Synthesis and Quantitative Structure-Activity Relationships of Anticonvulsant 2,3,6-Triaminopyridines

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The synthesis of 2,3,6-triaminopyridine derivatives, representing a unique chemical structure for anticonvulsants, is described. The synthetic program was performed (a) to identify more potent analogs, (b) to determine structural properties controlling potency as well as neurotoxicity, and (c) to reduce the requirements for animal testing. As a result, besides other structural properties, the overall molecular lipophilicity (log k', octanol-coated column) explained changes in anticonvulsant potency and neurotoxicity. Mimicking the interaction of the amphiphilic triaminopyridines with biological membranes, NMR experiments in the presence of lecithin vesicles were conducted in order to measure the phospholipid-binding parameter log $\Delta(1/T_2)$. Replacement of log k' with log $\Delta(1/T_2)$ in the correlation analysis afforded a more significant equation describing the anticonvulsant activity of 21 derivatives.

Introduction

The synthesis and biological evaluation of 2,3,6-triaminopyridines have been under extensive investigation in our laboratories over the past decade.[1-10] This resulted in the successful development of flupirtine maleate ([2-amino-6-[[(4-fluorophenyl)methyl]amino]-3-pyridinyl]carbamic acid ethyl ester maleate; Katadolon) as a centrally acting analgesic.

NHCO$_2$CH$_2$CH$_3$ · HC-COOH ‖ HC-COOH; NH, N, NH$_2$, F

Flupirtine maleate; Katadolon ®

Although flupirtine was originally developed as an analgesic, it exhibited potent antiepileptic properties against two major seizure models, the maximal electroshock (MES) and the subcutaneous pentylene tetrazol (Metrazol, ScMet) test systems, when screened in the Antiepileptic Drug Development Program of the National Institute of Neurological Disorders and Stroke. The potency in both seizure models and the established safety in humans rendered flupirtine an attractive candidate as a prototype worthy of further investigation. Compared to known anticonvulsants, the 2,3,6-triaminopyridines present a unique chemical structure wherein the only identifiable feature potentially in common is the presence of electron-donor groups which can be oriented to fall within an intramolecular distance that may relate to antiepileptic activity. The distance between the nitrogen atom of the free amino group and the carbonyl oxygen of the carbamic acid ester group in flupirtine is 2.91 Å as determined from the X-ray crystal structure.[2] This falls within the range postulated as important for the antiepileptic activity of established anticonvulsant drugs.[11] As shown in Figure 1, distances

Trihexyphenidyl 2.76 Å

Diazepam 3.35 Å

Flupirtine 2.91 Å

Phenacemide 4.13 Å

Phenobarbital 4.51 Å

Figure 1. Comparison of intramolecular distances of electron-donor groups in flupirtine and known anticonvulsants.

in these compounds vary from 2.76 Å for trihexyphenidyl to 4.51 Å for phenobarbital.

With these presumably essential structural features in mind, new flupirtine analogs were designed in which the central 6-N-benzyl-2,3,6-triaminopyridine nucleus was maintained intact. An obvious goal was to identify more potent flupirtine derivatives as potential anticonvulsant agents. Second was to determine structural properties controlling potency as well as those affecting toxicity. Finally, the continuous and concurrent employment of QSAR techniques was intended to increase the overall efficiency of the investigations, thus obviating unnecessary syntheses and reducing the requirements for extensive animal testing. The results of these recently concluded studies provide the basis for this report.

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Scheme 1

Chemistry

A synthetic scheme previously established for flupirtine[1,2] was employed for the generation of the new derivatives, Scheme 1. Reaction of 3-nitro-2,6-dichloropyridine (**I**) with ammonia yielded 2-amino-3-nitro-6-chloropyridine (**II**). Treatment of the pyridine with an appropriately substituted benzylamine in the presence of dioxane/triethylamine gave the corresponding 2-amino-6-[(R′-benzyl)amino]-3-nitropyridines **III** in 75–90% yield. Reduction of the 3-nitro group by catalytic hydrogenation followed by reaction with the desired chloroformate or carbonyl chloride then afforded the flupirtine analogs **1–24** (Table 1). Cyclization of the 2-bromoethyl carbamate in **V** proceeded smoothly in the presence of NaOH/methanol to give the oxazolidinone **25**. Chemical data for the final derivatives are summarized in Table 1.

Biology

All molecules were submitted to the Epilepsy Branch of the National Institute of Neurological Disorders and Stroke (USA) for evaluation in the maximal electroshock test as a measure of anticonvulsant activity.[12] The derivatives were also tested for neurotoxicity[19] at the same facility. Activities have been converted to a molar basis and are given in Table 2 in the form log $1/C$ (where $C = ED_{50}$ or I_{50}). A number of analogs exhibited improved potency over flupirtine. The observed range in anticonvulsant activity was approximately 60-fold with the 2,4,6-trimethyl analog **3** being the most active, while the 4-acetamido derivative **9** was least active. In contrast, the range in neurotoxicity was much larger, about 400-fold. The same trimethyl derivative, **3**, was most toxic, while the 4-sulfonamide **2** showed least toxicity. Preliminary qualitative evaluation of structure–activity relationships revealed that substitution with electron-withdrawing groups, such as $-CF_3$, -F, or -Cl has little effect on anticonvulsant activity or neurotoxicity, whereas introduction of lipophilic methyl groups, especially in the *ortho* position, caused significant increases in both types of activity. Modification of the carbamic ester moiety generally lowers the anticonvulsant activity but simultaneously gives rise to an even greater decrease in neurotoxicity, thus suggesting a means of improving the therapeutic index.

Quantitative Structure–Activity Relationships

The *in vivo* pharmacological test data on neurotoxicity and anticonvulsant activity were subjected to multiparameter regression analysis in an effort to elucidate chemical features affecting activity as well as to provide indications for improving selective action. Initial attempts to develop relationships between maximal electroshock data and phenyl ring substituent parameters such as Hansch π, Hammett σ, or molar refractivity, MR, did give statistically significant correlations; however, the quality was not as good as one might anticipate. It may be that the failure of straightforward substituent parameter approaches is a reflection of deviations in actual lipophilicities versus theoretical estimates as well as an indication of the importance of conformation for receptor interaction. Consequently, an alternative set of parameters was developed. HPLC capacity factors, log k', were determined for all derivatives using octanol-saturated columns. Since *ortho* substitution appeared to exert an additional effect on activity, the van der Waals volume, V_{ortho}, for the 2,6-positions of the benzyl group was examined as a potential parameter. To investigate possible conformational preferences, NMR spectra for selected derivatives were examined in some detail. In DMSO solution, it appears that two conformations predominate, on the basis of observed $-CH_2-NH-$ vicinal coupling constants.[13] In relation to the benzylic C–N torsion angle, these were a folded, pseudogauche conformer with the phenyl-C–N-pyridyl torsion angle falling in the 30–50° range and an extended, pseudotrans conformer with the same angle in the 180–210° range. The preferences for one or the other of the two dominant conformations, while obviously sensitive to changes in chemical structure, were also related to the degree of ionization of the pyridine ring. Certain derivatives such as the free base of the 2,4,6-trimethyl analog **3** were predominantly in the pseudogauche conformation. However, fully protonated derivatives appeared to exist as an equilibrium between the two major conformations. Thus possible conformations appear to depend not only upon the phenyl substitution patterns but also upon electronic factors present in the pyridyl ring. The type of substructures present in the pyridylurethane portion of the derivatives may therefore exert an indirect effect on conformation in this fashion. To test this possibility, molecular mechanics minimization[14] of all analogs, in their protonated form, was conducted using identical starting conformations (Figure 2) with the phenyl-C–N-pyridyl torsion angle set to 205°. Additionally, the urethane carbonyl was rotated such that hydrogen bonding could occur between the carbonyl oxygen and the 2-amino group.

This intramolecular hydrogen bond is supported by X-ray data on flupirtine (**1**) as well as by NMR data on **1** and a number of derivatives.[15] Finally, computations were carried out on protonated derivatives since these should predominate at biological pH values. Only the extended conformation was considered because this conformation is thought to be preferred in the interac-

Table 1. Characterization of Flupirtine Derivatives

no.	R	R′	R″	mp (°C)	formula	anal.[a]
1	4-F	H	NHCOOEt	210–212	$C_{15}H_{17}FN_4O_2 \cdot HCl$	C,H,N,Cl
2	4-SO_2NH_2	H	NHCOOEt	168–171	$C_{15}H_{19}N_5O_4S \cdot HCl$	C,H,N,Cl
3	2,4,6-Me_3	H	NHCOOEt	203–204	$C_{18}H_{24}N_4O_2 \cdot HCl$	C,H,N,Cl
4	4-$COCH_3$	H	NHCOOEt	203–206	$C_{17}H_{20}N_4O_3 \cdot HCl$	C,H,N,Cl
5	3-F	H	NHCOOEt	205	$C_{15}H_{17}FN_4O_2 \cdot HCl$	C,H,N,Cl
6	4-Cl	H	NHCOOEt	219–220	$C_{15}H_{17}ClN_4O_2 \cdot HCl$	C,H,N,Cl
7	H	H	NHCOOEt	208–209	$C_{15}H_{18}N_4O_2 \cdot HCl$	C,H,N,Cl
8	2,4-F_2	H	NHCOOEt	195–196	$C_{15}H_{16}F_2N_4O_2 \cdot HCl$	C,H,N,Cl
9	4-$NHCOCH_3$	H	NHCOOEt	211–212	$C_{17}H_{21}N_5O_3 \cdot HCl$	C,H,N,Cl
10	2-Me	H	NHCOOEt	204–205	$C_{16}H_{20}N_4O_2 \cdot HCl$	C,H,N,Cl
11	2-OH	H	NHCOOEt	195–197	$C_{15}H_{18}N_4O_3 \cdot HCl$	C,H,N,Cl
12	4-CF_3	H	NHCOOEt	220	$C_{16}H_{17}F_3N_4O_2 \cdot HCl$	C,H,N,Cl
13	3-Cl, 4-Me	H	NHCOOEt	216–218	$C_{16}H_{19}ClN_4O_2 \cdot HCl$	C,H,N,Cl
14	4-F	Me	NHCOOEt	160	$C_{16}H_{19}FN_4O_2 \cdot HCl$	C,H,N,Cl
15	2,4-Me_2	H	NHCOOEt	216–217	$C_{17}H_{22}N_4O_2 \cdot HCl$	C,H,N,Cl
16	2,6-Me_2	H	NHCOOEt	198	$C_{17}H_{22}N_4O_2 \cdot HCl$	C,H,N,Cl
17	H	H	NHCOOPh	190–191	$C_{19}H_{18}N_4O_2 \cdot HCl$	C,H,N,Cl
18	4-F	H	NHCOOPh	200	$C_{19}H_{17}FN_4O_2 \cdot HCl$	C,H,N,Cl
19	4-Cl	H	NHCOOPh	199–200	$C_{19}H_{17}ClN_4O_2 \cdot HCl$	C,H,N,Cl
20	4-F	H	$NHCOOCH_2CH{=}CH_2$	102–103	$C_{16}H_{16}FN_4O_2$	C,H,N
21	4-F	H	$NHCOO(CH_2)_2Cl$	205	$C_{15}H_{16}ClFN_4O_2 \cdot HCl$	C,H,N,Cl
2	4-F	H	NHCOOMe	227–230	$C_{14}H_{15}FN_4O_2 \cdot HCl$	C,H,N,Cl
23	4-F	H	$NHCOCH_3$	247–249	$C_{14}H_{15}FN_4O \cdot HCl$	C,H,N,Cl
24	4-F	H	NHCO– (Me, N, O, Me ring)	109–110	$C_{18}H_{18}FN_5O_2 \cdot HCl$	C,H,N,Cl
25	4-F	H	—N (O, O ring)	220	$C_{15}H_{15}FN_4O_2 \cdot HCl$	C,H,N,Cl

[a] Analyses for C, H, N, and Cl were correct within ±0.4%.

Table 2. Physicochemical Parameters and Biological Activities for Flupirtine Derivatives

	log 1/ED_{50}(MES)			log 1/I_{50}(NT)							
no.	obs[a]	calcd[b]	dev	obs[d]	calcd[e]	dev	log Δ(1/T_2)	log k'	C—N_{tors}	V_{ortho}	V_{ure}
1	4.128	4.113	0.015	3.956	3.887	0.069	1.668	1.85	207.38	6.80	114.40
2	3.572	3.592	−0.020	2.880	2.900	−0.020	0.806	−0.17	207.93	6.80	114.40
3	5.268	5.295	−0.027	5.525	5.459	0.066	2.318	3.06	208.53	27.34	114.40
4	3.831	3.807	0.024	3.693	3.576	0.117	1.170	1.21	207.58	6.80	114.40
5	4.297	4.121	0.176	4.018	3.930	0.088	1.652	1.86	207.84	6.80	114.40
6	4.388	4.372	0.016	4.174	4.029	0.145	2.069	2.10	207.55	6.80	114.40
7	4.214	4.106	0.108	3.850	3.993	−0.143	1.502	1.67	209.82	6.80	114.40
8	4.321	4.354	−0.033	4.166	4.196	−0.030	1.838	2.06	208.62	9.20	114.40
9	3.487	3.608	−0.121	3.426	3.289	0.137	0.847	0.63	207.69	6.80	114.40
10	4.872	4.602	0.270	4.762	4.370	0.392	1.794	1.77	208.53	17.07	114.40
11	3.896	3.772	0.124	*f*	*g*		1.089	1.61	206.87	11.44	114.40
12	4.146	*c*		3.709	*g*		*h*	2.67	207.64	6.80	114.40
13	4.083	*c*		3.901	*g*		*h*	2.90	208.19	6.80	114.40
14	3.938	*c*		4.076	*g*		*h*	1.75	210.86	6.80	114.40
15	4.530	4.918	−0.388	4.720	4.944	−0.224	2.164	2.55	210.69	17.07	114.40
16	5.010	4.926	0.084	4.991	4.990	0.001	1.769	2.52	207.94	27.34	114.40
17	3.942	4.037	−0.095	3.524	3.729	−0.205	1.822	2.23	197.14	6.80	163.97
18	3.724	3.768	−0.044	3.252	3.253	−0.001	1.939	2.67	194.41	6.80	163.97
19	3.507	*c*		*f*	*g*		*h*	2.66	199.63	6.80	163.97
20	4.483	4.277	0.206	4.292	4.031	0.261	1.931	2.14	207.23	6.80	130.52
21	4.459	4.362	0.097	4.088	4.011	0.077	2.034	2.02	207.84	6.80	134.36
22	4.110	4.025	0.085	3.979	3.698	0.281	1.509	1.43	207.69	6.80	104.12
23	3.567	3.843	−0.276	3.189	3.437	−0.248	1.204	0.88	207.94	6.80	100.77
24	3.940	4.182	−0.242	3.250	3.789	−0.539	1.741	1.57	203.04	6.80	169.82
25	4.065	4.014	0.051	3.339	3.409	−0.070	1.505	2.14	207.47	6.80	102.03

[a] Observed activity in maximal electroshock test where ED_{50} is molar. [b] Calculated by eq 2. [c] Not included in eq 2. [d] Observed neurotoxicity where I_{50} is molar. [e] Calculated by eq 5. [f] Not tested. [g] Not included in eq 5. [h] Not determined.

tion with phospholipids of biological membranes. The torsion angle resulting from minimization was then examined as a potential QSAR parameter, C—N_{tors}, providing an estimate of the effects of structural changes upon molecular conformation. Although not anticipated, it can be seen from the data in Table 2 that significant changes from the starting torsion angles have occurred in arriving at the minimum energy

Torsion Angle 1, C-C-C-N = 100
Torsion Angle 2, C-C-N-C = 205
Torsion Angle 3, C-N-C-N = 10
Torsion Angle 4, C-C-N-C = 40

Figure 2. Starting torsion angles employed in molecular mechanics minimization.

structures, especially for derivatives where the 3-urethane structure has been altered to -NHCOOPh. This effect may also be described by an indicator variable (I_{Ph}).

Correlation analysis employing the following parameters of the flupirtine derivatives: measured log k' for overall lipophilicity, V_{ortho} for specific *ortho* substituent effects, and I_{Ph} or C$-$N$_{tors}$ for conformational effects, afforded highly significant relationships given by eq 1.

$$\log 1/\mathrm{ED}_{50}(\mathrm{MES}) = 0.287(0.164)\log k' + 0.048(0.030)\mathrm{C{-}N_{tors}} + 0.037(0.019)V_{ortho} - 6.692(6.629) \quad (1a)$$

$$n = 25 \quad r^2 = 0.776 \quad s = 0.231 \quad F = 24.31$$

$$r^2_{cv} = 0.698 \qquad \mathrm{sqr(PRESS/n)} = 0.246$$

$$\log 1/\mathrm{ED}_{50}(\mathrm{MES}) = 0.259(0.072)\log k' - 0.559(0.155)I_{Ph} + 0.0405(0.0087)V_{ortho} + 3.36(0.124) \quad (1b)$$

$$n = 25 \quad r^2 = 0.789 \quad s = 0.223 \quad F = 26.18$$

$$r^2_{cv} = 0.694 \qquad \mathrm{sqr(PRESS/n)} = 0.247$$

In these equations, the numbers in parentheses are the 95% confidence intervals associated with the coefficients, n is the number of flupirtine derivatives employed, r^2 is the squared correlation coefficient corresponding to the fraction of observed variance accounted for, s is the standard deviation, F is the F-test value, r^2_{cv} is the cross-validated (leave-one-out) correlation coefficient, and PRESS is the sum of squared prediction errors (leave-one-out). The positive coefficient for log k' suggests that activity improves with increasing lipophilicity, while the positive coefficient associated with V_{ortho} indicates that the presence of *ortho* substituents on the benzyl ring also contributes beneficially. Although the positive coefficient for the phenyl-C$-$N-pyridyl torsion angle, C$-$N$_{tors}$, does imply that increases in this torsion angle will provide corresponding improvements in biological activity, it must be remembered that NMR-determined torsion angles for the pseudotrans conformation were found to lie in a limited range such that an angle of 210° may represent a practical maximum. The analysis of possible nonlinear dependence of the biological activity (ED_{50}/MES) on log k' or C$-$N$_{tors}$ did not lead to an increase in explained variance. C$-$N$_{tors}$ may explain the physicochemical effect of the phenyl group which is indicated by I_{Ph}. Therefore C$-$N$_{tors}$ has been used in all following equations.

Changes in overall molecular lipophilicity, as represented by log k', explained a significant portion of the observed changes in the activities from the maximal electroshock test. These nonspecific influences are often attributed to membrane transport phenomena; however, one would anticipate a nonlinear relationship between lipophilicity and *in vivo* CNS activity if transport in fact plays a limiting role. Consequently, additional effects arising from interaction with the lipophilic environment surrounding a membrane-bound receptor may be significant here. Neuroleptics are assumed to interact with the sodium channel, a membrane-embedded receptor. Moreover, it has been demonstrated that amphiphilic substances such as the flupirtines often interact strongly with biological membranes or phospholipids derived from such membranes.[16-18] In a number of cases, partitioning into membranes, or into membrane models, has been shown to differ considerably from octanol$-$water partitioning.[18] Therefore, it was of interest to determine, more directly, the effects of changes in flupirtine structure on interaction with membrane components. One such method involves determination of ligand$-$phospholipid binding by following changes in the NMR spectrum half-height line width for a specific ligand proton,[16-18] this being related to transverse relaxation rate, $1/T_2$. For the flupirtine analogs, this was carried out in the presence of a suspension of lecithin vesicles as the membrane model with measurement of the changes in the benzylamine methylene proton half-height line width over a range in phospholipid concentration. When conducted using an excess of ligand, the changes in peak line width with changes in phospholipid concentration can easily be followed and remain linear over a suitable concentration range such that the slope of the resulting plot can be accurately computed as a reflection of the degree of interaction. These measurements were conducted for 21 of the 25 analogs in Table 2. The logarithm of the slopes computed from plots of phospholipid concentration versus methylene half-height bandwidth was then examined as a potentially more relevant parameter to explain membrane interactions. Replacement of log k' with log $\Delta(1/T_2)$ in the correlation analysis yielded eq 2. Because of the low solubility of four derivatives, the NMR-derived parameters could only be measured for 21 molecules (see Table 2). Therefore eq 3, with log k' and the same 21 molecules, is given for direct comparison.

$$\log 1/\mathrm{ED}_{50}(\mathrm{MES}) = 0.629(0.224)\log \Delta(1/T_2) + 0.040(0.027)\mathrm{C{-}N_{tors}} + 0.035(0.014)V_{ortho} - 5.426(5.557) \quad (2)$$

$$n = 21 \quad r^2 = 0.885 \quad s = 0.174 \quad F = 43.796$$

$$r^2_{cv} = 0.825 \qquad \mathrm{sqr(PRESS/n)} = 0.193$$

$$\log 1/\mathrm{ED}_{50}(\mathrm{MES}) = 0.362(0.165)\log k' + 0.054(0.033)\mathrm{C{-}N_{tors}} + 0.028(0.019)V_{ortho} - 8.023(7.003) \quad (3)$$

$$n = 21 \quad r^2 = 0.844 \quad s = 0.203 \quad F = 30.652$$

$$r^2_{cv} = 0.781 \qquad sqr(PRESS/n) = 0.216$$

The enhancement in correlation through the use of a directly measured phospholipid-binding parameter, log $\Delta(1/T_2)$, implies that membrane interaction may indeed play a special role, possibly at the receptor site, in the observed anticonvulsant activity of the flupirtine analogs. It is evident that at least some components of this interaction are relatively specific and cannot be accounted for by partitioning processes as modeled by parameters such as the HPLC-derived log k'. It has been shown, however, that if one employs an HPLC column coated with phosphatidylserine or phosphatidylcholine, the log k' values very closely parallel the ligand–vesicle binding determined by NMR.[18] It is also worth noting that one can replace $C-N_{tors}$ in eq 2 with the van der Waals volume for the pyridylurethane substituent, V_{ure}, to give eq 4a. Thus, the role of $C-N_{tors}$ is possibly to reflect subtle but important conformational effects exerted by changes in the urethane moiety.

$$\log 1/ED_{50}(MES) = 0.696(0.259) \log \Delta(1/T_2) - 0.006(0.004)V_{ure} + 0.034(0.016)V_{ortho} + 3.453(0.595) \quad (4a)$$

$$n = 21 \quad r^2 = 0.869 \quad s = 0.185 \quad F = 37.841$$

$$r^2_{cv} = 0.809 \qquad sqr(PRESS/n) = 0.202$$

In contrast to the correlation with $C-N_{tors}$ (eq 2), a further slight improvement in the statistical significance is obtained if a parabolic dependence on the van der Waals volume for the urethane substituent is assumed.

$$\log 1/ED_{50}(MES) = 0.644(0.237) \log \Delta(1/T_2) + 0.0686(0.068)V_{ure} - 0.000272(0.000248){V_{ure}}^2 + 0.034(0.014)V_{ortho} - 1.414(4.478) \quad (4b)$$

$$n = 21 \quad r^2 = 0.902 \quad s = 0.165 \quad F = 37.05$$

$$r^2_{cv} = 0.824 \qquad sqr(PRESS/n) = 0.194$$

The degree of interaction of the derivatives with artificial membranes ($1/T_2$) is explaining a large fraction of the observed variance in biological activity (MES-test). Therefore this simple test system might offer a suitable screening system for selecting compounds for further animal tests.

In an effort to delineate requirements for selectivity, correlation analyses were similarly conducted using the neurotoxicity data, log $1/I_{50}$(NT), in Table 2. Remarkably, the same physicochemical features appeared to control toxicity, resulting in the derivation of eq 5 using log k' as a lipophilic parameter and eq 6 as the comparable relationship using log $\Delta(1/T_2)$.

$$\log 1/I_{50}(NT) = 0.511(0.191) \log k' + 0.081(0.038)C-N_{tors} + 0.042(0.022)V_{ortho} - 14.186(8.084) \quad (5)$$

$$n = 20 \quad r^2 = 0.899 \quad s = 0.233 \quad F = 47.770$$

$$r^2_{cv} = 0.868 \qquad sqr(PRESS/n) = 0.239$$

$$\log 1/I_{50}(NT) = 0.772(0.370) \log \Delta(1/T_2) + 0.057(0.042)C-N_{tors} + 0.055(0.023)V_{ortho} - 9.778(8.788) \quad (6)$$

$$n = 20 \quad r^2 = 0.863 \quad s = 0.271 \quad F = 33.865$$

$$r^2_{cv} = 0.820 \qquad sqr(PRESS/n) = 0.279$$

$$\log 1/I_{50}(NT) = 0.390(0.151) \log k' + 0.061(0.028)C-N_{tors} + 0.051(0.018)V_{ortho} - 9.982(6.014) \quad (7)$$

$$n = 23 \quad r^2 = 0.882 \quad s = 0.233 \quad F = 47.411$$

$$r^2_{cv} = 0.825 \qquad sqr(PRESS/n) = 0.282$$

Unlike the correlations of the maximal electroshock data, log k' is clearly a superior measure of the type of lipophilic interaction involved in neurotoxicity. Thus the more nonspecific partitioning parameter provides a better explanation of the processes involved in this toxic action of the flupirtine analogs. Equation 7 gives the correlation for all 23 derivatives for which neurotoxicity is available. Especially those derivatives bearing polar substituents at the aromatic ring system show different partitioning behavior in phospholipid buffer and octanol buffer systems as compared to derivatives bearing only nonpolar substituents.

Finally, as an indication of parameter covariance, pairwise correlations, r^2, are given in Table 3. The 20 analogs for which all parameters and all biological activities are available were used to compute these correlations. As expected, the covariance between the two biological activities is quite high, 90%. Similarly, the two lipophilic parameters are significantly intercorrelated at 85%, although they are clearly far from identical. The pairwise correlations between the biological activities and the physicochemical parameters show that V_{ortho} is highly correlated both with anticonvulsant activity and with neurotoxicity while lipophilicity is the second highest. Of greatest concern in multiple regression analysis is parameter covariance. Table 3 shows that intercorrelation between parameters used in the development of eqs 1–3 and 5–7 is not significant, with none greater than 32%.

In summary, it is evident that very few significant differences in anticonvulsant activity as compared to neurotoxicity exist for the flupirtine derivatives examined in this study. Nonetheless, anticonvulsant activity does seem to be governed by more specific membrane interactions, while neurotoxicity appears to be more sensitive to overall lipophilicity. These investigations do serve to further emphasize the importance of experimentally determining physicochemical characteristics of potential relevance to biological response. Moreover, differences observed in partitioning behavior as measured by HPLC (octanol-coated column) versus membrane binding have once more been demonstrated.

The derived equations may enable a rational optimization of activity, whereas the optimization of selectivity remains an unsolved problem. Generally QSAR equations with sufficient predictive power may be used to

Table 3. Covariance Matrix for Eqs 2, 3, 5, and 6

	log $1/I_{50}$(NT)	log $1/ED_{50}$(MES)	log k'	log $\Delta(1/T_2)$	V_{ortho}	C−N$_{tors}$
log $1/I_{50}$(NT)	1.00					
log $1/ED_{50}$(MES)	0.95	1.00				
log k'	0.71	0.70	1.00			
log $\Delta(1/T_2)$	0.67	0.74	0.88	1.00		
V_{ortho}	0.82	0.79	0.52	0.43	1.00	
C−N$_{tors}$	0.36	0.33	−0.23	−0.10	0.22	1.00

avoid the synthesis of inactive compounds and therefore assist in reducing the number of animal tests.

Experimental Section

Synthesis. General Procedure for the Synthesis of 3-Monoacylated 2,3,6-Triaminopyridines (Compounds 1−24, Table 1). A suspension of 0.1 mol of 2-amino-3-nitro-6-[(arylmethyl)amino]pyridine **III** and 5 g of Raney nickel catalyst in 280 mL of dioxane was hydrogenated at 5 bar and 50 °C. The suspension was cooled to 20 °C and the catalyst removed. To the solution was added 0.22 mol of acylating agent (acylating agents: **1−16**, ethyl chloroformate; **17−19**, phenyl chloroformate; **20**, 2-allyl chloroformate; **21**, 2-chloroethyl chloroformate; **22**, methyl chloroformate; **23**, acetyl chloride; **24**, 3,5-dimethylisoxazolyl-4-carbonyl chloride). Stirring was continued for 1 h, the dioxane solution was evaporated under reduced pressure to half of the volume, and the concentrated solution was kept at 4 °C: colorless crystals. Yield: 70−85%. Recrystallization from isopropyl alcohol.

General Procedure for 3-[2-Amino-6-[[(4-fluorophenyl)methyl]amino]-3-pyridinyl]-2-oxazolidinone Hydrochloride (Compound 25, Table 1). A suspension of 0.1 mol of 2-bromoethyl *N*-[2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl]carbamate hydrochloride (**V**) in 250 mL of methanol was treated with excess NaOH at 35 °C for 3 h. The crystalline product was isolated and washed with methanol followed by treatment in an acetonic solution of **25**−base with isopropanolic HCl to give the desired compound **25**.

MES−Maximal Electroshock Seizure Test.[12] Maximal electroshock seizures were elicited with a 60 cycle alternating current of 50 mA intensity (5−7 times that necessary to elicit minimal electroshock seizures) delivered for 0.2 s *via* corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extension component of the seizure is defined as protection, and results are expressed as number of animals protected/number of animals tested.

NT−Neurotoxicity. The Rotorod test was used to evaluate neurotoxicity.[19] The animal was placed on a 1 in. diameter knurled plastic rod rotating at 6 rpm. Normal mice can remain on a rod rotating at this speed indefinitely. Neurologic toxicity is defined as the failure of the animal to remain on the rod for 1 min and is expressed as number of animals exhibiting toxicity/number of animals tested.

Determination of Median Effective (ED_{50}) or Toxic Dose (I_{50}). All quantitative studies were conducted at the previously determined time of peak effect. Groups of at least eight mice (male Carworth Farms #1) were tested with various doses of the candidate drug until at least two points were established between the limits of 100% protection or minimal motor impairment (toxicity) and 0% protection or toxicity. The dose of drug required to produce the desired endpoint in 50% of the animals in each test, the 95% confidence interval, the slope of the regression line, and the SE of the slope were then calculated by a computer program based on the method described by Finney.[20] The lower value of the 95% confidence interval is on average about 15.7% (of ED_{50}) below the ED_{50} value and the upper about 21.8% above the ED_{50}.

Measurement of log k'. Capacity factors in the form of log k' were determined in a manner similar to previous studies[17] using a Waters HPLC instrument fitted with a Beckman 160 analytical UV detector and an octanol-coated ODS column (4 cm). The mobile phase consisted of octanol-saturated physiological PBS buffer, pH 7.4, containing 10 mM DMOA.

NMR−Binding Measurements. Stock solutions of lecithin (type X-E lecithin, Sigma catalog No. P-5394) were prepared by sonication as previously described.[17,21] All NMR spectra were recorded on a Bruker AM 360 L NMR spectrometer in deuterated 0.03 M phosphate buffer containing 10% DMSO-d_6, pH 4.9, at 22 °C with acetonitrile as internal standard. Relaxation rates, $1/T_2$, were calculated using the equation: $1/T_2$ obs $= \pi\Delta\nu_{1/2}$ where $\Delta\nu_{1/2}$ is the line width (Hz) of the proton signal (benzylic methylene group) at one-half maximum peak height. Line broadening was determined for a constant concentration of 4×10^{-3} M flupirtine analog in the presence of at least five different lecithin concentrations selected from the range affording a final concentration of 0.0−0.3 mg/500 μL. Line broadening versus lecithin concentration was then plotted and the slope calculated as a measure of the binding interaction.

Molecular Mechanics Minimization. All molecules were minimized using a modified MM2 force field, MMX87, within the microcomputer program PCMODEL.[14] The default parameters and convergence criteria were employed. Molecules, with a positive charge on the pyridyl nitrogen, were minimized once to establish standard bond lengths and angles. All were then placed in the same starting conformation and reminimized to obtain the desired torsion angle.

The extended conformation has been used because of the assumed interaction of the derivatives with phospholipids. In such an interaction, the extended conformation seems to be more likely.

The Conolly volumes, V_{urc}, have been calculated for the minimized structures using the GEPOL program from QCPE.

The *ortho* substituent volumes, V_{ortho}, have been calculated by addition of Bondi fragment volumes.[22]

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