N-aminosuccinimide hydrochloride (200 mg, 1.33 mmol). The mixture was stirred at 100 °C for 18 h, and the solvent was removed in vacuo. The residue was treated with saturated sodium bicarbonate solution and chloroform and filtered, and the filtrate was extracted with chloroform (three times). The combined organic phases were washed with brine, dried, and concentrated to afford an oil that was purified on a Sephadex column (LH-20, MeOH) to give **11** (49 mg, 24.5%), **2** (38 mg, 19%), and **6** (24 mg, 12%). Data for **11**: R_f 0.23 (CMA, 18:2:0.1); ^{1}H NMR ($CDCl_3$) δ 0.10 (2 H, m), 0.51 (2 H, m), 0.82 (1 H, m), 1.70–2.80 (complex pattern), 2.32 (3 H, s), 2.90–3.30 (complex pattern), 5.42 (2 H, s), 5.42 (2 H, s), 6.20–6.80 (complex pattern); FABMS, m/z 606.0 ($M^+ - 1$). Data for **11**·2HCl: R_f 0.40 (BAW, 2:1:1). Anal. ($C_{37}H_{38}O_6N_3$·2HCl·3.4H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-pyrrolomorphinan (12). To a stirred solution of naltrexone hydrochloride (**13**·HCl) (150 mg, 0.40 mmol) in benzene (10 mL) and DMF (10 mL) were added aminoacetaldehyde diethyl acetal (0.1 mL, 0.95 mmol) and methanesulfonic acid (0.05 mL, 0.5 mmol). The resulting mixture was stirred under reflux with Dean–Stark trap for 14 h. Ethyl acetate and saturated sodium bicarbonate solution were added to the mixture. The resulting mixture was filtered, and the filtrate was extracted with $CHCl_3$ (three times). The combined organic phases were washed with brine, dried, and concentrated to give a crude product that was purified by preparative TLC (silica gel, 10% MeOH–saturated NH_4OH–$CHCl_3$) to afford pure **12** (72.3 mg, 50%): R_f 0.06 (CMA, 19:1:0.1); IR (KBr, cm^{-1}) 3373, 2932, 1644, 1616; ^{1}H NMR (DMSO-d_6) δ 0.08 (2 H, m), 0.50 (2 H, m), 0.85 (1 H, m), 1.50 (1 H, m), 3.05 (1 H, d, J = 18.5 Hz), 3.18 (1 H, d, J = 5.3 Hz), 5.15 (1 H, s), 5.70 (1 H, t, J = 2.3 Hz), 6.45 (1 H, d, J = 8.1 Hz), 6.52 (1 H, d, J = 8.1 Hz), 6.68 (1 H, t, J = 2.6 Hz); CIMS, m/z 365 ($M^+ + 1$). Data for **12**·HCl: R_f 0.66 (BAW, 2:1:1); mp >330 °C dec. Anal. ($C_{22}H_{24}O_3N_2$·HCl·1.5H_2O) C, H, N, Cl.

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3-Phenoxypyridine 1-Oxides as Anticonvulsant Agents

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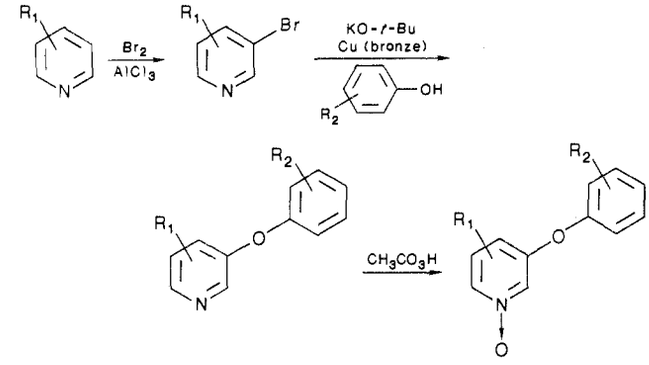
The anticonvulsant activity of a series of 3-phenoxypyridine 1-oxides is described. An investigation carried out to optimize the activity/side effect ratio provided 4-methyl-3-phenoxypyridine 1-oxide, **3**, as the derivative of choice. Overall, **3** has a pharmacological profile that is very similar to phenytoin. It exhibited significant anticonvulsant activity at doses that did not produce ataxia or sedation but caused increased spontaneous behavioral activity not seen with most anticonvulsants. The short duration of pharmacological effect of **3** was attributed to metabolic hydroxylation at the C-4 pyridine methyl group; however, structural modifications designed to inhibit this metabolic pathway were unsuccessful.

The search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry. Since currently available antiepileptic drugs are effective in only 60–80% of patients, there is a real need for improved agents.[1] Absence (petit mal) seizures are well treated in most instances, but significant therapeutic improvement is required for the treatment of partial-complex (focal) and generalized tonic–clonic (grand mal) seizures.[2] In addition, most marketed anticonvulsants suffer from a broad range of undesirable side effects[3] 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 these drugs before a satisfactory therapeutic dose is reached.

In collaboration with the NIH–NINCDS Antiepileptic Drug Development Program,[4] we have recently discovered the potent anticonvulsant effects of a series of substituted 3-phenoxypyridine 1-oxides.[5,6] While phenoxypyridine derivatives have been reported to possess a diverse range of biological properties,[7-13] the 3-phenoxypyridine 1-oxides described here have not previously been reported to possess anticonvulsant activity.

Initially, three compounds (**1**–**3**, Table I) were identified to possess anticonvulsant properties. While each of these compounds was effective in blocking seizures induced by maximal electroshock (an accepted model for generalized tonic–clonic seizures), **3** displayed the best overall profile. With 4-methyl-3-phenoxypyridine 1-oxide as a starting point, we systematically examined structural modifications

Scheme I



with the goal of increasing the potency, protective index, and duration of action of **3**.

† Department of Chemistry.
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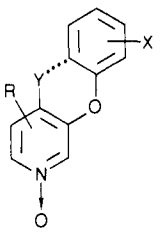
Table I. Structure/Anticonvulsant Activity of 3-Phenoxypyridine 1-Oxides



compd	R	X	Y	ref and exptl method[a]	overall yield, %	bp (Torr) or mp, °C	formula[b]	minimum effective dose[c] (MED)	minimum ataxic dose[d] (MAD)
1[e]	H	H		1, A	65	107–109	$C_{11}H_9NO \cdot H_2SO_4$	100	300
2	H	H		3, B	62	80–82	$C_{11}H_9NO_2$	100	300
3	4-CH_3	H		C, A, B	48	141–143	$C_{12}H_{11}NO_2 \cdot HCl$	30	100
4	6-CH_3[f]	H		5, B	20	125–127 (0.15)	$C_{12}H_{11}NO_2 \cdot 0.25H_2O$	100	300
5	5-CH_3	H		C, A, B	80	100–102	$C_{12}H_{11}NO_2$	100	300
6	2-CH_3	H		5, B	13	59–62	$C_{12}H_{11}NO_2$	300	300
7	4-Cl	H		B	25	129–130	$C_{11}H_8ClNO_2 \cdot HCl$	>300	300
8	4-CN	H		B	43	91–92	$C_{12}H_8N_2O_2$	300	300
9	4-CH_3	4-Cl		C, A, B	54	155–157 (0.2)	$C_{12}H_{10}ClNO_2$	100	100
10	4-CH_3	4-OCH_3		C, A, B	47	123–125	$C_{13}H_{13}NO_3 \cdot HCl$	300	300
11	4-CH_3	4-CH_3		C, A, B	41	102–104	$C_{13}H_{13}NO_2 \cdot HCl$	300	300
12[e]	4-CH_2OH	H			51	174–176	$C_{12}H_{11}NO_2 \cdot HCl$	300	300
13	4-CH_2OH	H		B	33	128–129	$C_{12}H_{11}NO_3$	300	30
14	4-CHO	H		B	10	95–96	$C_{12}H_9NO_3$	300	>300
15	4-COOH	H		B	12	176.5–178	$C_{12}H_9NO_4$	>300	>300
16	4-CH_2CH_3	H		D, B	43	oil	$C_{13}H_{13}NO_2$	30	100
17	4-$CH(CH_3)_2$	H		D, B	88	oil	$C_{14}H_{15}NO_2$	30	100
18	4-$C(CH_3)_3$	H		D, B	21	oil	$C_{15}H_{17}NO_2$	30	100
19	4-cyclohexyl	H		D, B	92	oil	$C_{17}H_{19}NO_2$	>300	300
20		H	C=O	6, B	10	238–239	$C_{12}H_7NO_3$	>300	300
21[e]		H	CH_2		8	91–93	$C_{12}H_9NO$	100	300

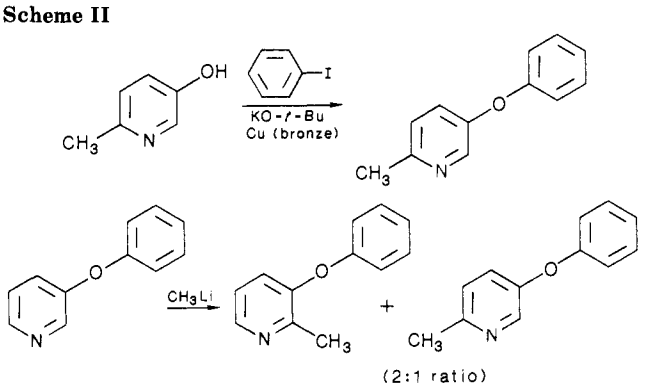
[a] The original reference is footnoted in the Experimental Section and indicated by numerals. The general methods are those described in the Experimental Section and are designated by letters. [b] Satisfactory analytical data ±0.4% obtained for all compounds; satisfactory halogen analysis for **3**, **7**, **9–12** and satisfactory water analysis (Karl Fischer) for 4. [c] Lowest dose level (10, 30, 100, 300, >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. [d] Lowest dose level (10, 30, 100, 300, >300 mg/kg) at which two or more mice (n = 5) exhibited ataxia using the inverted screen procedure at 30 min postadministration. [e] Des-1-oxide. [f] Named as 2-methyl-5-phenoxypyridine 1-oxide by Chemical Abstracts Service.

Since metabolism studies suggested that rapid hydroxylation occurred at the 4-methyl group to afford a much less active derivative, compounds were prepared with increased steric bulk and altered oxidation states at C-4 along with conformationally restrained analogues.

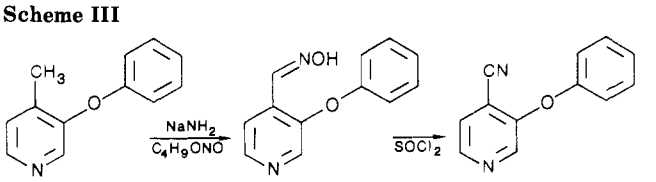
Chemistry

3-Phenoxypyridine was prepared by reaction of 3-hydroxypyridine with bromobenzene in the presence of K_2CO_3.[14] Conversion to the corresponding 1-oxide was accomplished by reaction with 30% peracetic acid.[15]

Scheme II



Scheme III



The route employed to prepare the various methyl-substituted pyridine 1-oxides, **3–6** (Table I), varied with the availability of the starting materials. 3- and 4-methylpyridine were selectively brominated[16] in the 3- or

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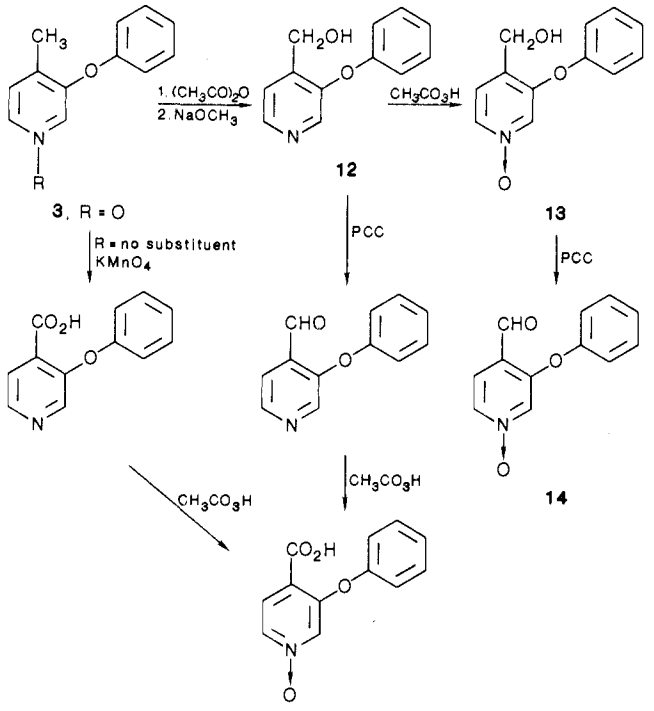
Scheme IV



5-position, and the resulting bromine was displaced by phenoxide under copper catalysis[14] to give either 4-[12] or 5-methyl-3-phenoxypyridine (Scheme I).

6-Methyl-3-phenoxypyridine was obtained from commercially available 3-hydroxy-6-methylpyridine and iodobenzene.[8] Finally, the 2-methyl derivative was obtained by reaction of 3-phenoxypyridine with methyllithium. Addition of the methyl group was followed by rearomatization to afford the desired 2-methyl-3-phenoxypyridine along with the 6-methyl isomer (Scheme II).

Each of the above pyridines was converted to the corresponding 1-oxide, **3–6**, by reaction with 30% peracetic acid.

The 4-chloro derivative **7** was prepared by chlorination of **1** almost exclusively in the 4-position followed by treatment with peracetic acid.[17]

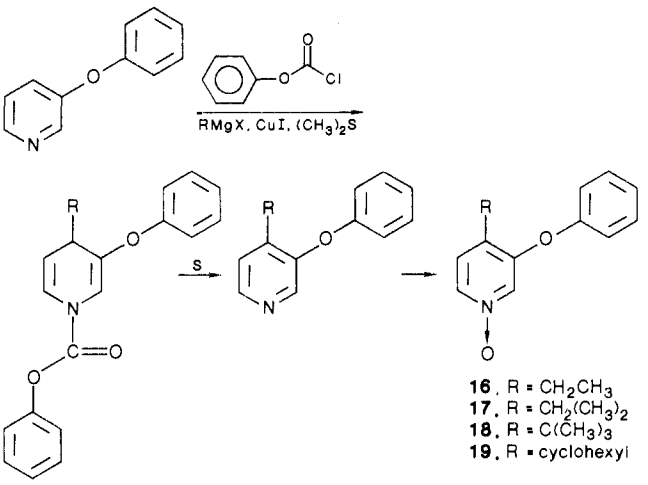
Reaction of 3-phenoxypyridine with sodium amide and butyl nitrite afforded the hydroxylamine derivative (Scheme III), which was dehydrated with thionyl chloride to give the nitrile, which was further transformed to **8**.

The preparation of analogues with substituents on the phenyl ring, **9–11**, was accomplished by the route described in Scheme I.

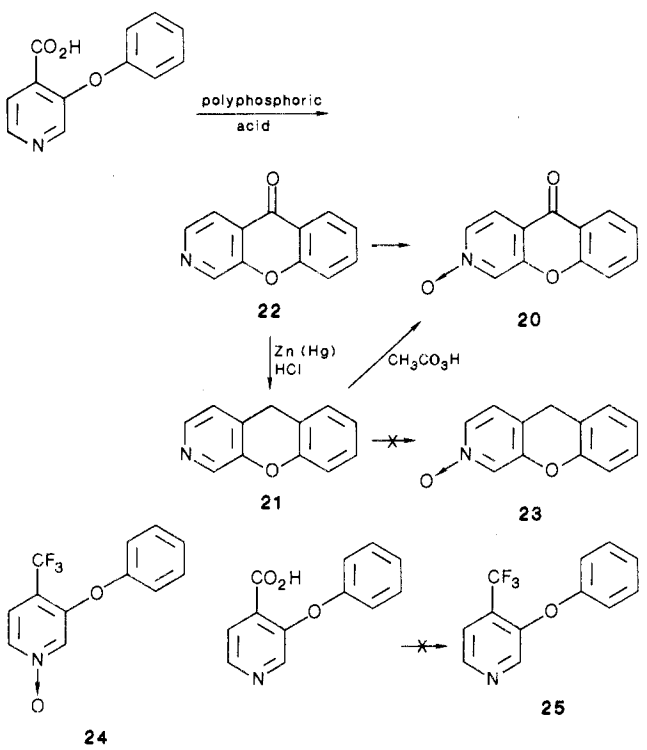
Upon treatment of **3** with acetic anhydride, the 4-acetoxymethyl derivative[18] was obtained, which was converted to **12** via basic hydrolysis. Oxidation with 30% peracetic acid gave **13** (Scheme IV).

Conversion of alcohol **12** to the corresponding aldehyde could be accomplished by oxidation with either activated MnO_2 or pyridinium chlorochromate. The former reagent gave the aldehyde in 40% isolated yield while the latter gave the desired product in 84% yield and was used for larger scale preparation. Unfortunately, this material could not be converted into the 1-oxide with peracetic acid due to concomitant oxidation of the aldehyde to afford **15**. Compound **14** was prepared by direct oxidation of **13** with pyridinium chlorochromate. While the preparation of **15** could be accomplished as described above, the direct oxidation of the aldehyde with chromium-based reagents did not give useful yields of the desired acid. An alternate route to **15**, which worked well on a large scale, was to oxidize 3-phenoxy-4-methylpyridine directly to the acid with potassium permanganate, followed by 1-oxide formation with 30% peracetic acid.

Scheme V



Scheme VI



The preparation of the 4-alkyl derivatives **16–19** was readily accomplished by the method of Comins[19] (Scheme V).

Several tricyclic analogues were prepared as shown in Scheme VI. Treatment of 3-phenoxypyridine-4-carboxylic acid with hot polyphosphoric acid gave the desired tricyclic ketone **22** in high yield.[11] Conversion of **22** to **20** was carried out without complications. Compound **21** was formed by treating the keto compound **22** with zinc amalgam in HCl/ethanol. However, we were unable to

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prepare the corresponding 2-oxide, **23**, by treatment of **21** with peracetic acid since these reaction conditions resulted in both *N*-oxide formation and reoxidation of the methylene carbon to the carbonyl to give **20**.

Considerable effort was directed toward the preparation of the 4-trifluoromethyl analogue, **24**. Conversion of carboxylic acids to the trifluoromethyl group has been accomplished by reaction with SF_4 in liquid HF.[20,21] Isonicotinic acid, when treated under these conditions, readily gave 4-(trifluoromethyl)pyridine. When analogous conditions were applied to 3-phenoxypyridine-4-carboxylic acid, the reaction afforded only intractable tars instead of the desired **25**.

An alternate route, bromination of 4-(trifluoromethyl)pyridine under Lewis acid ($AlCl_3$) catalysis to afford the 3-bromo derivative, was also unsuccessful.

Reaction of pyridine 1-oxides with *p*-toluenesulfonyl chloride has been reported to introduce the tosyloxy group into the 3-position of the pyridine ring,[22,23] but attempts to carry out this reaction on 4-(trifluoromethyl)pyridine 1-oxide were also unsuccessful.

Lastly, we tried to prepare **24** by reacting 4-lithio-3-bromopyridine with an electrophilic source of the CF_3 group. The lithio derivative was prepared by reaction of 3-bromopyridine with lithium diisopropylamide, but reaction with CF_3I at low temperature gave only lithium–halogen exchange to afford 4-iodo-3-bromopyridine. The presence of three highly electronegative fluorine atoms makes CF_3I a poor electrophilic substrate. To date we have been unsuccessful in our attempts to prepare **24**.

Discussion

The previously described pharmacological activity of 3-phenoxypyridines[8,9] suggested that they be examined for anticonvulsant activity. Of the three compounds (**1–3**, Table I) found to possess anticonvulsant activity by the NIH–NINCDS Antiepileptic Drug Development Program, **3** showed the best overall profile and represented a starting point for a detailed structure–activity study on a series of 3-phenoxypyridine 1-oxides.

Since the 4-methyl derivative **3** displayed a more desirable profile of activity than 3-phenoxypyridine itself, we chose to explore the effect of placing a methyl group in other positions of the pyridine nucleus. These compounds, **4–6**, were less active than **3**. We next replaced the methyl group with an electron-withdrawing substituent in the 4-position of the pyridine nucleus. Both the 4-Cl derivative **7** and the 4-CN analogue **8** were weak anticonvulsant agents, demonstrating that an electron-donating substituent in the 4-position of the pyridine ring is necessary for the desired pharmacological effect.

Substitution on the phenyl ring was next examined with substituent choice guided by the operational scheme of Topliss.[24] The 4-chlorophenyl derivative **9** was found to be slightly less active than **3**, dictating that the 4-methoxyphenyl and 4-methylphenyl analogues **10** and **11** should subsequently be prepared. Both were much less active than **9** suggesting that further phenyl substitutions were not warranted.

While the potency/ataxia profile of **3** was sufficient for further development, the duration of anticonvulsant action was less than optimal. Studies suggested that **3** was rapidly metabolized to 3-phenoxy-4-(hydroxymethyl)pyridine (**12**). Testing of synthetically prepared **12** revealed it to be much less active than **3**, confirming the short half-life of **3** was due to rapid metabolic conversion to a much less active product.

Several compounds were prepared in an attempt to block the metabolism of **3** and these can be divided into three classes: (1) change in the oxidation state of the 4-pyridine substituent, (2) variation in the size of the 4-alkyl group, and (3) tricyclic systems.

The 4-CHO and 4-CO_2H derivatives, **14** and **15**, were found to possess no significant anticonvulsant activity. Both of these groups are electron withdrawing and support our earlier observation that this electronic effect is undesirable. The carboxylic acid **15** showed no activity or toxicity, suggesting that **15** is too polar to enter the central nervous system (CNS).

The size of the 4-alkyl substituent was sequentially increased in compounds **16–19** with the hope that the increasing steric bulk would hinder metabolic inactivation, resulting in a longer duration of action. The ethyl, **16**, and isopropyl, **17**, analogues displayed good activity without ataxia, but as with **3** this activity was gone by 2 h postdosing. Increasing the bulk further with a *tert*-butyl group furnished **18**, which had a similar pharmacological profile with some activity now present at 2 h. The improvement in overall profile, however, was not enough to warrant further testing. The 4-cyclohexyl derivative **19** showed no anticonvulsant activity at any of the doses tested, suggesting that the limits of steric bulk had been exceeded.

The tricyclic compound **21** was prepared to explore the effect of holding the molecule in a rigid conformation with the two aromatic rings nearly planar. Unfortunately, while we were not able to prepare the 1-oxide of **21** for direct comparison with **3**, we were able to compare **21** with 3-phenoxy-4-methylpyridine (compound **1**). Both compounds **21** and **1** exhibited short-lived anticonvulsant action after a dose of 100 mg/kg to mice, suggesting that the 1-oxide derivative of **21** would show similar activity to that seen with **3**. Compound **21** was not investigated further because of its lack of potency and relatively short duration of action.

Finally, we had hoped to evaluate 4-(trifluoromethyl)-3-phenoxypyridine 1-oxide, **24**, since the trifluoromethyl group is not susceptible to the observed metabolic pathway. Unfortunately, attempts to synthesize **24** were unsuccessful.

Since our studies identified **3** as possessing the most desirable in vivo profile for a potential anticonvulsant drug, it was selected for a detailed pharmacological and toxicological evaluation.

Compound **3** was submitted to the Antiepileptic Drug Development Program[4] of the National Institute of Neurological and Communicative Disorders and Stroke (ADD Program) for further testing. Tonic extensor seizures from maximal electroshock[25] were blocked in a dose-related manner, with testing done at the approximate time of peak effect, and the calculated ED_{50} values were 23 mg/kg (ip) and 85 mg/kg (po) in mice and 11 mg/kg (po) in rats. Anticonvulsant effects lasted for less than 2 h after a single ip or po dose to mice, and 4 or more h after a single po dose to rats. Behavioral side effects (ataxia, sedation) were assessed by the rotorod procedure, and a dose-related increase in ataxia was observed. The calculated ED_{50} values from these tests are listed in Table II.

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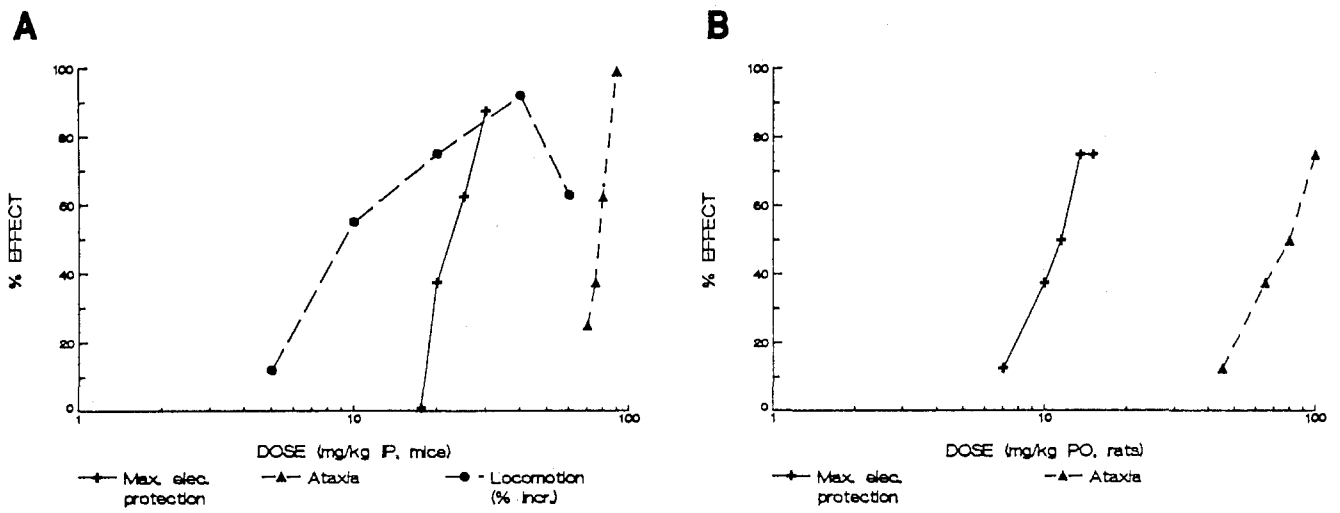

Figure 1. Anticonvulsant activity, locomoter stimulation, and ataxia produced by compound **3**. A: Mice were tested 0.5 h after intraperitoneal dosing with eight mice per dose in each test. Maximal electroshock data (max. elec. protection) represents fraction of mice protected from tonic extensor seizures. Ataxia denotes fraction of mice falling from rotorod. Locomotion (% incr.) represents the spontaneous locomotor activity of four mice measured in a light-beam chamber compared to locomotor activity of vehicle-treated mice. B: Rats were tested 1.0 h after oral dosing, with eight rats per dose. Electroshock and ataxia as in A, except ataxia was by subjective evaluation.

Table II. Anticonvulsant Activity and Ataxia Produced by Compound **3** in Mice and Rats

species	dose route	time of test (h) post-dosing	ED_{50},[a] mg/kg MES[b]	ED_{50},[a] mg/kg ROT[c]
mouse	ip	0.5	23 (20–26)[d]	76 (71–81)
mouse	po	0.5	85 (69–103)	300 (250–344)
rat	po	1.0	11 (9–14)	75[e] (58–95)

[a] Calculated by probit analysis of quantal data from four dose groups of eight animals each. [b] MES = maximal electroshock test. [c] ROT = rotorod ataxia test. [d] 95% confidence intervals. [e] Ataxia determined by visual observation.

The fractions of mice showing anticonvulsant activity, ataxia, or increased locomotion at a number of different doses are shown in Figure 1. The data also indicate that **3**, at doses of 100 mg/kg (ip) or less (like phenytoin), did not significantly protect mice from clonic seizures caused by threshold doses of subcutaneous pentylenetetrazol, bicuculline, picrotoxin, or strychnine.

Interestingly, **3** and several related compounds produced dose-related increases in spontaneous locomotor activity in mice (Table III, Figure 1) and increases in electrical self-stimulation of the brain.[26] Most anticonvulsants have little effect on spontaneous locomotion or self-stimulation unless high doses are given and then they cause decreased activity. Compound **3** and the other related compounds listed in Table III produced dose-related changes in spontaneous behavior that were grossly similar, although to a lesser degree than those of D-methamphetamine. The pharmacological mechanism for this behavioral stimulation is not known, but compound **1** has also been reported to cause stimulation of spontaneous behavior,[26] as has hydergine, a nootropic.

Cardiovascular effects of **3** after iv infusion to pentobarbital-anesthetized dogs included moderate increases in myocardial contractility and heart rate at a dose of 31 mg/kg infused over 8 min. Significant effects were not observed after a similar dose of 10 mg/kg iv. Compound **3** at 30 mg/kg iv also potentiated the increase in blood pressure and heart rate caused by epinephrine, norepinephrine, or tyramine, but not to acetylcholine, angiotensin II, or carotid occlusion in anesthetized, vagotomized dogs. These results suggest that **3** potentiates sympathetic autonomic responses after high doses.

In biochemical studies, **3** did not significantly displace radioligand binding in dopamine, muscarinic-cholinergic, α_1-, α_2-, β_1-, and β_2-adrenergic, serotonin-1, serotonin-2, and γ-aminobutyric acid receptors. In each assay, **3** was tested at concentrations up to 10^{-5} M.

Compound **3** was also evaluated for toxicological effects after oral acute administration to rats and dogs. The

Table III. Stimulation of Spontaneous Locomotor Behavior by Several Compounds during the First Hour after Intraperitoneal Administration to Mice

compd	dose, mg/kg	increase spontaneous locomotion activity,[a] %	ataxia (inverted screen test[b])
1	10	—	—
	20	28	—
	40	53	—
	100	15	—
2	10	19	—
	30	45	1/9
	100	122	—
3	5	12	—
	10	55	—
	20	75	1/12
	40	92	1/12
	60	63	6/12
4	10	32	—
	30	96	—
	100	134	3/9
5	10	87	—
	30	186	—
	100	228	—
6	10	48	—
	30	25	—
	100	184	2/9

[a] Represents percent change from saline-treated mice in locomotor activity during a 1-h period beginning 20 min after drug injection (nine or more mice per dose group). [b] Represents number of mice falling from an inverted wire mesh during a 1-min test period, 20 min after drug injection (dashes signify that no animals fell).

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findings included greatly increased locomotor activity, agitation, and emesis in dogs (80 mg/kg and higher). In rats, moderate doses caused increased activity (10–60 mg/kg) and higher doses caused ataxia, behavioral depression, and convulsions (250 mg/kg and above). Behavioral effects occurred during the first day after drug administration.

These data suggest that **3** and related derivatives possess significant anticonvulsant activity at doses that do not produce ataxia or sedation; however, significant stimulation of locomotor activity occurred at doses below and including those that had anticonvulsant effects.

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 with TMS as the internal reference standard and deuteriochloroform or DMSO-d_6 as solvents. Purity was determined by microanalysis and by TLC with use of 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 with use of KBr pellets. All compounds possessed microanalytical and spectral data consistent with the structures proposed.

Method A. General Procedure for Synthesis of 3-(Aryloxy)pyridines. The method used was that of Renshaw and Conn.[14]

Method B. General Procedure for Synthesis of 3-(Aryloxy)pyridine 1-Oxides. The method used was that of Ochiai and Sai.[15]

Method C. General Procedure for Synthesis of 3-Bromopyridines. The method used was that of Pearson.[16]

Method D. General Procedure for Synthesis of 4-Alkyl-3-phenoxypyridines. 3-Phenoxypyridine (**1**) was reacted by the method of Comins.[19]

2-Methyl-5-phenoxypyridine 1-oxide (4) and 2-methyl-3-phenoxypyridine 1-oxide (6) were prepared as described by Butler.[8]

4-Chloro-3-phenoxypyridine 1-Oxide (7). One mole of 3-phenoxypyridine 1-oxide was added portionwise to 90 °C $POCl_3$ (500 mL) with stirring. The mixture was then heated and stirred at reflux temperature for 4 h, cooled, concentrated in vacuo, and poured into a stirred mixture of 100 mL of concentrated HCl, 100 mL of water, and 300 g of ice. The product was extracted into $CHCl_3$ (3 × 300 mL). The combined extracts were stirred with 100 mL of concentrated NH_4OH, dried over $MgSO_4$, and concentrated in vacuo to an oil, which was distilled at 0.2 Torr to give 80 g (39%) of 97% pure (by VPC) 4-chloro-3-phenoxypyridine (contaminated with 3% of 2-chloro-3-phenoxypyridine). Oxidation by method B afforded **7** in 25% yield.

4-Cyano-3-phenoxypyridine 1-Oxide (8). An ether solution (100 mL) of 4-methyl-3-phenoxypyridine[11] (21.4 g, 0.116 mol) was added dropwise with stirring to sodium amide (0.283 mol) [prepared by portionwise addition of sodium (6.5 g) to liquid NH_3 (1 L) containing a catalytic amount of ferric nitrate]. A solution of butyl nitrite (0.14 mol) in ether (100 mL) was added dropwise with stirring during 30 min. Stirring was continued for 2 h, ether (500 mL) was added, and the NH_3 was allowed to slowly evaporate. A solution of ammonium sulfate (20 g, 0.151 mol) in water (400 mL) was added, and the resultant mixture was stirred for 10 min. The aqueous layer was washed with CH_2Cl_2 (2 × 100 mL). These washes were combined with the organic layer, dried over $MgSO_4$, and concentrated in vacuo to afford 17.6 g of solid 4-[(hydroxyimino)methyl]-3-phenoxypridine (71%), mp 160–161 °C.

A solution of the (hydroxyimino)methyl compound (17.3 g, 0.081 mol) in CH_2Cl_2 (200 mL) was added in a slow, steady stream to a solution of $SOCl_2$ (120 g, 1 mol) dissolved in CH_2Cl_2 (200 mL). The mixture was heated and stirred at reflux temperature for 48 h, cooled, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed with cold, concentrated NH_4OH (5 × 100 mL), dried over $MgSO_4$, and concentrated in vacuo to afford an oil, which was distilled at 88 °C (0.55 Torr) to give 10.6 g (67%) of 4-cyano-3-phenoxypyridine. Oxidation by method C gave **8** in 67% yield, mp 91–92 °C.

3-Phenoxy-4-pyridinemethanol (12) was prepared from **3** according to the following procedure. A solution of **3** (13 g, 0.065 mol) in HOAc (20 mL) was added to 100 mL of stirred, refluxing acetic anhydride over a 30-min period. The reaction mixture was stirred at reflux temperature for 30 h and cooled, and MeOH (150 mL) was added over a 1-h period.

After being stirred for an additional 10 min, the mixture was concentrated in vacuo to an oil and was purified via column chromatography (silica gel; MeCN) to afford 10 g (64%) of (3-phenoxy-4-pyridinyl)methyl acetate.

A solution of (3-phenoxy-4-pyridinyl)methyl acetate (10 g, 0.041 mol) in MeOH (150 mL) was treated with a solution of sodium metal (1.2 g, 0.052 mol) in MeOH (100 mL). The mixture was stirred at reflux temperature for 24 h, cooled, acidified to pH 7.3, and concentrated in vacuo to an oil. This oil was treated with water (50 mL) and extracted into $CHCl_3$ (3 × 100 mL). The combined extracts were washed with water, dried over Na_2SO_4, and concentrated in vacuo to furnish an oil (7 g). This was purified via column chromatography (silica gel; EtOAc) to afford 4.2 g (51%) of **12**.

3-Phenoxy-4-pyridinecarboxaldehyde 1-oxide (14) was prepared by dissolving **13** (5.4 g, 0.0249 mol) in CH_2Cl_2 (100 mL) and treating with NaOAc (8 g, 0.1 mol) followed by portionwise addition of pyridinium chlorochromate (PCC, Aldrich) (8 g, 0.0374 mol). The reaction mixture was stirred at room temperature for 4 h, at which time an additional 3 g of PCC was added. After being stirred for an additional 16 h, the mixture was poured into 1 L of ether and filtered through Florisil, and the filtrate was concentrated in vacuo to an oil. Purification via flash column chromatography (silica gel; 75% EtOAc in hexane) afforded 1.54 g (30%) of **14**, mp 95–96 °C.

3-Phenoxypyridine-4-carboxylic Acid 1-Oxide (15). 4-Methyl-3-phenoxypyridine (206.5 g, 1.12 mol) was suspended in 15 L of water and heated to 70 °C with stirring. $KMnO_4$ (530 g) was added portionwise during 5 h, followed by stirring at 70 °C for an additional 24 h. The insoluble materials were filtered, and the filtrate was concentrated on a steam bath to a volume of 2 L. The cooled solution was acidified with glacial HOAc and then treated with excess saturated copper(II) sulfate solution. The copper salt was filtered (135 g), suspended in 1 L of water, and heated on the steam bath while H_2S was bubbled rapidly through the mixture for 12 h. The black solid was filtered, suspended in 2-propanol and treated with a slight excess of 20% HCl dissolved in 2-propanol. The mixture was boiled and filtered, and the filtrate was concentrated in vacuo to give 55 g (23%) of analytically pure material, mp 237–238 °C. Oxidation by method B afforded **15** in 85% yield, mp 173–175 °C.

2-Azaxanthene (21) was prepared by treating commercially available 2-azaxanthone **22** (1 g, 0.005 mol) with 3.33 g of Zn(Hg) (freshly prepared from a suspension of 12 g of mossy zinc in 20 mL of water being treated with 1.2 g of mercuric chloride and 0.6 mL of HCl), 5 mL water, and 5 mL of EtOH and subsequent vigorous stirring of the reaction mixture as it was heated to reflux temperature. Heating was continued for 3 h, and then the mixture was cooled, poured into 100 g of ice, and made basic with the addition of 50% NaOH. The product was extracted into CH_2Cl_2 (2 × 100 mL), and the extracts were dried over $MgSO_4$ and concentrated in vacuo to a yellow solid, mp 91–93 °C, in 89% yield.

Pharmacological Methods. The test compounds were dissolved in water or suspended in 0.29% Methocel 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 25–32 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 at three time points (0.5, 2, and 4 h).

The mice were subjected to 90 mA, 1 ms, monophasic pulses at 100 Hz, delivered through ear clips for 0.2 s. This current strength was approximately 4 times that required to produce seizures in 99% of mice tested and reliably produces 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, which untreated mice easily clung to but from which impaired mice fell[27] or by measuring the ability of

the test animal to remain on a rotating rod (rotorod test).[28] In addition, spontaneous locomotor activity of mice was measured by an automated procedure.[29] Rats were tested for drug-induced alterations of electrical self-stimulation of the brain (methods of ref 26a). Rats were tested against maximal electroshock (methods of ref 25) and in a subjective assessment of behavioral impairment. Median effective doses were determined by a probit analysis.[30]

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Registry No. 1 des-1-oxide, 2176-45-6; 1·H_2SO_4 (des-1-oxide), 65846-21-1; **2**, 32967-12-7; **3**, 81911-41-3; **3**·HCl, 112945-84-3; **3** des-1-oxide, 54629-96-8; **4**, 112945-85-4; **4** des-1-oxide, 75580-04-0; **5**, 112945-86-5; **6**, 112945-87-6; **6** des-1-oxide, 76167-46-9; **7**, 112946-02-8; **7**·HCl, 112945-88-7; **7** des-1-oxide, 73406-90-3; **8**, 112945-89-8; **8** des-1-oxide, 78790-74-6; **9**, 112945-90-1; **9** des-1-oxide, 28232-56-6; **10**, 112946-03-9; **10**-HCl, 112945-91-2; **10** des-1-oxide, 112968-85-1; **11**, 112946-04-0; **11**-HCl, 112945-92-3; **11** des-1-oxide, 28232-60-2; **12**, 112946-05-1; **12**-CHCl, 112945-93-4; **13**, 112945-94-5; **14**, 112945-95-6; **15**, 112945-96-7; **15** des-1-oxide, 54629-99-1; **16**, 112945-97-8; **16** des-1-oxide, 112946-08-4; **17**, 112945-98-9; **17** des-1-oxide, 112946-09-5; **18**, 112945-99-0; **18** des-1-oxide, 112946-10-8; **19**, 112946-00-6; **19** des-1-oxide, 112946-11-9; **20**, 112946-01-7; **21**, 38674-06-5; **22**, 54629-29-7; isonicotinic acid, 55-22-1; 2-chloro-3-phenoxypyridine, 73406-93-6; 4-((hydroxyimino)methyl)-3-phenoxypyridine, 112946-06-2; (3-phenoxy-4-pyridyl)methyl acetate, 112946-07-3; 5-methyl-3-phenoxypyridine, 73571-20-7; 4-(trifluoromethyl)pyridine, 3796-24-5.

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Synthesis, Molecular Modeling, DNA Binding, and Antitumor Properties of Some Substituted Amidoanthraquinones

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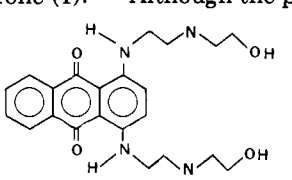
A series of 1- and 1,4-substituted amidoanthraquinones have been prepared, with side chains possessing basic nitrogen atoms. Computer modeling and energy calculations have shown that all eight compounds can bind intercalatively to DNA and that there are significant differences in the additional nonbonded and electrostatic interactions possible at the DNA binding site. Solution DNA binding and closed-circular DNA unwinding studies confirmed intercalative interactions, and the predicted differences in strength of interactions between mono- and disubstituted compounds were found. All compounds were modestly cytotoxic to L1210 cells in culture. In vivo activity against L1210 and S180 tumors was not found for the monosubstituted compounds, whereas the four disubstituted ones had varying levels of measurable, though low, activity.

The clinical utility of the anthracycline antitumor antibiotics, especially the broad spectrum activity of Adriamycin, is hampered by their severe dose-limiting, cumulative cardiotoxicity.[1] There has accordingly been a continuing search for analogues with diminished toxicity, as well as with improved therapeutic indices.[2] An area of particular activity and promise is concerned with aminoalkyl-substituted anthraquinones[3-6] with the demonstration of clinical activity for the 1,4-disubstituted compound mitoxantrone (**1**).[7,8] Although the precise mode of action in vivo for these compounds has not been fully elucidated, a number of studies have shown that they interact intercalatively with DNA in vitro[9-15] and that therefore DNA is a major biological target for mitoxantrone.



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The present study examines the DNA-interactive abilities, both calculated the experimental, of a new series of 1-mono- and 1,4-disubstituted alkylamido-substituted anthraquinones.[1-8] These are examined in relation to their cytotoxic and antitumor properties. Earlier studies by Palumbo et al. have shown direct relationships between DNA-binding parameters and at least some biological

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