethyl-3-methyl-2,5-pyrrolidinedione), or valproic acid (2-propylpentanoic acid)[19] since compound **37** failed to prevent clonic seizures from threshold doses of pentylenetetrazole in mice.

Other pharmacological effects of **37** after ip administration to mice included decreased spontaneous locomotor activity (ED_{50} = 36 mg/kg) measured by an automated device (see Pharmacological Methods). After 100 mg/kg ip, decreased activity, hypothermia, mydriasis, and Straub tail were seen in all mice, with loss of righting reflex in a fraction of mice. These symptoms are similar to those seen with other anticonvulsants at high doses.

The pharmacological similarities between phenytoin and **37** in these animal models suggest that **37** will be useful in the treatment of generalized tonic–clonic and partial seizures but not absence seizures. Compound **37** was selected for Phase 1 clinical trials and designated CI-953.

Experimental Section

All melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Varian EM-390 NMR spectrometer using Me_4Si as the internal reference standard and deuteriochloroform or Me_2SO-d_6 as solvent. Purity was determined by microanalysis and by TLC with 0.25-mm-thick plates coated with silica gel G as the stationary phase. IR spectra were recorded with a Nicolet XS-20 FT-IR spectrometer using KBr pellets. All compounds possessed microanalytical and spectral data consistent with the proposed structures.

Method A. General Procedure for Synthesis of Aryl Isocyanates. The method used was that of Hardy.[11] A refluxing solution of the substituted aniline (0.1 mol) in toluene (100 mL; 1.0 M) was saturated with HCl(g). Phosgene was then passed through the solution until a clear solution was obtained. Careful removal of the toluene by distillation resulted in isolation of the desired isocyanate.

Method B. General Procedure for Synthesis of Ureas. These compounds were prepared by treating a solution (0.25 M) of the aminopyridine (or amine, Table IV) in anhydrous THF with an equivalent amount of the appropriate isocyanate. The resultant mixture was stirred for 0.5–24 h at room temperature to reflux temperature under nitrogen. Upon cooling, the product was filtered and dried to give analytical material, or if it did not precipitate upon cooling, the reaction was concentrated in vacuo, and the residue was recrystallized from aqueous ethanol to afford analytically pure material.

***N*-(2-Chloro-6-methylphenyl)-*N'*-(4-pyridinyl)urea Monohydrate (37).** A solution of commercially available 2-chloro-6-methylphenyl isocyanate (8.4 g, 0.05 mol) in anhydrous THF (50 mL) was treated with a solution of commercially available 4-aminopyridine (4.7 g, 0.05 mol) in anhydrous THF (150 mL). The mixture was heated and stirred under a nitrogen atmosphere at reflux temperature for 4 h, cooled, filtered, and dried in vacuo at 45 °C for 24 h. The material was recrystallized from aqueous ethanol to afford (11.2 g, 80%) analytically pure product as the monohydrate, mp 210–212 °C.

NMR (Me_2SO-d_6) δ 9.30 (1 H, s, NH), 8.3–8.4 (2 H, m, Py), 8.2 (1 H, s, NH), 7.15–7.5 (5 H, m, Py, phenyl), 3.3 (2 H, s, H_2O), 2.2 (3 H, s, CH_3).

2-[[(4-Pyridinylamino)carbonyl]amino]benzoic Acid (9). A methanol solution (6 mL) of **10** (1.5 g, 0.0053 mol) was treated with 1 N LiOH (4 mL), and the mixture was stirred for 48 h, concentrated in vacuo, and acidified with 1 N HCl. The cooled mixture was filtered, and the product was dried to afford the product (0.75 g, 55%) as the hydrochloride hemihydrate, >300 °C.

***N*-(2-Hydroxyphenyl)-*N'*-(4-pyridinyl)urea (13).** A solution of **15** (6.4 g, 0.02 mol) in THF (50 mL) was diluted to a volume of 120 mL with methanol. To this was added 5% Pd/C (0.6 g) followed by evacuation of oxygen. It was treated with excess hydrogen and stirred for 24 h under an atmosphere of hydrogen. The hydrogen was evacuated, and the mixture was warmed on a steam bath for 20 min, filtered, and concentrated to a volume where product began to crystallize. It was allowed to cool, and the crystals were filtered and recrystallized from methanol to afford the product (3.5 g, 76%), mp 155 °C.

***N*-(2-Aminophenyl)-*N'*-(4-pyridinyl)urea (16).** A solution of **8** (5 g, 0.019 mol) in methyl-Cellosolve (50 mL) was hydrogenated at 50 psi over Raney Nickel at room temperature until the required pressure change was recorded. Two equivalents of dry hydrogen chloride (1.4 g, 0.038 mol) dissolved in 2-propanol were placed in the receiving filter flask, and the reaction mixture was filtered through Supercel. The filtrate was diluted with ethyl ether to turbidity and allowed to stand at room temperature for 2 h. The solid was filtered, dried in vacuo at 35 °C for 4 h, dissolved in water (50 mL), and made basic with 1 N NaOH. The solid was filtered, washed with cold water (3 × 25 mL), and dried in vacuo at 80 °C to give 3.6 g (84%) of product, mp 188–189 °C.

***N*-[2-(Dimethylamino)phenyl]-*N'*-(4-pyridinyl)urea (17).** An *N,N*-dimethylformamide solution (10 mL) of commercially available isonicotinic acid (3 g, 0.024 mol) was treated with diphenyl phosphorazidate[12] (10 g, 0.36 mol). The mixture was stirred at room temperature for 30 min, poured into 50 mL of water, and extracted into (3 × 25 mL) ethyl ether. The extracts were dried over $MgSO_4$ and concentrated in vacuo to give 2.72 g of the azide. A toluene solution (50 mL) of the azide was treated with *N,N*-dimethyl-1,2-benzenediamine (2.5 g, 0.018 mol), and the mixture was stirred at reflux temperature for 30 min and then concentrated in vacuo to a solid. A solution of the solid in 10% HCl (250 mL) was washed with ethyl ether (2 × 100 mL) and neutralized with $NaHCO_3$. The solid was filtered, washed with water (3 × 50 mL), dried, dissolved in hot 2-propanol (10 mL), and treated with hydrogen chloride dissolved in 2-propanol. The solution was diluted to turbidity with ethyl ether, cooled, filtered, washed with ethyl ether (50 mL), and dried in vacuo to give the product as the partial hydrate, mp 136–140 °C.

***N*-(4-Aminophenyl)-*N'*-(4-pyridinyl)urea (31)** was prepared from **24** by using the method described for **16**.

***N*-(2-Chloro-4,6-dimethylphenyl)-*N'*-(4-pyridinyl)urea (39).** A solution of 1-chloro-3,5-dimethyl-2-nitrobenzene[14] (0.5 g, 2.7 mmol) in methanol (25 mL) containing water (25 mL) was treated with sodium dithionite (3.1 g, 17.8 mmol), and the mixture was stirred for 24 h at 60 °C. The reaction mixture was cooled to 25 °C and concentrated to half volume in vacuo, and the residue was diluted with water (25 mL). The product was extracted into ethyl ether (2 × 25 mL), and the combined extracts were dried over $MgSO_4$ and concentrated in vacuo to give 0.4 g (96%) of the desired aniline. The hydrochloride was prepared by dissolving the aniline in 2-propanol (1 mL) and treating with one equivalent of hydrogen chloride dissolved in 2-propanol followed by trituration with ethyl ether to turbidity. The crystals were filtered, washed with ethyl ether (2 × 5 mL), and dried in vacuo at 35 °C. This material was reacted by using procedure A, followed by procedure B to give the desired product.

***N*-(2,6-Dimethyl-4-methoxyphenyl)-*N'*-(4-pyridinyl)urea (41).** 3,5-Dimethyl-4-nitrophenol[15] was alkylated with methyl iodide. An ethyl acetate solution (50 mL) of 5-methoxy-1,3-dimethyl-2-nitrobenzene (5 g, 0.027 mol) was treated with stannous chloride dihydrate (31 g, 0.137 mol), and the mixture was stirred at reflux temperature for 1.5 h. An additional amount of stannous chloride dihydrate (20 g, 0.089 mol) was added, and the mixture was stirred for 30 h and then poured onto ice (300 g) and neutralized with $NaHCO_3$. The product was extracted into ethyl acetate (3 × 75 mL), and the combined extracts were dried over $MgSO_4$ and concentrated in vacuo to give an oil. A solution of the oil in 2-propanol was treated with an equivalent of dry hydrogen chloride dissolved in 2-propanol to give the hydrochloride salt, 1 g (20%). This was reacted according to procedure A,

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followed by procedure B to give 0.9 g of product, mp 196–197°C.

N-[Chloro-6-(hydroxymethyl)phenyl]-N'-(4-pyridinyl)-urea (43). 3-Chloro-2-nitrobenzenemethanol was prepared from 3-chloro-2-nitrobenzoic acid in 74% yield.[16] This was silylated with *tert*-butyldimethylsilyl chloride in 67% yield. Subsequent hydrogenation over Raney nickel in THF gave the desired aniline, which was converted to the hydrochloride and isocyanate according to procedure A, followed by Procedure B to give 1 g of the silyl derivative. This was converted to the product in 92% yield by using 1.25 equiv of tetra-*n*-butylammonium fluoride in THF (0.2 M) at room temperature for 5 h.

N-[2-Chloro-6-(trifluoromethyl)phenyl]-N'-(4-pyridinyl)urea (44). A neat mixture of 2-chloro-6-methylbenzenamine (10 g, 0.07 mol) and phthalic acid (11.7 g, 0.07 mol) was heated at 190–220 °C, and the water was removed by distillation as it was formed. When the requisite amount of water (2.6 mL) was collected, the reaction was cooled and the residue was recrystallized from aqueous ethanol to give 12.3 g of the phthalimide in 64% yield.

The phthalimide was warmed to 220 °C to give a melt, which was treated with excess chlorine delivered via a fritted glass rod while irradiating with visible light. The reaction was monitored by GC and was complete after 10 h. The reaction was cooled, dissolved in CH_2Cl_2 (100 mL), and concentrated to dryness in vacuo. The residue was pumped to constant weight to afford 15 g (88%) of the 2-(trichloromethyl)-6-chlorophenyl phthalimide. This material (0.04 mol) was added to anhydrous HF (11 g, 0.55 mol) at 0 °C in a stainless steel vessel and then warmed to 150 °C and followed by GC. The reaction was cooled and vented through aqueous KOH, and the vessel was washed with CH_2Cl_2 (3 × 100 mL). The combined extracts were poured into water (1 L), washed with water (5 × 200 mL), dried over $CaCl_2$, and concentrated in vacuo to give a beige solid which gave a microanalysis consistent with the desired product.

The 2-chloro-6-(trifluoromethyl)benzenamine was prepared from this phthalimide according to the Groves procedure[17] by treating a suspension of the phthalimide (7.7 g, 23.6 mmol) in water (40 mL) with hydrazine (2 g, 62.5 mmol) and heating at reflux temperature for 1 h. An additional amount of hydrazine (1 g, 31 mmol) was added dropwise, and heating was continued for 1 h. The product was steam distilled, extracted into ethyl ether (4 × 50 mL), dried, and concentrated in vacuo to 3.5 g of a low-boiling oil (76%), 100% by GC and analytically pure.

The aniline (1 g, 5.1 mmol) was dissolved in dioxane (20 mL), warmed to 60 °C, and treated with trichloromethyl chloroformate[13] (1.0 g, 5.1 mmol). The reaction was stirred at 60 °C for 5 h, treated with 4-aminopyridine (0.75 g, 8 mmol), and stirred for 20 h. The mixture was concentrated to dryness in vacuo and purified by using flash chromatography (silica, 10% methanol in chloroform) to give the desired product in 71% yield, mp 140–142 °C.

Pharmacological Methods. The test compounds were dissolved in water or suspended in 0.2% (hydroxymethyl)cellulose and evaluated for their ability to prevent the tonic extensor component of maximal seizures induced in male Swiss-Webster mice by electroshock (MES test). The mice ranged in weight from 25 to 32 g and were allowed food and water prior to testing. Doses of the drugs were calculated as the free base.

Drugs were administered intraperitoneally (ip). Five mice were tested at each of three doses (30, 100, and 300 mg/kg) and three times (0.5, 2, and 4 h). The mice were subjected to electrical current delivered through ear clips for 0.2 s (90 mA, 1-ms monophasic pulses at 100 Hz). This current strength was approximately 4 times that required to produce seizures in 99% of mice and reliably produced seizures in 100% of control mice. Prevention of tonic hind limb extension was taken as an anticonvulsant effect.

Behavioral side effects were measured in mice by inversion of a square of wire mesh to which untreated mice easily clung but from which impaired mice fell.[20] In addition, spontaneous locomotor activity of mice was measured by an automated procedure.[21] Rats were tested against maximal electroshock (methods of ref 19) and in a subjective assessment of behavioral impairment. Median effective doses were determined by a probit analysis.[22]

Acknowledgment. We thank Dr. F. A. MacKellar and his associates for IR and NMR spectra as well as for the elemental analyses, log *P*, and pK_a' values. We acknowledge M. Vartanian, P. Mickevicius, and B. Stieber (Warner-Lambert) for pharmacological test results. We gratefully acknowledge the help of the Anticonvulsant Drug Discovery Program, Epilepsy Branch, NINCDS (H. J. Kupferberg and G. Gladding), in the pharmacological evaluation of several of these compounds.

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Glycolipids as Host Resistance Stimulators

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6-(5-Cholesten-3β-yloxy)hexyl 1-thio-β-D-mannopyranoside (L-644,257) enhances natural host resistance in cyclophosphamide-treated mice against *Pseudomonas aeruginosa* in a dose-dependent manner. It is active sc, im, and ip but not orally. L-644,257 is substantially more protective against *P. aeruginosa* than its α anomer. The β-L-fucose glycolipid is more effective when given im and ip than sc. The lactose and β-D-glucose glycolipids were only marginally effective to nonprotective. The 17β-steroidal side chain of L-644,257 can be modified without substantial loss of protective activity.

Opportunistic infections arising from trauma and stress in immunocompromised patients such as surgery patients, severe burn victims, and cancer patients receiving chemotherapy are the leading cause of their morbidity and mortality.[1-3] The opportunistic pathogens may include bacteria, viruses, fungi, protozoa, and mycoplasma and are thus difficult to treat with antibiotic therapy or conventional active or passive immunization. A more desirable method of treatment is to use an agent that acts prophylactically and/or therapeutically to stimulate nonspecific host resistance in immunocompromised patients. A num-

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