8f, 109334-67-0; PhC(Cl)=NNHPh, 15424-14-3; m-PhC(Cl)=NNHC$_6$H$_4$Cl, 50802-12-5; m-PhC(Cl)=NNHC$_6$H$_4$Br, 109334-48-7; (E)-o-NO$_2$C$_6$H$_4$CH=CHCO$_2$Me, 39228-29-0; PhNHNH$_2$·HCl, 59-88-1; m-ClC$_6$H$_4$NHNH$_2$·HCl, 2312-23-4; m-BrC$_6$H$_4$NHNH$_2$·HCl, 27246-81-7; m-MeC$_6$H$_4$NHNH$_2$·HCl, 637-04-7; p-ClC$_6$H$_4$NHNH$_2$·HCl, 1073-70-7; p-MeC$_6$H$_4$NHNH$_2$·HCl, 637-60-5; Et$_3$MeN$^+$OH$^-$, 109334-81-8; methyl 1,3-diphenyl-5-(2-nitrophenyl)-4-pyrazolinecarboxylate, 109334-49-8; methyl 1-(3-chlorophenyl)-5-(2-nitrophenyl)-3-phenyl-4-pyrazolinecarboxylate, 109334-50-1; methyl 1-(3-bromophenyl)-5-(2-nitrophenyl)-3-phenyl-4-pyrazolinecarboxylate, 109334-51-2; methyl 1,3-diphenyl-4-(2-nitrophenyl)-5-pyrazolinecarboxylate, 109334-52-3; methyl 1-(3-chlorophenyl)-4-(2-nitrophenyl)-3-phenyl-5-pyrazolinecarboxylate, 109334-53-4; methyl 1-(3-bromophenyl)-4-(2-nitrophenyl)-3-phenyl-5-pyrazolinecarboxylate, 109334-54-5.

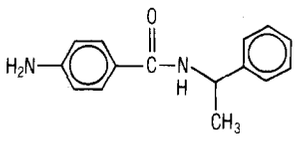
Discovery and Anticonvulsant Activity of the Potent Metabolic Inhibitor 4-Amino-*N*-(2,6-dimethylphenyl)-3,5-dimethylbenzamide[1]

David W. Robertson,* J. David Leander, Ron Lawson, E. E. Beedle, C. Randall Clark,† Brian D. Potts, and C. John Parli

Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, and School of Pharmacy, Auburn University, Auburn University, Alabama 36849. *Received March 27, 1987*

Compound **2** [4-amino-*N*-(2,6-dimethylphenyl)benzamide] is an effective anticonvulsant in several animal models. For example, following oral administration to mice, it antagonized maximal electroshock (MES) induced seizures with an ED_{50} of 1.7 mg/kg. During drug disposition studies with **2**, we found that it was rapidly metabolized by N-acetylation. Thirty minutes after oral administration of 1.7 mg/kg of **2** to mice, plasma concentrations of parent drug and the *N*-acetyl metabolite **5** were 1.09 and 0.41 μg/mL, respectively. Six hours postadministration the concentrations were 0.23 and 0.22 μg/mL, respectively. In order to sterically preclude or diminish the rate of metabolic N-acetylation, we synthesized analogues of **2** possessing either one (**3**) or two (**4**) methyl groups ortho to the 4-amino substituent. Both compounds antagonized MES-induced seizures after administration to mice; oral ED_{50} values for **3** and **4** were 3.5 and 5.6 mg/kg, respectively. Compound **3** was rapidly metabolized by N-acetylation. However, **4** provided exceptionally high and long-lived plasma concentrations of parent drug; no *N*-acetyl metabolite could be detected. While **2** and **3** had no pharmacologically relevant effects on hexobarbital-induced sleeping time in mice, **4** was a potent, dose-dependent potentiator of sleeping time. Oral administration of 375 μg/kg led to a 61% increase in sleeping time relative to control values. Thus, **4** represents one of the most potent potentiators of hexobarbital-induced sleeping time described to date.

The 4-aminobenzamides represent a fertile series for the discovery of anticonvulsants. Compound **1** [4-amino-*N*-(α-methylbenzyl)benzamide] inhibited maximal electroshock (MES) induced seizures in mice, and the ED_{50} following oral administration was 10.3 mg/kg.[2] Compound



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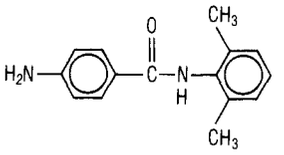
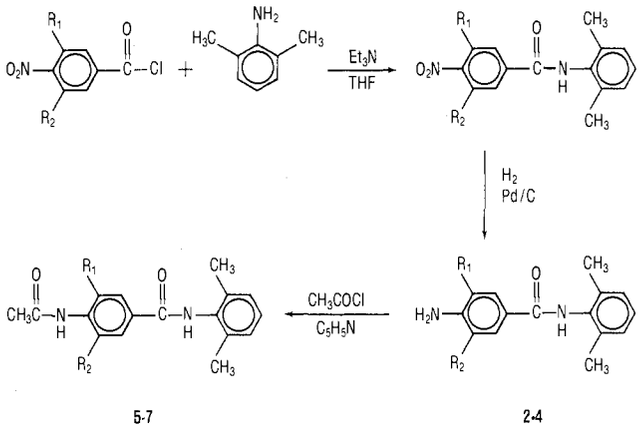


2

1 was resolved, and the racemate and its two enantiomers were evaluated in a variety of anticonvulsant assays. Surprisingly, both enantiomers were less active than the racemate in the horizontal screen test for ataxia; the ED_{50} values were 140, 640, and 94 for the *S* enantiomer, the *R* enantiomer, and the racemate, respectively.[3]

Compound **2** [4-amino-*N*-(2,6-dimethylphenyl)benzamide] was more potent than **1** against MES-induced seizures in mice (oral ED_{50} values = 1.7 and 10.3 mg/kg, respectively).[4,5] In contrast to **1**, compound **2** was unable to antagonize any of the standard chemically induced seizures. Thus, in these animals models, **2** displayed a phenytoin-like profile. On the basis of extensive pharmacological studies, **2** (LY201116)[6] is being developed for treatment of generalized tonic–clonic and partial seizures.

Scheme I



5-7 **2-4**

During metabolism studies involving **2**, we found that this compound was metabolically inactivated by N-

* Address correspondence to: David W. Robertson, Chemical Research Division, MC705, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

† Auburn University.

(1) Portions of this work have been presented previously: Robertson, D. W.; Parli, C. J.; Beedle, E. E.; Leander, J. D.; Clark, C. R. *Abstracts of Papers*, 193rd National Meeting of the American Chemical Society, Denver, CO; American Chemical Society: Washington, DC, 1987. Robertson, D. W.; Parli, C. J.; Beedle, E. E.; Leander, J. D. 10th International Congress of Pharmacology, Sydney, Australia, August 1987.

(2) For the SAR and synthesis of **1**, see: Clark, C. R.; Wells, M. J. M.; Sansom, R. T.; Norris, G. N.; Dockens, R. C.; Ravis, W. R. *J. Med. Chem.* **1984**, *27*, 779.

(3) For preliminary aspects of the pharmacology of the two enantiomers of **1**, see: Robertson, D. W.; Beedle, E. E.; Leander, J. D.; Rathbun, R. C. *Pharmacologist* **1985**, *27*, 231.

(4) For the SAR and synthesis of **2**, see: Clark, C. R.; Sansom, R. T.; Lin, C.-M.; Norris, G. N. *J. Med. Chem.* **1985**, *28*, 1259.

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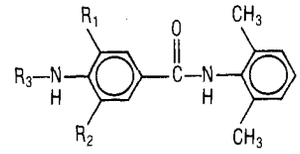
Table I. Structures and Physical Properties of Benzamide Anticonvulsants



no.	R_1	R_2	R_3	recrystn solvent	mp, °C	formula	anal.
3	CH_3	H	H	CH_3OH/H_2O	269–270	$C_{16}H_{18}N_2O$	C, H, N
4	CH_3	CH_3	H	CH_3OH/H_2O	167–169	$C_{17}H_{20}N_2O$	C, H, N
5	H	H	$COCH_3$	DMF/H_2O	293–295	$C_{17}H_{18}N_2O_2$	C, H, N
6	CH_3	H	$COCH_3$	DMF/H_2O	283–285	$C_{18}H_{20}N_2O_2$	C, H, N
7	CH_3	CH_3	$COCH_3$	DMF/H_2O	283–284	$C_{19}H_{22}N_2O_2$	C, H, N

Table II. Biological Activities of Benzamide Anticonvulsants after Oral Administration to Mice

compd	TPE,[a] h	MES[b] ED_{50}, mg/kg	HS[c] ED_{50}, mg/kg	PI[d]
2	0.5	1.7 (1.46–1.93)[e]	23 (20.6–25.7)	13
3	1.0	3.5 (2.79–4.41)	84 (64.2–109.8)	24
4	1.0	5.6 (4.64–6.76)	64 (55.0–74.5)	11
5	2.0	3.8 (3.11–4.59)	NA[f] [300]	–
6	1.0	4.5 (3.57–5.66)	56 (42.5–73.7)	12
7	2.0	170 (139–207)	NA [160]	–
phenytoin	1.0	9.4 (8.5–10.4)	210 (160–275)	22

[a] TPE = time to peak anticonvulsant effect. [b] MES = maximal electroshock assay. [c] HS = horizontal screen test. [d] PI = protective index (HS ED_{50}/MES ED_{50}). [e] Values in parentheses represent 95% confidence limits. [f] NA = not active at the highest dose tested as indicated in brackets.

acetylation. This paper details efforts to preclude metabolic N-acetylation by incorporating either one or two methyl substituents ortho to the 4-amino moiety. This led to the synthesis of **4** [4-amino-*N*-(2,6-dimethylphenyl)-3,5-dimethylbenzamide, LY201409], a potent anticonvulsant and metabolic inhibitor.

Results and Discussion

Chemistry. The compounds employed in these studies were prepared by well-known and straightforward synthetic methods (Scheme I).[2,4] Reaction of the appropriate 4-nitrobenzoyl chlorides with 2,6-dimethylaniline in the presence of triethylamine led to formation of the 4-nitrobenzanilides. Catalytic reduction of the 4-nitro group furnished the target anticonvulsants **2-4** (Table I). Finally, N-acetylation using acetyl chloride in the presence of pyridine provided compounds **5-7**.

Metabolism and Anticonvulsant Activity. Compound **2** effectively antagonized MES-induced seizures. In this series of experiments, the MES ED_{50} was 1.7 mg/kg following oral administration to mice (Table II). Moreover, the effect of the compound on the central nervous system (CNS) was fairly selective; the ED_{50} in the horizontal screen test, an indication of the compound's propensity to produce ataxia or neurological deficit, was 23 mg/kg, affording a protective index of 13 (Table II). Phenytoin was included as a reference drug, and the MES and horizontal screen test ED_{50} values were 9.4 and 210 mg/kg, respectively.

During drug disposition studies with **2**, we found that it was rapidly metabolized by N-acetylation, and results from a typical experiment are displayed in Figure 1 and

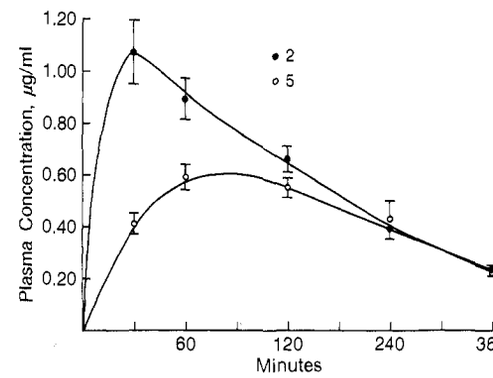


Figure 1. Compound **2** (1.7 mg/kg) was administered orally to three groups of two mice, and at the indicated times, plasma samples were collected and analyzed by HPLC as described in the Experimental Section. Filled circles refer to the parent compound, whereas unfilled circles refer to the *N*-acetyl metabolite. Each point is the mean ± SEM of experimental values.

Table III. After oral administration of 1.7 mg/kg to mice, plasma concentrations of parent drug reached a maximum of 1.09 μg/mL at 30 min and diminished slowly to 0.23 μg/mL 6 h postadministration. The major metabolite of **2**, the *N*-acetyl analogue **5**, reached its maximum plasma concentration of 0.59 μg/mL 60 min postadministration, and concentrations diminished slowly and parallel to the decay curve for **2** during the course of the experiment. Qualitatively similar results were obtained in rats and monkeys (data not shown). This rapid N-acetylation was not unanticipated on the basis of studies involving other compounds possessing a 4-aminobenzamide substructure. For example, significant quantities of the antiarrhythmic drug procainamide [4-amino-*N*-[2-(diethylamino)ethyl]-benzamide] are metabolized and excreted as *N*-acetylprocainamide (NAPA).[7-9]

The *N*-acetyl metabolite **5**, after oral administration to mice, proved to be a potent anticonvulsant and displayed an ED_{50} of 3.8 mg/kg (Table II). However, oral bioavailability appeared to be limited at higher doses since no effects in the horizontal screen test were detected after administration of 300 mg/kg. Similar dose-dependent decreases in oral bioavailability have been demonstrated for other anticonvulsant drugs, including phenytoin,[10] and such a decrease was not unexpected in view of the limited solubility of **5**. Although oral administration of **5** indicated

(5) For preliminary aspects of the pharmacology of **2**, see: Robertson, D. W.; Leander, J. D.; Rathbun, R. C.; Clark, C. R. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 676.
(6) Compound **2** was extensively evaluated by the Epilepsy Branch of the NINCDS; their code number for this substance is ADD 75073.
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(8) Woosley, R. L.; Drayer, D. E.; Reidenberg, M. M.; Niles, A. S.; Carr, K.; Oates, J. A. *N. Engl. J. Med.* **1978**, *298*, 1157.
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Table III. Plasma Concentrations of Benzamide Anticonvulsants and Their *N*-Acetyl Metabolites following Oral Administration to Mice[a]

	drug and metabolite concentrations, ng/mL					
	2		3		4	
time, min	2	5	3	6	4	7
30	1088 ± 146	412 ± 38	ND[b]	79 ± 3.9	1477 ± 82	ND
60	893 ± 81	587 ± 50	ND	97 ± 8.2	1160 ± 29	ND
120	656 ± 51	545 ± 45	ND	91 ± 8.0	1060 ± 34	ND
240	394 ± 46	425 ± 81				
360	299 ± 12	225 ± 14				

[a] Each drug was orally administered to three groups of two mice at its previously determined ED_{50} value: **2**, 1.7 mg/kg; **3**, 3.5 mg/kg; and **4**, 5.6 mg/kg. Plasma concentrations of parent benzamides **2–4** and *N*-acetyl metabolites **5–7** were determined at the indicated time points by HPLC as described in the Experimental Section. The concentrations represent the mean ± SEM of experimental values. [b] ND = not detected at the limit of assay sensitivity (25 ng/mL).

that the substance was a potent anticonvulsant, iv time-course studies in mice revealed that it does not possess intrinsic anticonvulsant activity, but is metabolically hydrolyzed to sufficient quantities of **2** to produce the observed anticonvulsant effects.[11]

In order to sterically preclude or diminish the rate of metabolic N-acetylation and inactivation, we synthesized analogues of **2** possessing either one (compound **3**) or two (compound **4**) methyl substituents ortho to the 4-amino group. Both compounds were potent antagonists of MES-induced seizures. ED_{50} values of **3** and **4** were 3.5 and 5.6 mg/kg, respectively, following oral administration to mice (Table II). Whereas **4** had a protective index comparable to that of **2** (11 and 13, respectively), the protective index of **3** was considerably greater (24) because of the compound's relative impotency in the horizontal screen test (ED_{50} = 84 mg/kg, Table II).

The metabolic profiles of both methylated analogues were probed. Absolute bioavailability of **3** appeared to be low (Table III): after oral administration of 3.5 mg/kg to mice, plasma concentrations of parent drug could not be detected 30, 60, or 120 min postadministration (detection limit = 25 ng/mL). There was, however, rapid appearance and maintenance of *N*-acetyl metabolite (**6**) plasma concentrations, which achieved 97 ng/mL 1 h postadministration. The metabolic profile of **4** differed significantly (Table III). After oral administration of 5.6 mg/kg to mice, plasma concentrations of parent drug rose rapidly and reached their maximum 30 min postadministration. Peak plasma concentrations of 1.48 μg/mL were greater than observed with **2** or **3**. Moreover, plasma concentrations of parent drug had diminished to only 1.06 μg/mL at the 2-h measurement. Thus, both peak plasma concentrations and apparent half-life of parent drug were increased when two flanking methyl groups were used to sterically encumber the 4-amino substituent; the potential *N*-acetyl metabolite **7** was not detected.

Compound **6**, the *N*-acetyl metabolite of **3**, was an effective anticonvulsant in mice, with an oral ED_{50} of 4.5 mg/kg (Table II), probably by virtue of its ability to be converted metabolically to **3**.[11] However, **7**, the *N*-acetyl analogue of **4**, was impotent in the MES assay, with an oral ED_{50} of 170 mg/kg (Table II). Thus, **7** is 45- and 38-fold less potent than **5** and **6**, respectively, which probably reflects the inability of plasma amidases to efficiently hydrolyze **7** to **4**.

Effects on Hexobarbital-Induced Sleeping Time. Drug interactions are of paramount importance in the pharmacological management of epilepsy since several antiepileptic drugs are often administered concomitantly. Therapeutically important, well-established interactions include the ability of valproic acid to increase serum concentrations of phenobarbital by 50–100%, leading to intoxication with the latter compound.[12] Addition of phenytoin to a carbamazepine regimen causes the plasma concentrations of the latter drug to fall as much as 50%.[13] Recently, several imidazole-based anticonvulsants, including denzimol, nafimidone, and LY177165, were shown to cause profound increases in hexobarbital-induced sleeping time by virtue of their ability to inhibit cytochrome P-450 mediated metabolism of the barbiturate.[14,15] Clinical studies with denzimol[16,17] and nafimidone[18] have revealed that this drug interaction in animals is reflected in an impaired metabolism of phenytoin and carbamazepine in humans, leading to toxicities associated with ele-

Table IV. Effects of **2** on Hexobarbital-Induced Sleeping Time in Mice[a]

dose, mg/kg	sleeping time ± SEM, min
0	59.6 ± 9.58
1.6	67.9 ± 5.49 (14)[b]
3.2	69.3 ± 5.81 (16)
6.4	75.0 ± 4.14 (26)
12.8	76.4 ± 3.84 (28)

[a] A hypnotic dose of hexobarbital (100 mg/kg, ip) was administered 0.5 h following the oral doses of **2**, and reported sleeping time values are the mean ± SEM for 10 animals. [b] Values in parentheses are percent increases relative to hexobarbital control.

Table V. Effects of **3** on Hexobarbital-Induced Sleeping Time in Mice[a]

dose, mg/kg	sleeping time ± SEM, min
0	66.4 ± 7.02
3.8	85.6 ± 12.54 (29)[b]
7.6	97.9 ± 13.69 (47)
15.2	85.5 ± 2.92*[c] (29)
30.4	133.8 ± 24.83* (102)

[a] A hypnotic dose of hexobarbital (100 mg/kg, ip) was administered 1.0 h following the oral doses of **3**, and reported sleeping time values are the mean ± SEM for 10 animals. [b] Values in parentheses are percent increases relative to hexobarbital control. [c] (*) $p < 0.05$.

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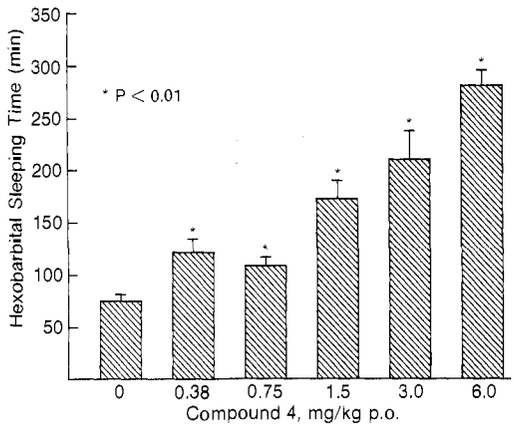

Figure 2. Dose-dependent effects of **4** on hexobarbital-induced sleeping time in mice. A hypnotic dose of hexobarbital (100 mg/kg, ip) was administered 1 h following oral administration of the indicated doses of **4**. The duration of loss of righting reflex was monitored. Values are the mean ± SEM for 10 animals. In this experiment control animals slept for 75 ± 5.8 min.

vated serum concentrations of the latter compounds. To determine if the 4-aminobenzamide anticonvulsants employed in this study would share this liability, we studied their ability to potentiate hexobarbital-induced sleeping time, and the results are displayed in Tables IV and V and Figure 2.

Compound **2** failed to produce a pharmacologically relevant increase in hexobarbital-induced sleeping time. A dose of 12.8 mg/kg, 8-fold greater than the anticonvulsant ED_{50}, did not result in a statistically significant interaction (Table IV). Compound **3** increased sleeping time (Table V), and the effect reached statistical significance at the 15.2 and 30.4 mg/kg doses (4 and 8 times the anticonvulsant ED_{50}). However, the magnitude of the effect was considerably less than that observed with imidazole anticonvulsants[14,15] and is comparable to that produced by phenytoin.[11]

Compound **4** was a potentiator of hexobarbital-induced sleeping time. At the approximate anticonvulsant ED_{50} of 6.0 mg/kg, a 272% increase in sleeping time was produced (Figure 2). The effect was dose-dependent and highly significant ($p < 0.01$) even at the lowest dose tested (375 μg/kg). Thus, **4** represents one of the most potent potentiators of hexobarbital-induced sleeping time described to date.

One possible explanation for the increased potency of **4** relative to **2** or **3** is its inability to undergo metabolic N-acetylation. As previously discussed, both **2** and **3** are rapidly N-acetylated, thereby precluding prolonged interaction of the 4-amino substituent with the heme iron atom of cytochrome P-450. Since **4** affords considerably higher and more long-lived plasma concentrations than either **2** or **3**, this pharmacokinetic phenomenon may explain the large potency differences among these compounds in the sleeping-time assay. However, electronic effects cannot be excluded. The two ortho methyl substituents would be expected to increase the basicity of the 4-amino substituent of **4**, thereby enhancing its ability to coordinate to the fifth or sixth ligand position of the heme iron.

Conclusions. In this work we have demonstrated that **2** is rapidly metabolized by N-acetylation. This metabolic reaction appears to be reversible, although the equilibrium of the reaction is in favor of **5** in most species.[11] Thus the metabolite **5** may serve as a long-lived reservoir of the active anticonvulsant **2**. Attempts to preclude or decrease the rate of metabolism led to synthesis of **3** and **4**, compounds that possess either one or two methyl substituents flanking the 4-amino group. Both these compounds were potent anticonvulsants in the MES assay. Whereas **3** underwent rapid metabolic N-acetylation, **4** provided exceedingly high and long-lived plasma concentrations of parent drug following oral administration to mice. Although **4** is an effective anticonvulsant with a good pharmacological profile, its ability to potentiate hexobarbital-induced sleeping time in mice diminishes its attractiveness as a drug candidate. In future papers we will further delineate the pharmacology and metabolism of **4** and **2**, the cytochrome P-450 interactions of **4**, and the SAR of benzamide anticonvulsants.

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories; only symbols of elements analyzed are given, and they were within 0.4% of theoretical values unless indicated otherwise.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation". Compound **2** was prepared as previously described,[4] as was the intermediate, 3,5-dimethyl-4-nitrobenzoic acid.[19] The synthetic methods employed in this study are illustrated by the following examples.

3-Methyl-4-nitro-*N*-(2,6-dimethylphenyl)benzamide. A mixture of 3-methyl-4-nitrobenzoic acid (31.9 g, 176 mmol) and thionyl chloride (250 mL) was refluxed for 3 h. The reaction mixture was cooled, and excess thionyl chloride was removed in vacuo. Dry benzene was added and removed in vacuo thrice. A solution of this acid chloride in ca. 200 mL of THF was added dropwise to a solution of 2,6-dimethylaniline (21.3 g, 176 mmol) and triethylamine (25 mL, 176 mmol) in 400 mL of THF at room temperature. The reaction mixture was stirred at room temperature overnight and cooled to 0 °C, and the triethylamine hydrochloride was removed by filtration. Evaporation of the THF, product isolation (chloroform, 1 N hydrochloric acid, 1 N sodium hydroxide, water, brine, Na_2SO_4), and recrystallization from methanol afforded 35.7 g (71%) of product: mp 177–178 °C. Anal. ($C_{16}H_{16}N_2O_3$) C, H, N.

4-Amino-3-methyl-*N*-(2,6-dimethylphenyl)benzamide (3). A mixture of 3-methyl-4-nitro-*N*-(2,6-dimethylphenyl)benzamide (5 g, 17.6 mmol) and 0.5 g of 5% Pd/C in 97 mL of absolute ethanol was stirred under 60 psi of hydrogen until the theoretical amount had been consumed. The reaction mixture was filtered through Celite. Removal of solvent under reduced pressure and recrystallization from methanol/water gave 3.13 g (70%) of **3** as white crystals: mp 269–270 °C. Anal. ($C_{16}H_{18}N_2O$) C, H, N.

4-(Acetylamino)-3-methyl-*N*-(2,6-dimethylphenyl)benzamide (6). Acetyl chloride (1.7 mL, 23.6 mmol) was added dropwise to a solution of pyridine (1.6 mL, 19.6 mmol) and **3** (5.0 g, 19.6 mmol) in ca. 100 mL of DMF at room temperature. The reaction mixture was stirred for 2 h at room temperature and for 2 h at 50 °C. The reaction mixture was diluted with water and cooled to 0 °C, and 5 g (86%) of **6** was collected by filtration: mp 283–285 °C. Anal. ($C_{18}H_{20}N_2O_2$) C, H, N.

Pharmacological Methods. Effects on Horizontal Screen (HS).[20] Groups of four male mice (Crl: $CF1^RBR$; Charles River; 20–25 g) were fasted for 90 min and were then administered a range of oral doses of each compound as a suspension in 5% acacia/water. Treated animals were placed individually on top of a square (13 cm × 13 cm) wire screen (no. 4 mesh) that was

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mounted on a metal rod. The rod was rotated 180°, and the number of mice that returned to the top of the screen within 1 min was determined. This measurement was performed 0.5, 1, 2, 3, and 4 h posttreatment to determine the approximate time of peak effect. Each compound was then retested at the estimate time of peak effect by using at least four doses with 12 mice at each dose. The HS ED_{50} was calculated according to the method of Litchfield and Wilcoxon.[21]

Inhibition of MES-Induced Seizures. Groups of four mice were fasted for 90 min and were then administered a range of oral doses of each compound as a suspension in 5% acacia/water. The time to peak effect (TPE) was determined by challenging the mice with MES 0.5, 1, 2, 3, and 4 h posttreatment. Electroshock (40 mA, 0.1 s, ac) was administered through corneal electrodes, and the mice were observed for clonic, tonic-flexor, and tonic-extensor convulsions. Each compound was then retested at the estimated time of peak effect by using at least four doses with 12 mice at each dose. The MES ED_{50} for the prevention of tonic-extensor convulsions was calculated according to the method of Litchfield and Wilcoxon.[21]

Effects on Hexobarbital-Induced Sleeping Time. Groups of 10 fasted mice were treated orally with various doses of each compound in 5% acacia/water. At the previously determined time of peak effect, hexobarbital (100 mg/kg, ip) was administered to the mice. The hexobarbital was solubilized with stoichiometric quantities of sodium hydroxide, and the volume of administration for all test compounds, acacia, and hexobarbital was 10 mL/kg. Sleeping time (the time of loss of righting reflex) was determined to the nearest minute for each mouse.

Benzamide Anticonvulsant Plasma Assay. A Model 5000 liquid chromatograph (Varian Associates) equipped with a Spectraflow 783 absorbance detector (Kratos Analytical) and a Dupont Instruments autosampler was used. This instrument was fitted with a 250 mm × 4.6 mm 5-μm C18 column (Alltech Associates). The detection output signal was interfaced to an HP 1000 laboratory chromatography system.

Standard curves were prepared by adding the compounds, dissolved in 10% methanol, to drug-free plasma and serially diluting with plasma to give final concentrations of 50–2000 ng/mL. These samples were assayed, and standard curves were constructed by plotting compound concentration vs. compound/internal standard peak-height ratio. The least-squares regression lines for all standard curves gave correlation coefficients of greater than 0.999.

Plasma samples were obtained from three groups of two mice dosed orally with each compound suspended in 5% acacia. The mice were anesthetized with methoxyfluorane, and blood was collected by cardiac puncture into heparinized syringes. The blood was promptly centrifuged and the plasma separated and stored at −30 °C until analysis.

An endcapped CN solid-phase extraction column was conditioned by passing 1 column volume of methanol through the column, followed by 1 column volume of distilled, deionized water. The plasma sample (200 μL) and 50 μL of a 10 μg/mL solution of the internal standard, 4-(acetylamino)-*N*-(α-methylbenzyl)-benzamide (**8**), were placed on the column to assay compounds **2** and **4**. To assay compound **3**, we used 50 μL of a 1 μg/mL solution of 4-amino-*N*-(2-methylphenyl)benzamide (**9**) as the internal standard. The remainder of the column was filled with deionized water, the mixture was pulled through the column, and the column was washed with 2 volumes of water. Compounds were eluted with 500 μL of 0.1 M ammonium acetate/methanol/acetonitrile/acetic acid/diethylamine (50:25:25:2:0.5). A 100-μL sample was injected into the liquid chromatograph. Isocratic elution was performed by using a mobile phase of 0.1 M ammonium acetate/methanol/acetonitrile/diethylamine (470:380:150:0.6) at a flow rate of 0.7 mL/min. Approximate retention times for **2**, **3**, **4**, **5**, **6**, **8**, and **9** were 540, 650, 850, 630, 680, 670, and 500 s, respectively.

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Registry No. **2**, 787-93-9; **3**, 109306-93-6; **4**, 109306-94-7; **5**, 794-98-9; **6**, 107634-10-6; **7**, 109306-95-8; 2,6-dimethylaniline, 87-62-7; 4-nitrobenzoyl chloride, 122-04-3; 3-methyl-4-nitrobenzoyl chloride, 35675-46-8; 3,5-dimethyl-4-nitrobenzoyl chloride, 3558-73-4; 4-nitro-*N*-(2,6-dimethylphenyl)benzamide, 64594-44-1; 4-nitro-3-methyl-*N*-(2,6-dimethylphenyl)benzamide, 109306-96-9; 4-nitro-3,5-dimethyl-*N*-(2,6-dimethylphenyl)benzamide, 109306-97-0; cytochrome P-450, 9035-51-2.

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Synthesis of Potential Anticancer Agents: Imidazo[4,5-*c*]pyridines and Imidazo[4,5-*b*]pyridines

Carroll Temple, Jr.,* Jerry D. Rose, Robert N. Comber, and Gregory A. Rener

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255-5305. *Received March 18, 1987*

The 1,2-dihydropyrido[3,4-*b*]pyrazines (**1**) are mitotic inhibitors with significant antitumor activity in mice. Also, the active imidazo[4,5-*b*]pyridine **3** was shown to cause the accumulation of cells at mitosis. Routes were developed for the synthesis of congeners of **3** by cyclization of 4-(substituted amino)-5,6-diaminopyridines with ethyl orthoformate. Oxidative cyclization of either 4,5- or 5,6-diaminopyridines with aryl aldehydes produced the [4,5-*c*] and [4,5-*b*] imidazopyridine ring systems, respectively. The latter reaction with 6-(substituted amino)-4,5-diaminopyridines gave imidazo[4,5-c]pyridine ring analogues of **1**. Biological studies indicated that the target compounds were less active than **1** and **3**.

The 1,2-dihydropyrido[3,4-*b*]pyrazines (e.g., **1**)[1,2] and 2*H*-pyrido[4,3-*b*][1,4]oxazines (e.g., **2**)[3] have shown significant antitumor activity in mice (see Scheme I). These compounds appear to interfere with cell division by binding to a cellular protein, tubulin, at a site that is different from that at which the clinically used antimitotic drug vincristine reacts.[4,5] Since the 1,2-dihydropyrido-[3,4-*b*]pyrazines are subject to air oxidation to the corresponding inactive heteroaromatic compounds,[2] metabolic

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