Synthesis and Anticonvulsant and Neurotoxic Properties of Substituted *N*-Phenyl Derivatives of the Phthalimide Pharmacophore

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A series of compounds including 4-amino (**1**), 3-amino (**2**), 4-nitro (**3**), 2-methyl-3-amino (**4**), 2-methyl-3-nitro (**5**), 2-methyl-4-amino (**6**), 2-methyl-4-nitro (**7**), 2-methyl-5-amino (**8**), 2-methyl-5-nitro (**9**), 2-methyl-6-amino (**10**), 2-methyl-6-nitro (**11**), 2,6-dimethyl (**12**), 2-methyl-3-carboxy (**13**), 2-methoxycarbonyl (**14**), 2-methyl-4-methoxy (**15**), 2,4-dimethoxy (**16**), 2-chloro-4-amino (**17**), and 2-chloro-4-nitro (**18**) *N*-phenyl substituents of phthalimide were evaluated along with *N*-[3-methyl-(2-pyridinyl)]phthalimide (**19**), *N*-(3-amino-2-methylphenyl)succinimide (**20**), and phenytoin for anticonvulsant and neurotoxic properties. Initial screening in the intraperitoneal (ip) maximal electroshock-induced seizure (MES) test and the subcutaneous pentylenetetrazol-induced seizure (scPtz) test in mice led to the selection of **1**, **2**, **4**, **10**, **12**, **17**, and **19** for oral MES evaluation in rats. The resultant ED_{50} values for **4**, **10**, **17**, and phenytoin were 8.0, 28.3, 5.7 and 29.8 mg/kg, respectively. In the batrachotoxin affinity assay, IC_{50} values for **17** and phenytoin were 0.15 and 0.93 μM, respectively, and in the recently validated magnesium deficiency-dependent audiogenic seizure test, ED_{50} values of 5.2 and 23 mg/kg were obtained for **17** and phenytoin, respectively. Electrophysiology studies on compound **17** point out its ability to (i) potentiate GABA-evoked current responses with a failure to directly activate the GABAA receptor and (ii) to affect, at 100 μM excitatory non NMDA, but not NMDA, receptors with a 25% block of kainate-evoked response. Electrophysiology measurements on voltage-gated sodium channels in N1E-115 neuroblastoma cells confirm voltage-dependent block of these channels by compound **17**. In view of its interaction with multiple ion channels, one would predict that compound **17** might be active in a wide range of seizure models.

Introduction

Using the 4-aminobenzoylamino template, Clark and co-workers demonstrated through a series of successive works the significant anticonvulsant potential in animal epilepsy models for the amino-substituted benzamides derived from alkyl-, arylalkyl-, and arylamines.[1−4] SAR studies showed that optimal antiepileptic activity was found in those amides having a primary amine in the 4-position of the benzamide moiety and an aromatic N-substitution of the 4-aminobenzamide pharmacophore. From this work emerged ameltolide which exhibits a phenytoin-like profile, i.e., it is quite potent in the maximal electroshock seizure (MES) test and is inactive in the subcutaneous pentylenetetrazol (scPTZ) test.[5] More recently, *N*-phenylphthalimide derivatives were shown to possess a similar degree of anticonvulsant potency also associated with a phenytoin-like profile, leading to the design of the phthalimide counterpart of ameltolide: the 4-amino-*N*-(2,6-dimethylphenyl) phthalimide.[6−9]

Our interest in developing phenytoin-like compounds relies with the need of alternative new drugs because of well-established side effects of phenytoin which include in humans neurologic signs (ataxia, nystagmus, sedation or irritability, oro-facial dyskinesia), along with hematologic (leucopenia), immunologic (reduction of IgA, lupus syndrome), endocrinologic (hirsutism), and cell growth (gingival hypertrophy) dysfunctions. 4-Amino-*N*-(2,6-dimethylphenyl)phthalimide was previously designed from the models of ameltolide and thalidomide (see ref 10 for the anticonvulsant properties of thalidomide) (Figure 1, design 1). In a recent work using the batrachotoxin affinity assay, it was noted that the 4-amino moiety played an important role in the molecular recognition process at the level of the receptor modulating the voltage-dependent sodium channel status.[11] This moiety was therefore preserved in most pharmacomodulations. The question could then be raised whether this functionality not present in thalidomide could be omitted in the phthaloyl moiety (Figure 1, design 2). In a previous series, one such compound was relatively active,[11] and in an effort to extend the SAR of *N*-phenylphthalimide possessing no substitution

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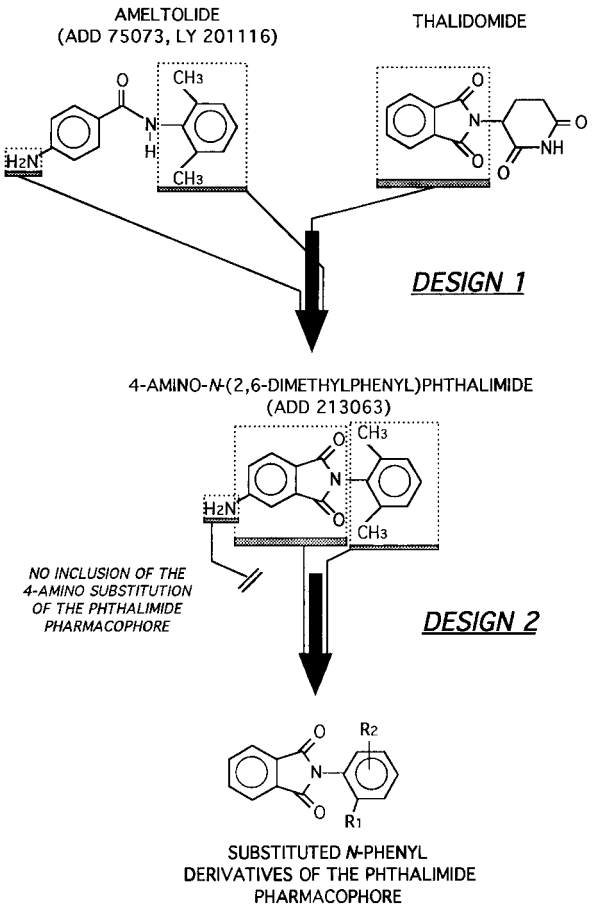

Figure 1. Anticonvulsant compounds designed from ameltolide and thalidomide. Design 1 has previously generated 4-amino-*N*-phenylphthalimide compounds. The prototype of this design is 4-amino-*N*-2,6-dimethylphthalimide and represents the phthalimide counterpart of the benzamide ameltolide; the former may be considered as a rigidified analogue of the latter. Design 2 is the basis of the present work and, by reference to thalidomide, deals with the omission of the 4-amino substituent of the phthalimide pharmacophore. Structures and chemistry of compounds synthesized according to this design are illustrated by Figure 2.

in the phthaloyl moiety, we undertook to study a series of 20 derivatives including methyl, amino, chloro, and nitro substituents of the *N*-phenyl moiety of *N*-phenylphthalimide pharmacophore (these substituents were those giving the most active compounds against animal seizure models [in MES testing] from an earlier series[2,4,5,8,9,11] on phenylbenzamides and phenylphthalimides). The compounds were evaluated for their antiepileptic and neurotoxic properties through the Antiepileptic Drug Development (ADD) Program developed by the National Institutes of Health.[12−15] These evaluations were completed by testing the anticonvulsant profile of the most potent compound in the recently validated[16] magnesium deficiency-dependent audiogenic seizure (MDDAS) model. The selected compound (i.e., *N*-(2-chloro-4-aminophenyl)phthalimide) was found to be more potent than phenytoin in the batrachotoxin affinity assay performed on rat brain synaptosomes. Furthermore, electrophysiology studies point out interactions of this compound with multiple ion channels and present a promising pharmacological profile for the treatment of the various types of seizure disorders.

Chemistry

The target compounds (Figure 2) were synthesized by condensing phthalic anhydride with the appropriate aniline derivative in acetic acid at reflux temperature (compounds **3**, **5**, **7**, **9**, **11**−**16**, and **18**). The amino derivatives were obtained by reducing the nitro precursor through transfer hydrogenation at reflux temperature using cyclohexene as hydrogen donor, palladium on charcoal as catalyst, and 2-propanol as a solvent (compounds **1**, **2**, **4**, **6**, **8**, **10**, and **17**).

Compound **19** was generated by reacting phthalic anhydride with 2-amino-3-picoline. Compound **20**, a succinimide derivative, was obtained by reducing the nitro group of the compound resulting from the condensation of succinic anhydride with 2-methyl-3-nitroaniline. Most of the evaluations on compound **12** and phenytoin were previously reported in refs 11 and 13, respectively.

Results

Anticonvulsant and Neurotoxicity Screening Data in Mice Dosed Intraperitoneally (ip). Table 1 provides screening data for the activity of compounds in animal testing of seizures induced by electroshock (MES test) and pentylenetetrazol (scPtz test) and neurotoxicity determined in the rotorod test. Compounds were administered to mice by intraperitoneal route 30 min or 4 h before evaluation of their activities in these tests. Comparison with data recorded under the same conditions on phenytoin, the reference prototype antiepileptic drug, is further provided.

1. MES and Rotorod Tests. At doses tested (30, 100, and 300 mg/kg), compounds **3**, **5**, **13**, **16**, and **18** were found to be devoid of activity in the MES test and presented no neurotoxicity (neurotoxicity is defined in the Experimental Section) at any of the doses administered. Anti-MES activity at 300 mg/kg was recorded for compounds **7**, **11**, and **14**, with neurotoxicity expressed only by compound **14**. Compounds **1**, **2**, **4**, **8**, **9**, **10**, **12**, **15**, and **19** were active in the MES test at 30 min using 100 mg/kg in the MES test. Compounds **1**, **8**, **10**, **12**, **15**, and **19** were active at the same dose at the 4 h time point while compounds **2**, **4**, and **9** had protective effects at both time points. The most neurotoxic of these compounds were **9** (30 mg/kg) and **2** (100 mg/kg). A lesser degree of neurotoxicity was recorded for compounds **1**, **10**, **14**, and **19**, whereas compounds **4**, **8**, **12**, and **15** were devoid of any neurotoxicity at doses up to 300 mg/kg. Anti-MES activity at 30 mg/kg was obtained for compounds **6**, **17**, and phenytoin. Regarding their degree of neurotoxicity, the order of compounds (from none to high neurotoxicity) was **17**, phenytoin, and **6**.

2. scPtz Test. Some activity was recorded in the scPtz test for compounds **2** and **9** (active at 30 mg/kg at 30 min), **15** and **17** (active at 100 mg/kg at 4 h and 30 min, respectively), and **19** (at 300 mg/kg). But only compounds **2**, **15**, and **17** were active in this model at non-neurotoxic doses.

Anticonvulsant and Neurotoxic Properties of Compounds in Rats Dosed Orally. Compounds **1**, **2**, **4**, **10**, **12**, **17**, **19**, and phenytoin were selected for oral evaluation of anti-MES and neurotoxic activity in rats (Table 2). Compounds were administered per os, and

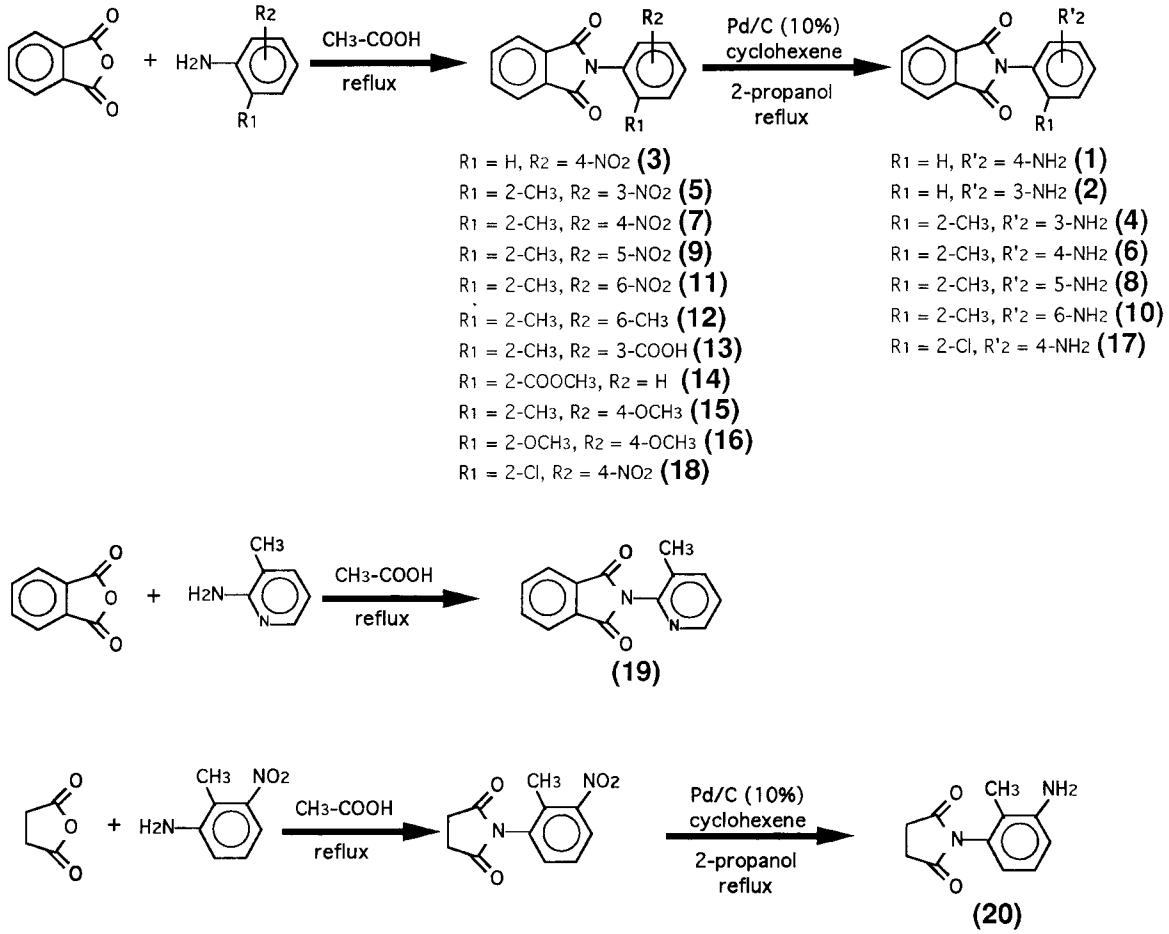

Figure 2. Chemistry of 20 compounds related to *N*-phenyl substituents of the phthalimide pharmacophore. Compounds **1**−**18** represent substituted phenyl N-derivatives of phthalimide. Compounds **19** and **20** are alternatively designed compounds which correspond to *N*-piperidinyl and *N*-phenyl derivatives of phthalimide and succinimide pharmacophores, respectively.

their effects in rats were studied 15, 30, 60, 120, and 240 min after ingestion of either 30 mg/kg (compounds **1**, **2**, **10**, **17**, and **19**) or 50 mg/kg (**4**, **12**, and phenytoin) of experimental drug. At doses given orally to rats, none of the compounds were neurotoxic. The most active compounds in the MES test were **4**, **17**, and phenytoin, giving protection of 100% of animals (4/4) at most time points. Compound **2** gave 50 to 100% protection, depending on the time point. The other compounds (**1**, **10**, **12**, and **19**) were active but in a lower percentage of animals.

Quantitative Evaluation on Selected Compounds in Rats Dosed Orally. Pharmacological quantitations were performed for compounds **4**, **10**, **17**, and phenytoin in the MES and scPtz tests. Table 3 summarizes the main results of determinations performed in rats dosed orally, including ED_{50} (effective dose in 50% of animals tested), TD_{50} (neurotoxic dose in 50% of animals tested), time to peak effect (TPE), and protective index (PI = ratio between TD_{50} and ED_{50} values). None of the selected compounds were active in the scPtz test at the doses administered. In the MES test, the best ED_{50} value was recorded for compound **17** (5.7 mg/kg) which also offered a good protection index (>88). Compound **4** was also highly active with an ED_{50} of 8.0 mg/kg and a PI greater than 50. Compound **10** and phenytoin were much less active with ED_{50} values approximating 30 mg/kg and the PIs exceeding 17 and 100, respectively. TPEs were 30 min (compound **10**), 1 h (compound **4**), 2 h (compound **17**), and 4 h (phenytoin).

Evaluation of Compound 17 in Batrachotoxin Affinity Assay and Magnesium Deficiency-Dependent Audiogenic Seizure Model. The ability of **17** to interact with neuronal voltage-dependent sodium channels was studied in the batrachotoxin affinity assay. The apparent IC_{50} (i.e., the concentration inhibiting by 50% the binding to rat synaptosomes of [3*H*]-batrachotoxinin-A-20α-benzoate) of this compound was found to be 0.15 ± 0.02 μM. The apparent IC_{50} recorded in the same experimental conditions for phenytoin, as a standard reference drug interacting with Na channels, was about 0.93 ± 0.11 μM.

Quantitative evaluation of **17** and phenytoin in the MES test in mice dosed intraperitoneally indicated ED_{50} values of 10.5 and 9.5 mg/kg, respectively. Compound **17** provided a TD_{50} value greater than 500 mg/kg which exceeded that for phenytoin (65.4 mg/kg), giving rise to PI exceeding 48 for compound **17** and about 7 for phenytoin. Determination of ED_{50} values was also carried out in adult mice in the MDDAS test. Compound **17** presented an ED_{50} of 5.2 mg/kg, and phenytoin ED_{50} amounted to 22 mg/kg. The two drugs were further tested using their ED_{50} by recording their effect on the duration of the four phases of MDDAS. The MDDAS component modulation profile of **17** was close to that of phenytoin, reducing the durations of wild running,

Table 1. Anticonvulsant and Neurotoxicity Screening Data in Mice Dosed Intraperitoneally with Compounds[a]

	MES test		scPtz test		toxicity	
compounds	30 min	4 h	30 min	4 h	30 min	4 h
1	++	−	−	−	+	−
2	++	++	+++	−	++	+
3	−	−	−	−	−	−
4	++	++	−	−	−	−
5	−	−	−	−	−	−
6	+++	++	−	−	++	++
7	−	+	−	−	−	−
8	++	−	−	−	−	−
9	++	++	+++	+	+++	+
10	++	+	−	−	+	−
11	−	+	−	−	−	−
12	++	−	−	−	−	−
13	−	−	−	−	−	−
14	+	−	−	−	+	−
15	++	−	+	++	−	−
16	−	−	−	−	−	−
17	+++	−	++	+	−	−
18	−	−	−	−	−	−
19	++	−	+	−	+	−
20	+	−	−	−	−	−
phenytoin	+++	++	−	−	+	−

[a] The anticonvulsant (MES and scPtz tests) and neurotoxicity activities were determined 30 min and 4 h after the administration of compounds. The symbols +++, ++, and + signify activity at 30, 100, and 300 mg/kg, respectively; − denotes no activity observed at 300 mg/kg. Toxicity was determined by the rotorod test. Abbreviations are as follows: MES, maximal electroshock seizures; scPtz, subcutaneous pentylenetetrazol.

Table 2. Anticonvulsant [anti-MES] and Toxicity Screening Data in Rats Dosed Orally with Selected Compounds

compounds	15 min	30 min	1 h	2 h	4 h	toxicity
1	−	+	+++	++	+	−
2	++	++++	+++	+++	++++	−
4	++++	++++	++++	++++	++++	−
10	−	−	+++	+	+	−
12	++	++	−	+	++	−
17	+++	++++	++++	++++	++++	−
19	+	++	++	++	−	−
phenytoin	++++	++++	++++	++++	+	−

[a] At each time point, rats were given a single dose of either 30 mg (**1**, **2**, **10**, **17**, and **19**) or 50 mg (**4**, **12**, and phenytoin) of compound per kilogram of body weight, and anticonvulsant activities were determined in the maximal electroshock seizures (MES) test. No toxicity was observed at this dose, except for compound **13** at the 4 h time point. Symbols are as follows: ++++, activity in 75−100% of administered animals; +++, in 50−75% of animals; ++, in 25−50% of animals; +, 0−25% of animals; and −, no activity or toxicity.

convulsion, and recovery phases by factors 4, 2, and 7 (versus factors 3, 2, and 8 for phenytoin) (Table 4). On the other hand, an effect not induced by other anticonvulsants tested in previous studies was the 43% decrease by compound **17** of the latency period for wild running (Table 4). Phenytoin induced a 5-fold increase of this latency period. Like phenytoin, GABAergic compounds such as diazepam increase this latency period but to a lesser extent (by 60−70%).[16] Ethosuximide does not alter this latency period.[16]

Electrophysiology Measurements of Compound 17. Whole-cell electrophysiology studies were performed using patch clamp technology[17] for compound **17**. These methods were employed in an effort to facilitate a better understanding of potential interactions with receptors' gated ion channels.[12,13] Anticonvulsant effects are often associated with either excitatory (NMDA and kainate) or inhibitory (GABAA) neurotransmission. In addition, voltage-gated Na channel effects were evaluated using N1E-115 neuroblastoma cells.

Table 3. Quantitative Anticonvulsant Data in Rats Dosed Orally[a]

compound	ED_{50} MES test (mg/kg)	ED_{50} scPtz (mg/kg)	TD_{50} (mg/kg)	PI MES test (TD_{50}/ED_{50})	TPE MES test (h)
4	8.0 ± 3.0 (6.2−10.6)	>200	>400	>50	1
10	28.3 ± 3.8 (26.0−32.0)	>250	>500	>17	0.5
17	5.7 ± 1.6 (4.0−8.0)	>250	>500	>88	2
phenytoin	29.8 ± 4.3 (21.9−38.9)	>800	>3000	>100	4

[a] Pharmacological values given in this table are as follows: the ED_{50}, dose of drug required to assure anticonvulsant protection in 50% of animals; the TD_{50}, dose eliciting minimal neurological toxicity in 50% of animals; the PI, protection index (PI = TD_{50}/ED_{50}), and the time to peak effect (TPE). ED_{50} and TD_{50} values are expressed as mg/kg, and TPE as hours. Data on phenytoin are from ref 13.

Table 4. Modulation by Compound **17** and Phenytoin of the Various Components of the Seizure Episode in the MDDAS Model[a]

administration of drugs	latency for wild running	duration of wild running	convulsion phase time-period	recovery phase time-period
no drugs	11.2 ± 1.1	20.0 ± 1.6	5.4 ± 0.6	52.4 ± 1.6
PHT (22 mg/kg)	55.7 ± 3.2*	6.7 ± 0.4*	2.3 ± 0.6*	6.8 ± 0.9*
17 (5 mg/kg)	6.4 ± 0.7*	5.1 ± 0.5*	2.9 ± 0.6*	7.8 ± 0.8*

[a] The various time period determinations are expressed as seconds in convulsing animals at drug dosing ensuring an anticonvulsant protection in 50% and no protection in the other 50% of tested animals. Adult OF1 mice receiving a magnesium-deficient (50 ppm for 42 days) were utilized. *$p < 0.01$ (vs magnesium-deficient mice having received no drugs).

Compound **17** potentiated GABA-evoked current responses. At the highest concentration tested (100 μM), it failed to directly activate the GABAA receptor (Table 5, A). NMDA-evoked potentials were not affected by compound **17** at 100 μM (Table 5, B). By contrast, the compound (at 100 μM) was somewhat active at non-NMDA receptors, producing a 25% block of the kainate-evoked response (Table 5, C). As expected, compound **17** produced a voltage-dependent block of voltage-gated sodium channels (Table 5, D). This activity was found to be of the same order of magnitude as activities recorded for phenytoin, carbamazepine, and lamotrigine in similar experimental conditions (see legend to Table 5).

Discussion

The present design rests on *N*-phenyl derivatives of the phthalimide pharmacophore devoid of the 4-amino substitution (see design 2, Figure 1) which was utilized in previous series.[6−9] Despite omission of this 4-substituent on the phthalimide nucleus, the design results in a series of compounds with highly potent leads active notably in the MES test. SAR studies disclose that, in these conditions, the best patterns require amino substitution of the *N*-phenyl moiety. Combination of the amino and methyl substitutions of the *N*-phenyl group gives apparently more potent compounds with optimal anti-MES properties conveyed by compound **4** (combined 2-methyl and 3-amino substitutions of the *N*-phenyl ring). When the same combined substitutions of the

Table 5. Electrophysiology Experiments of Compound **17**[a]

	type of current responses	concentration of compound **17** (μM)	number of cells (i.e., number of experiments on isolated cells)	current responses recorded during incubation with compound **17** (expressed as % of control current responses [mean ± SEM])
	Whole-Cell Currents of Mouse Cortical Neurons			
A	evoked by 1 μM GABA	100	10	118 ± 4
		30	6	142 ± 6
		10	5	174 ± 9
B	evoked by 10 μM NMDA + 1 μM glycine	100	6	99 ± 2
C	evoked by 100 μM kainate	100	7	74 ± 1
	Voltage-Dependent Sodium Channels in N1E-115 Neuroblastoma Cells			
D	evoked by depolarizing voltage steps			
	- from −60 mV	100	3	49 ± 9
	- from −90 mV	100	5	62 ± 8

[a] Results from several individual cells were averaged, SEM was calculated, and statistical significance was determined by Student's *t*-test. All percentages of control current response values (last vertical column) were significantly different than control at $p < 0.001$. Additional data concerning voltage-dependent sodium channels in N1E-115 neuroblastoma cells include the percentage of control current response values evoked by −90 mV for reference antiepileptic drugs at 100 mM; in these conditions, carbamazepine, lamotrigine, and phenytoin induced values of 78 ± 2, 67 ± 5, and 78 ± 2% of control current responses, respectively. For extensive protocols, see the Experimental Section.

N-phenyl ring are associated with succinimide (compound **20**) instead of phthalimide (compound **4**) as the pharmacophore, only little anticonvulsant protection may be offered in the MES test. The most potent compound of our series might be compound **17**. It combines 2-chloro and 4-amino substitutions of the *N*-phenyl ring. This better potency might be accounted for by a more favorable log *P* (calculated to be 2.24 for **17** versus 1.17 for **1** and 2.08 for phenytoin).

Compared to phenytoin, a prototype antiepileptic drug largely utilized in human clinics,[18] compound **17** was found at least as efficient as phenytoin in all determinations performed in mice and rats. In vivo determinations in rats indicate that compound **17** is severalfold more potent than phenytoin in the MES test (ED_{50} of 5.7 mg/kg versus 29.8 mg/kg for phenytoin). In vitro determinations disclose that **17** is 6-fold more efficacious than phenytoin in counteracting the binding of batrachotoxinin-A-20α-benzoate to rat brain voltage-dependent sodium channels (apparent IC_{50} of 0.15 μM versus 0.93 μM for phenytoin). In mice submitted to the MES test, the protection offered by **17** was similar to that recorded on phenytoin, with ED_{50} values approximating 10 mg/kg. PI offered in these conditions was better for **17** (PI superior to 48 versus 6.9 for phenytoin). Similar activities of **17** and phenytoin in mice were also noticed during electrophysiology studies dealing with voltage-dependent sodium channels.

Activity of **17** in the MES test, batrachotoxin affinity assay, and electrophysiology evaluations indicates that reduction of seizure spread by blockade of the neuronal voltage-dependent Na^+ channels contributes to the anticonvulsant protection given by this compound. This mechanism is therefore common to **17** and phenytoin. Results obtained in the MDDAS test confirm this phenytoin-like mode of action of compound **17**. But in this model, the effects of phenytoin and **17** on the initial component (i.e., latency period) of the audiogenic seizure test are, however, opposite, suggesting that underlying mechanisms for anticonvulsant protection are not strictly the same for the two drugs. Actually, anticonvulsant mechanisms identified for **17** further include potentiation of GABA-evoked current responses demonstrated by electrophysiology studies (this effect may be a coherent explanation for the activity of **17** recorded in the scPtz test in mice dosed intraperitoneally) and inhibition of kainate-evoked response. Therefore, compound **17** interacts with multiple ion channels and may be of interest in a wide range of seizure models and neurologic disorders as explained in the remaining part of this discussion.

Epilepsy is diagnosed on the basis of clinical and electroencephalogram criteria. In this respect, epilepsy covers a wide range of distinct clinical disorders which share in common episodes of synchronous neuronal hyperdischarge. The synchronous neuronal hyperdischarge episode may result from neuronal recruitment initiated by a neuronal irritative thorn and leading to well localized epileptic focus (e.g., symptomatic localization-related epilepsies) or from general lowering of the seizure threshold (e.g., status epilepticus). Simply stated, presently utilized animal seizure models for the former and latter situations are MES (the electroshock acts as a general inducer of irritative thorns which through recruitment result in multiple epileptic foci) and scPtz (GABA-directed inhibitory toxins results in lowering seizure threshold) tests, respectively. The former and latter cited human disorders/animal models respond well to neuronal voltage-dependent sodium channel inhibitors (the drugs reduce seizure spread) and GABAergic compounds (these drugs inhibit neuronal firing by enhancing seizure threshold), respectively. These above cited human/animal epileptic conditions only account for a part of the epileptic disorders. The reality is complicated by the fact that in practice (i) both excessive neuronal firing and seizure spread may concur in a same patient, (ii) focalised epilepsies may secondarily generalize (see the importance of long-term potentiation and kindled animal models), and (iii) the seizure threshold is not governed only by GABA receptors but represents a balance between GABAergic and excitoaminoacidergic pathways. All these considerations explain why polytherapy has developed in the field of human epilepsy, reducing the number of CNS surgery indications (i.e., the number of pharmacoresistant cases). Alternatively to (but not ruling out) polytherapy, the recent development of antiepileptic drugs with multiple cerebral targets has also been successful in reducing the

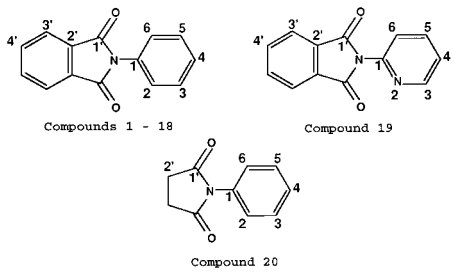

Figure 3. Number attribution of atom positions in formula of compounds submitted to NMR studies.

number of pharmacoresistant epilepsies, avoiding a pool of epileptic patients to undergo CNS surgery. Lamotrigine (see references 19 and 20 for the mechanisms of actions and clinical use, respectively) is a good example of a compound with multiple modes of action and its clinical use presents promising activity in some human epilepsies refractory to other antiepileptic drugs. In this respect, one of the major contributions of the present work is the demonstration that the emerging compound **17** acts on both seizure spread (inhibition of neuronal voltage-dependent sodium channels) and seizure threshold. It is noteworthy that lowering of seizure threshold by compound **17** is mediated by interaction with both the GABAergic (potentiation effect) and excitoaminoacidergic (inhibition effect) pathways. Therefore, compound **17** enters adequately the current strategy devoted to treating pharmacoresistant epilepsies and reducing epilepsy-related CNS surgery indications.

Experimental Section

Materials. Reagents were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France; Bornem, Belgium). Solvents were obtained from Merck (Darmstadt, Germany). The [3*H*]-batrachotoxinin-A-20α-benzoate was obtained from Amersham International plc, Buckinghamshire, England.

Analytical Procedures. Melting points (uncorrected) were determined in open capillary tubes on a Totolli apparatus. IR spectra were recorded on KBr disks using a Spectrum 2000 Perkin-Elmer FT-IR spectrophotometer (FT-IR spectrophotometer, model spectrum 2000). NMR spectra were measured at 25 °C on compounds in solution in DMSO-d_6 at 200 MHz for ^{1}H and 50 MHz for ^{13}C, on a BRUKER AC 200 apparatus. Chemical shifts are expressed relative to the resonance of $(CD_3)_2SO$ at δ 2.49 for ^{1}H NMR and 39.7 for ^{13}C NMR. The NMR data presented below for each compound refer to atom positions for which individual attribution number on formula of compounds is given in Figure 3. HPLC analyses of compounds were performed on a Lichrocart 125-R Licrospher RP 18 column with a flow of 1 mL/min of a methanol−water (65/35, v:v) mixture as elution solvent and detection at 260 nm. Elemental analyses (C, H, N) were performed on a Carlo-Erba EA 1108 elemental analyzer. All compounds had IR and ^{1}H and ^{13}C NMR spectra consistent with their assigned structure. Their microanalytical data were within ±0.4% of the calculated figures. TLC data were obtained using aluminum backed sheets with silica gel 60 F_{254}.

Chemistry. The nitro compounds **3**, **5**, **7**, **9**, and **18** produce the respective amino compounds **1**, **4**, **6**, **8**, **10**, and **17**. The general procedure for the synthesis of the aminophenyl-substituted phthalimides **1**, **4**, **6**, **8**, **10**, and **17** is illustrated for the preparation of *N*-(4-aminophenyl)phthalimide (**1**) using *N*-(4-nitrophenyl)phthalimide (**3**) as the nitro intermediate.

***N*-(4-Aminophenyl)phthalimide (1) and *N*-(4-Nitrophenyl)phthalimide (3).** A mixture of 4-nitroaniline (6.7 g, 49 mmol) and phthalic anhydride (6.0 g, 41 mmol) in acetic acid (30 mL) was stirred and heated under reflux for 5 h; the product of this reaction (i.e., compound **3**) was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield of **3**, 79.7%; mp 274.5−246.5 °C; TLC R_f 0.49 (acetone−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 7.80 (d, 2.0 H, 9.1 Hz, H-2 + H-6), 7.98 (m, 4.0 H, H-3′ + H-4′), 8.41 (d, 2.0 H, 8.9 Hz, H-3 + H-5); ^{13}C NMR (DMSO-d_6) δ ppm 123.8 (C-3′), 124.3 (C-3 and C-5), 127.9 (C-2 and C-6), 131.6 (C-2′), 135.1 (C-4′), 137.9 (C-1), 146.2 (C-4), 166.5 (C-1′); IR (ν, cm^{-1}) 1782.7, 1732.1 (C=O phthalimide), 1610.3, 1597.6, 1496.1, 1466.4 (phenyl), 1522.3, 1345.3 (NO_2). Anal. ($C_{14}H_8N_2O_4$) C, H, N.

Reduction of **3** was performed using cyclohexene as hydrogen donor, palladium on charcoal (Pd/C, 10%) as catalyst, and 2-propanol as a solvent. The resulting *N*-(4-aminophenyl)-phthalimide compound was obtained pure after being recrystallized from ethanol−water (1/1, v:v): yield of **1**: 74.7%; mp 183−185 °C; TLC R_f 0.33 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 5.30 (s, 1.6 H, NH_2), 6.4−6.7 (m, 3.0 H, H-2 + H-4 + H-6), 7.12 (t, 1.0 H, 7.8 Hz, H-5), 7.90 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 112.9, 113.8 and 114.7 (C-2 + C-4 + C-6), 123.5 (C-3′), 129.3 (C-5), 131.7 (C-2′), 132.6 (C-1), 134.8 (C-4′), 149.4 (C-3), 167.3 (C-1′); IR (ν, cm^{-1}) 3420.7, 3346.1, 1634.7 (NH_2), 1765.7, 1704.5 (C=O phthalimide), 1605.9, 1497.8, 1468.4 (phenyl). Anal. ($C_{14}H_{10}N_2O_2$) C, H, N.

***N*-(3-Aminophenyl)phthalimide (2).** Using a procedure similar to that of **1**, 3-nitroaniline (6.7 g, 49 mmol) and phthalic anhydride (6.0 g, 41 mmol) provided the title compound, after recrystallization from ethanol−water (1/1, v:v): yield 73.7%; mp 244−246 °C; TLC R_f 0.35 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 5.35 (s, 1.7 H, NH_2), 6.64 (d, 2.0 H, 8.7 Hz, H-3 + H-5), 7.01 (d, 2.0 H, 8.4 Hz, H-2 + H-6), 7.88 (m, 4.0 H, H-3′ + H-4′). ^{13}C NMR (DMSO-d_6) δ ppm 113.7 (C-3), 119.8 (C-1), 123.4 (C-3′), 128.4 (C-2), 131.8 (C-2′), 134.7 (C-4′), 149.0 (C-4), 167.8 (C-1′); IR (ν, cm^{-1}) 3462.2, 3369.3, 1625.7 (NH_2), 1779.1, 1708.5 (C=O phthalimide), 1611.3, 1516.5, 1462.5 (phenyl). Anal. ($C_{14}H_{10}N_2O_2$) C, H, N.

***N*-(3-Amino-2-methylphenyl)phthalimide (4) and *N*-(2-Methyl-3-nitrophenyl)phthalimide (5).** Using a procedure similar to that for **1**, 2-methyl-3-nitroaniline (9.0 g, 59 mmol) and phthalic anhydride (8.0 g, 54 mmol) provided the nitro intermediate **5** after recrystallization from 95% ethanol: yield 92.5%; mp 179−181 °C; TLC R_f 0.52 (acetone−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.34 (s, 3.0 H, CH_3), 7.48 (t, 1.0 H, 8.1 Hz, H-5), 7.53−7.85 (m, 5.0 H, H-3′ + H-4′ + H-6), 7.92 (d, 1.0 H, 7.5 Hz, H-4); ^{13}C NMR (DMSO-d_6) δ ppm 13.9 and 14.2 (CH_3), 123.9 (C-3′), 126.7 (C-5), 129.9 (C-2′ or C-1), 135.8 (C-4′), 151.1 (C-3); IR (ν, cm^{-1}) 1782.8, 1731.7 (C=O phthalimide), 1600.8, 1575.8 (phenyl), 1524.5, 1354.0 (NO_2). Anal. ($C_{15}H_{10}N_2O_4$) C, H, N.

Reduction of **5** provided the title compound **4** after recrystallization from ethanol: yield 38%; mp 246−248 °C; TLC R_f 0.39 (acetone−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 1.79 (s, 3.0 H, CH_3), 5.12 (s, 1.67 H, NH_2), 6.50 (dd, 1.0 H, 7.7 and 1.1 Hz, H-4), 6.73 (dd, 1.0 H, 8.1 and 1.2 Hz, H-6), 6.99 (t, 1.0 H, 7.9 Hz, H-5), 7.92 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 12.2 (CH_3), 114.8 (C-4), 116.7 (C-6), 119.9 (C-2), 123.6 (C-3′), 126.4 (C-5), 131.4 and 131.8 (C-1 and C-2′), 134.9 (C-4′), 147.9 (C-3), 167.4 (C-1′); IR (ν, cm^{-1}) 3444.9, 3352.8, 1632.9 (NH_2), 1784.2, 1769.7, 1705.9 (C=O phthalimide), 1584.8, 1475.8, 1468.4 (phenyl). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

It has been observed that compound **5** was not absolutely stable, and when solubilized in DMSO for 24 h, part of this compound gave rise to the corresponding 2-carboxy-benzamide derivative obtained by opening of the phtahlimide ring. For experimental determinations including anticonvulsant and neurotoxic evaluation, compound **5** was utilized immediately after puting the powder in solution, avoiding the formation of the hydrolyzed product before use. Hydrolyzed product has not yet been checked.

***N*-(4-Amino-2-methylphenyl)phthalimide (6) and *N*-(2-Methyl-4-nitrophenyl)phthalimide (7).** Using a procedure similar to that for **1**, 2-methyl-4-nitroaniline (10.0 g, 66 mmol) and phthalic anhydride (9.5 g, 64 mmol) provided the nitro

intermediate **7** recrystallized from 95% ethanol: yield 77.8%; mp 193−195 °C; TLC R_f 0.52 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.28 (s, 3.0 H, CH_3), 7.70 (d, 1.0 H, 8.6 Hz, H-6), 7.96 (m, 4.0 H, H-3′ + H-4′), 8.20 (dd, 1.0 H, 8.6 and 2.4 Hz, H-5), 8.30 (d, 1.0 H, 2.5 Hz, H-3); ^{13}C NMR (DMSO-d_6) δ ppm 17.8 (CH_3), 121.8 (C-3), 123.9 (C-3′), 125.6 (C-6), 130.8 (C-5), 131.9 (C-2′), 135.1 (C-4′), 137.2 (C-2), 139.0 (C-1), 147.6 (C-4), 166.5 (C-1′); IR (ν, cm^{-1}) 1788.9, 1728.2 (C═O phthalimide), 1615.7, 1587.1, 1489.8 (phenyl), 1518.8, 1340.1 (NO_2). Anal. ($C_{15}H_{10}N_2O_4$) C, H, N.

Reduction of **7** provided the title compound **6** recrystallized from ethanol: yield 77.8%; mp 179−181 °C; TLC R_f 0.39 (ethyl acetate-chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 1.92 (s, 3.0 H, CH_3), 5.29 (s, 1.4 H, NH_2), 6.45 (dd, 1.0 H, 8.4 and 2.4 Hz, H-5), 6.51 (d, 1.0 H, 2.2 Hz, H-3), 6.89 (d, 1.0 H, 8.4 Hz, H-6), 7.91 (m, 4.0, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 17.7 (CH_3), 111.9 (C-5), 115.2 (C-3), 118.8 (C-1), 123.5 (C-3′), 129.7 (C-6), 131.8 (C-2′), 134.8 (C-4′), 136.5 (C-2), 149.6 (C-4), 167.8 (C-1′); IR (ν, cm^{-1}) 3451.2, 3363.9, 1623.4 (NH_2), 1776.5, 1756.9, 1722.8, 1697.6 (C═O phthalimide), 1583.2, 1507.2, 1465.8 (phenyl). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

***N*-(5-Amino-2-methylphenyl)phthalimide (8) and *N*-(2-Methyl-5-nitrophenyl)phthalimide (9).** Using a procedure similar to that for **1**, 2-methyl-5-nitroaniline (9.6 g, 65 mmol) and phthalic anhydride (10.0 g, 66 mmol) provided the nitro intermediate **9** recrystallized from 95% ethanol: yield 71.9%; mp 232−235 °C; TLC R_f 0.52 (ethyl acetate-chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.28 (s, 3.0 H, CH_3), 7.71 (d, 1.0 H, 8.6 Hz, H-3), 7.96 (m, 4.0 H, H-3′ + H-4′), 8.26 (dd, 1.0 H, 8.6 and 2.5 Hz, H-4), 8.41 (d, 1.0 H, 2.5 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ ppm 18.0 (CH_3), 123.8 (C-3′), 124.0 and 124.7 (C-4 and C-6), 131.9 (C-3′), 132.1 (C-3), 135.0 (C-4′), 145.4 (C-2), 146.2 (C-5), 166.8 (C-1′); IR (ν, cm^{-1}) 1780.6, 1717.8 (C═O phthalimide), 1595.0, 1519.7, 1468.2 (phenyl), 1519.7, 1343.4 (NO_2). Anal. ($C_{15}H_{10}N_2O_4$) C, H, N.

Reduction of **9** provided the title compound **8**: 70.6% after recrystallization from ethanol; mp 166−169 °C; TLC R_f 0.32 (ethyl acetate-chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 1.90 (s, 3.0 H, CH_3), 5.12 (s, 1.6 H, NH_2), 6.46 (d, 1.0, 2.2 Hz, H-6), 6.60 (dd, 1.0 H, 8.1 and 2.2 Hz, H-4), 7.00 (d, 1.0 H, 8.2 Hz, H-3), 7.92 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 16.5 (CH_3), 114.4 and 115.1 (C-4 and C-6), 122.5 (C-2), 123.6 (C-3′), 130.9 (C-3), 131.3 (C-1), 131.7 (C-2′), 134.9 (C-4′), 147.5 (C-5), 167.2 (C-1′); IR (ν, cm^{-1}) 3442.3, 3362.3, 1622.4 (NH_2), 1782.7, 1765.8, 1714.1 (C═O phthalimide), 1510.6, 1464.9 (phenyl). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

***N*-(6-Amino-2-methylphenyl)phthalimide (10) and *N*-(2-Methyl-6-nitrophenyl)phthalimide (11).** Using a procedure similar to that for **1**, 2-methyl-6-nitroaniline (10.0 g, 66 mmol) and phthalic anhydride (9.6 g, 65 mmol) provided nitro intermediate **11**: 31% after recrystallization from 95% ethanol; mp 171−173 °C; TLC R_f 0.53 (ethyl acetate-chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.29 (s, 3.0 H, CH_3), 7.71 (t, 1.0 H, 8.0 Hz, H-4), 7.87 (d, 1.0 H, 7.2 Hz, H-3 or H-5), 8.0 (m, 5.0 H, H-3′ + H-4′ + [H-5 or H-3]). ^{13}C NMR (DMSO-d_6) δ ppm 18.4 (CH_3), 124.3 (C-5), 125.0 (C-3′), 131.3 (C-4), 132.2 (C-2′), 136.4 (C-4′), 137.3 (C-3), 141.3 (C-2), 147.5 (C-6), 167.5 (C-1′); IR (ν, cm^{-1}) 1780.8, 1742.4, 1713.8 (C═O phthalimide), 1605.0, 1579.3, 1466.9 (phenyl), 1529.5, 1369.2 (NO_2). Anal. ($C_{15}H_{10}N_2O_4$) C, H, N.

Reduction of **11** provided the title compound **10**: 53.7% after recrystallization from ethanol; mp 189−190 °C; TLC R_f 0.44 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 1.88 (s, 3.0 H, CH_3), 5.30 (s, 1.2 H, NH_2), 6.46 and 6.59 (d, 1.0 H, 7.3 and 7.2 Hz, H-3 and H-5), 7.01 (t, 1.0 H, 7.6 Hz, H-4), 7.91 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 17.6 (CH_3), 113.3 (C-5), 115.2 (C-1), 117.0 (C-3), 123.4 (C-3′), 129.6 (C-4), 132.3 (C-2′), 134.5 (C-4′), 137.0 (C-2), 146.9 (C-6), 167.6 (C-1′); IR (ν, cm^{-1}) 3435.7, 3345.4, 1623.2 (NH_2), 1780.5, 1760.4, 1736.6, 1708.9 (C═O phthalimide), 1597.5, 1487.3, 1473.9 (phenyl). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

***N*-(2,6-Dimethylphenyl)phthalimide (12).** A mixture of 2,6-dimethylaniline (5.0 g, 41 mmol) and phthalic anhydride (18.3 g, 123 mmol) in acetic acid (30 mL) was stirred and heated under reflux for 4−5 h. The solvent was evaporated in vacuo, and the residual material was recrystallized from methanol−water (1/1, v:v) giving rise to pure compound: yield 65.8%; mp 205−208 °C; TLC R_f 0.68 (acetone−chloroform−acetic acid, 49/49/2, v:v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.0 (s, 6.0 H, CH_3), 7.28−7.4 (m, 3.0 H, H-3 + H-4 + H-5), 7.97 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 17.7 (CH_3), 123.9 (C-3′), 128.4 (C-3 and C-5), 129.4 (C-4), 130.1 (C-1), 131.4 (C-2′), 135.2 (C-4′), 136.8 (C-2 and C-6), 167.6 (C-1′); IR (ν, cm^{-1}) 1779.6, 1759.1, 1738.9, 1709.5 (C═O phthalimide), 1595.8, 1469.8 (phenyl). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

***N*-(3-Carboxy-2-methylphenyl)phthalimide (13).** A mixture of 3-amino-2-methylbenzoic acid (5.0 g, 33 mmol) and phthalic anhydride (4.75 g, 32 mmol) in acetic acid/dimethylformamide [DMF] (50 mL/30 mL)) was stirred and heated under reflux for 6 h. The resulting intermediate was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield, 80.9%; mp 307−310 °C; TLC R_f 0.43 (ethyl acetate−chloroform−acetic acid, 49/49/2, v:v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.1 (s, 3.0 H, CH_3), 7.72 (t, 1.0 H, 7.6 Hz, H-5), 7.79 (d, 1.0 H, 7.6 Hz, H-4 or H-6), 7.9 (m, 5.0 H, H-3′ + H-4′ + [H-6 or H-4]), 13.1 (s, 0.4 H, COOH); ^{13}C NMR (DMSO-d_6) δ ppm 15.4 (CH_3), 123.8 (C-3′), 126.6 (C-5), 130.8 (C-4), 131.8 (C-3), 132.4 (C-2′), 132.8 (C-6), 133.1 (C-1), 135.0 (C-4′), 137.2 (C-2), 167.2 and 168.7 (C-1′ + COOH); IR (ν, cm^{-1}) 2500−3100, 1687.8 (COOH), 1778.9, 1757.8, 1717.3 (C═O phthalimide), 1594.6, 1468.9 (phenyl). Anal. ($C_{16}H_{11}NO_4$) C, H, N.

***N*-(2-Methyloxycarbonylphenyl)phthalimide (14).** A mixture of 2-methyloxycarbonyl-aniline (6.1 g, 40 mmol) and phthalic anhydride (5.0 g, 34 mmol) in acetic acid (30 mL) was stirred and heated under reflux for 5 h. The resulting intermediate was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: Yield 72.2%; mp 158−160 °C; TLC R_f 0.52 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 3.65 (s, 3.0 H, CH_3−O-[C═O]-), 7.58 and 7.66 (dd and d,t, 1.0 H, 7.6 and 2 Hz, [H-3 or H-4] + [H-5 or H-2]), 7.81 (t,d, 1.0 H, 7.6 and 2 Hz, H-4 or H-3), 7.96 (m, 4.0 H, H-3′ + H-4′), 8.05 (dd, 1.0 H, 7.6 and 2 Hz, H-2 or H-5); ^{13}C NMR (DMSO-d_6) δ ppm 52.6 (CH_3-O-[C═O]-), 123.8 (C-3′), 128.0 (C-6), 129.6 and 131.0 and 131.1 (C-2 + C-4 + C-5), 131.8 (C-2′ + C-1), 133.7 (C-3), 135.1 (C-4′), 165.1 and 167.2 (C-1′ + [C═O]-O-); IR (ν, cm^{-1}) 1776.6, 1766.6, 1712.9 (C═O phthalimide), 1601.6, 1496.6 (phenyl), 1712.9, 1276.0, 1122.8 ($COOCH_3$). Anal. ($C_{16}H_{11}NO_4$) C, H, N.

***N*-(4-Methoxy-2-methylphenyl)phthalimide (15).** A mixture of 4-methoxy-2-methylaniline (6.0 g, 44 mmol) and phthalic anhydride (6.5 g, 44 mmol) in acetic acid (75 mL) was stirred and heated under reflux for 5 h. The resulting intermediate was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield 44.5%; mp 189−190 °C; TLC R_f 0.56 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.07 (s, 3.0 H, CH_3), 3.79 (s, 3.0 H, CH_3−O-), 6.87 (dd, 1.0 H, 8.6 and 2.5 Hz, H-5), 6.95 (d, 1.0 H, 2.2 Hz, H-3), 7.25 (d, 1.0 H, 8.6 Hz, H-6), 7.92 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 17.8 (CH_3), 55.5 (CH_3-O-), 112.2 (C-5), 115.8 (C-3), 123.6 (C-3′ + C-1), 130.4 (C-6), 131.8 (C-2′), 134.8 (C-4′), 137.8 (C-2), 159.7 (C-4), 167.4 (C-1′); IR (ν, cm^{-1}) 1780.8, 1717.2, 1697.2 (C═O phthalimide), 1617.5, 1583.6, 1507.1 (phenyl), 1253.2 (O−CH_3). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

***N*-(2,4-Dimethoxyphenyl)phthalimide (16).** A mixture of 2,4-dimethoxyaniline (9.5 g, 62 mmol) and phthalic anhydride (9.1 g, 61 mmol) in acetic acid (50 mL) was stirred and heated under reflux for 5 h. The resulting intermediate was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield 75.7%; mp 228−231 °C; TLC R_f 0.52 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 3.74 (s, 3.0 H, CH_3−O-), 3.80 (s, 3.0 H, CH_3−O-), 6.62 (dd, 1.0 H, 8.2 and 2.5 Hz, H-5), 6.73 (d, 1.0 H, 2.2 Hz, H-3), 7.25 (d, 1.0 H, 8.0 Hz, H-6), 7.92 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 55.6 (CH_3−O-), 55.9 (CH_3−O-), 99.3 (C-3), 105.2 (C-5), 112.9 (C-1), 123.5 (C-3′), 130.9 (C-6), 131.6 (C-2′), 134.8 (C-4′), 156.3 (C-2), 161.3 (C-4), 167.3 (C-1′); IR (ν, cm^{-1}) 1781.6, 1761.9, 1730.9, 1710.5 (C═O phthalimide),

1613.3, 1588.9, 1514.6 (phenyl), 1216.4 (−O−CH_3). Anal. ($C_{16}H_{13}NO_4$) C, H, N.

***N*-(4-Amino-2-chlorophenyl)phthalimide (17) and *N*-(2-Chloro-4-nitrophenyl)phthalimide (18).** Using a procedure similar to that for **1**, 2-chloro-4-nitroaniline (12.0 g, 70 mmol) and phthalic anhydride (10.2 g, 69 mmol) provided nitro intermediate **18** recrystallized from 95% ethanol: yield 52.8%; mp 180−182 °C; TLC R_f 0.52 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 7.8−8.2 (m, 5.0 H, H-6 + H-3′ + H-4′), 8.39 (dd, 1.0 H, 8.7 and 2.5 Hz, H-5), 8.54 (d, 1.0 H, 2.5 Hz, H-3); ^{13}C NMR (DMSO-d_6) δ ppm 123.4 (C-5), 124.2 (C-3′), 125.2 (C-3), 131.6 (C-2′), 132.6 (C-6), 133.6 (C-2), 135.4 (C-4′), 135.7 (C-1), 148.5 (C-4), 165.9 (C-1′); IR (ν, cm^{-1}) 1785.7, 1733.0, 1714.2 (C═O phthalimide), 1590.6, 1597.1, 1481.5, 1466.5 (phenyl), 1524.0, 1338.9 (NO_2). Anal. ($C_{14}H_7ClN_2O_4$) C, H, N.

Reduction of **18** provided the title compound **17**, after recrystallization from acetic acid: yield 17.3%; mp 205−207 °C; TLC R_f 0.44 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 5.70 (s, 1.24 H, NH_2), 6.60 (dd, 1.0 H, 8.6 and 1.5 Hz, H-5), 6.76 (d, 1.0 H, 1.4 Hz, H-3), 7.14 (d, 1.0 H, 8.6 Hz, H-6), 7.93 (m, 4.0 H, H-3′ + H-4′); ^{13}C NMR (DMSO-d_6) δ ppm 112.9 and 113.5 (C-3 + C-5), 116.35 (C-1), 123.7 (C-3′), 131.6 (C-6), 132.5 (C-2′), 135.1 (C-4′), 151.2 (C-4), 167.3 (C-1′); IR (ν, cm^{-1}) 3433.9, 3352.5, 1646.6 (NH_2), 1778.8, 1758.8, 1725.6, 1703.8 (C═O phthalimide), 1600.0, 1502.0, 1466.2 (phenyl). Anal. ($C_{14}H_9ClN_2O_2$) C, H, N.

***N*-[3-Methyl-(2-pyridinyl)]phthalimide (19).** A mixture of 2-amino-3-picoline (5.1 g, 47 mmol) and phthalic anhydride (7.6 g, 51 mmol) in acetic acid (30 mL) was stirred and heated under reflux for 5−6 h. The resulting intermediate was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield 36.4%; mp 169−171 °C; TLC R_f 0.42 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 2.18 (s, 3.0 H, CH_3), 7.48 (dd, 1.0 H, 7.6 and 4.7 Hz, H-4), 7.96 (m, 5.0 H, H-3′ + H-4′ + H-5), 8.46 (dd, 1.0 H, 4.7 and 1.5 Hz, H-3); ^{13}C NMR (DMSO-d_6) δ ppm 17.4 (CH_3), 124.8 (C-3′), 125.9 (C-5 or C-4), 132.2 and 133.3 (C-2′ + [C-1 or C-6]), 136.1 (C-4′), 141.1 (C-4 or C-5), 145.7 (C-6 or C1), 148.2 (C-3), 167.4 (C-1′); IR (ν, cm^{-1}) 1786.3, 1763.8, 1714.5 (C═O phthalimide). Anal. ($C_{14}H_{10}N_2O_2$) C, H, N.

***N*-(3-Amino-2-methylphenyl)succinimide (20).** A mixture of 2-methyl-3-nitroaniline (9.0 g, 59 mmol) and succinic anhydride (6.3 g, 63 mmol) in acetic acid (50 mL) was stirred and heated under reflux for 5 h. The resulting intermediate was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol. Reduction of the nitro group was performed using cyclohexene (hydrogen donor) and palladium on charcoal (Pd/C, 10%) (catalyst) in 2-propanol (solvent). The resulting *N*-(3-amino-2methylphenyl)succinimide compound was obtained pure after being recrystallized from ethanol: yield 50.6%; mp 178−180 °C; TLC R_f 0.15 (ethyl acetate−chloroform, 1/1, v:v); ^{1}H NMR (DMSO-d_6) δ ppm 1.75 (s, 3.0 H, CH_3), 2.79 (m, 4.0 H, CH_2−CH_2), 5.04 (s, 1.6 H, NH_2), 6.30 (d, 1.0 H, 7.6 Hz, H-4), 6.67 (d, 1.0 H, 8.0 Hz, H-6), 6.95 (t, 1.0 H, 7.8 Hz, H-5); ^{13}C NMR (DMSO-d_6) δ ppm 12.0 (CH_3), 28.7 (CH_2−CH_2), 114.5 (C-4), 116.0 (C-6), 119.3 (C-2), 126.25 (C-5), 132.4 (C-1), 147.8 (C-3), 177.2 (-[C═O]-N-); IR (ν, cm^{-1}) 3433.3, 3355.9, 1624.9 (NH_2), 2991.4, 2938.2, 2911.2, 2863.0 (CH_3, CH_2), 1781.0, 1703.3 (C═O succinimide), 1587.6, 1491.5, 1478.5 (phenyl). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

In Vivo and in Vitro Experiments. Anticonvulsant evaluations in the MES and scPtz tests, determination of neurotoxicity (i.e., minimal neurotoxicity evaluated by the rotarod test), and batrachotoxin affinity assay procedure were performed as described previously.[11] MDDAS test was also described elsewhere.[16]

Whole-cell electrophysiology studies were performed using patch clamp technology.[17] Whole-cell voltage clamp data recordings were obtained using cortical cells cultured from 15-gestational-day-old mouse fetuses used 2−3 weeks after plating.[21,22]

Recordings were carried out at room temperature (23 °C) in a control bathing solution containing 142 mM NaCl, 1.5 mM KCl, 1 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, and 20 mM sucrose (320 mOsm, pH 7.4).[17] The bathing solution also contained 200 nM strychnine to block the glycine receptor and 200−500 nM tetrodotoxin to block voltage-gated sodium channels. For experiments in which GABA receptor currents were examined, the bathing solution also contained 1 mM $MgCl_2$ to block NMDA receptor currents.

For experiments on glutamate receptor current, the bathing solution contained 1 μM glycine (a required co-agonist at the NMDA receptor), and 10 μM picrotoxin was added to block GABAA channels. Whole-cell voltage clamp recordings were obtained with an Axopatch 200 amplifier (Axon instruments) using patch electrodes (2−3 Ω) filled with an intracellular solution containing 153 mM CsCl, 10 mM EGTA, 10 mM HEPES, and 4 mM $MgCl_2$ (290 mOsm, pH 7.4). Currents were filtered at 1−2 kHz, digitally sampled at 1 kHz, and acquired on computer using Axotape software (Axon instruments). Currents were also acquired on a chart recorder.

Cells were voltage clamped at −60 mV. Agonist and test compound were applied using a rapid perfusion system that consisted of a gravity fed multibarreled microperfusion pipet that was positioned 200−400 cm from the cell. Solution exchange was controlled by solenoid valves with a valvebank 8 controller (Automate Scientific). GABAA receptor currents were evoked using 1 μM GABA, whereas ionotropic glutamate currents were evoked using 10 μM NMDA and 100 μM kainate. Agonists were applied for 1−5 s and separated by a 20−30 s wash period. Within this protocol, GABA and glutamate receptor currents were relatively stable for the duration of the recording period.

The effects of compound **17** on GABA currents were evaluated using a pretreatment paradigm which allows measurement of the maximal effects of the compound without the confounding effects of GABA receptor desensitization. Following at least two control applications of GABA, the cell was perfused with compound **17** (100 μM) for 20−30 s prior to application of GABA plus the drug. Following the combined GABA and drug application, the perfusate was then switched back to drug alone. This sequence (drug/drug + agonist/drug) was repeated until at least two consistent currents were observed. The perfusate was then returned to the control bathing solution, and additional GABA responses were obtained to monitor return of the GABA response to control levels. This protocol allowed measurement of both the direct and modulatory actions of the test compound at GABAA receptors. An identical protocol to that described for GABA was employed to assess the interaction of compound **17** with the ionotropic glutamate receptors gated by NMDA and kainate.

The agonist-evoked current values were measured using the axotape software package or obtained directly from the chart-paper records. The effect of compound **17** on agonist-evoked currents was determined by comparing the agonist currents in the presence of drug with control agonist responses in the absence of drug.

Voltage-Gated Na^+ Channel. The effect of compound **17** on voltage-gated Na^+ channels was assessed using N1E-115 neuroblastoma cells and whole-cell voltage clamp recording techniques. The N1E-115 neuroblastoma cell line was maintained at 35 °C in Dulbecco's modified Eagles Medium supplemented with 5% fetal calf serum, 20 mM HEPES, 80 μg/mL gentamycine, and 4 mM glutamine. Prior to electrophysiological studies, cells were plated and incubated for 3−5 days in a differentiation medium similar to above with reduced (2.5%) fetal calf serum and 2% DMSO. Recordings were carried out at room temperature in a bathing solution containing 130 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 5 mM HEPES. The buffer also contained 0.1 mM $CdCl_2$ and 25 mM tetraethylamonium chloride to block voltage-gated Ca^{2+} and K^+ channels, respectively. Whole-cell recordings were obtained using patch electrodes (1−2 MΩ) filled with the intracellular solution described above. The currents were filtered at 5 kHz and acquired on computer using PClamp 6 (Axon Instruments). Series resistance and capacitive currents

were compensated using the internal clamp circuitry. The series resistance was 3−5 MΩ, and 80−90% of the series resistance was compensated. Cells were voltage clamped at −70 mV, and compound **17** was applied using the perfusion system described above. To activate voltage-gated sodium channels, cells were hyperpolarized to −90 mV for 90 ms and then depolarized to potentials from −80 to +60 mV in 10 mV increments in control and at 1 and 2 min following incubation with test compound. The process was repeated, hyperpolarizing at −60 mV.

In the electrophysiology experiments, the results from several individual cells were averaged, the SEM was calculated, and statistical significance was determined using the Student's *t*-test. Significance was taken to be in the range of *p* values $p < 0.01$.[23]

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References

(1) Clark, C. R.; Wells, M. J. M.; Sansom, R. T.; Norris, G. N.; Dockens, R. C.; Ravis, W. R. Anticonvulsant activity of some 4-aminobenzamides. *J. Med. Chem.* **1984**, *27*, 779−782.

(2) Clark, C. R.; Sansom, R. T.; Lin, C. M.; Norris, G. N. Anticonvulsant activity of some 4-aminobenzanilides. *J. Med. Chem.* **1985**, *28*, 1259−1262.

(3) Clark, C. R.; Lin, C. M.; Sansom, R. T. Anticonvulsant activity of 2- and 3-aminobenzanilides. *J. Med. Chem.* **1986**, *29*, 1534−1537.

(4) Clark, C. R.; Davenport, T. W. Synthesis and anticonvulsant activity of analogues of 4-amino-N-(1-phenylethyl)benzamide. *J. Med. Chem.* **1987**, *30*, 1214−1218.

(5) Clark C. R. Comparative anticonvulsant activity and neurotoxicity of 4-amino-N-(2,6-dimethylphenyl)benzamide and prototype antiepileptic drugs in mice and rats. *Epilepsia* **1988**, *29*, 198−203.

(6) Bailleux, V.; Vallée, L.; Nuyts, J. P.; Vamecq, J. Anticonvulsant activity of some 4-amino-N-phenylphthalimides and N-(3-amino-2-methylphenyl)phthalimides. *Biomed. Pharmacother.* **1994**, *48*, 95−101.

(7) Bailleux, V.; Vallée, L.; Nuyts, J. P.; Vamecq, J. Synthesis and anticonvulsant activity of some N-phenylphthalimides. *Chem. Pharm. Bull.* **1994**, *42*, 1817−1821.

(8) Bailleux, V.; Vallée, L.; Nuyts, J. P.; Hamoir, G.; Poupaert, J. H.; Stables, J. P.; Vamecq, J. Comparative anticonvulsant activity and neurotoxicity of 4-amino-N-(2,6-dimethylphenyl)-phthalimide and prototype antiepileptic drugs in mice and rats. *Epilepsia* **1995**, *36*, 559−565.

(9) Poupaert, J.; Hamoir, G.; Barbeaux, P.; Lambert, D.; Hénichart, J. P. Anticonvulsant activity of some N-phenylphthalimide derivatives in rats and mice. *J. Pharm. Pharmacol.* **1995**, *47*, 89−91.

(10) Friderichs, E. Studies on the CNS activity profile of thalidomide analogues. *Arzneimittelforschung* **1982**, *32*, 613−620

(11) Vamecq, J.; Lambert, D.; Poupaert, J. H.; Masereel, B.; Stables, J. P. Anticonvulsant activity and interactions with neuronal voltage-dependent sodium channel of 15 analogues of ameltolide, a novel antiepileptic drug. *J. Med. Chem.* **1998**, *41*, 3307−3313.

(12) White, H. S.; Johnson, M.; Wolf, H. H.; Kupferberg, H. J. The early identification of anticonvulsant activity: role of the maximal electroshock and subcutaneous pentylenetetrazol seizure models. *Ital. J. Sci.* **1995**, *16*, 73−77.

(13) White, H. S.; Woodhead, J. H.; Franklin, M. R. General Principles: Experimental Selection, Quantification, and Evaluation of Anticonvulsant Drugs. In *Antiepileptic drugs*, 4th ed.; Levy, R., Mattson, R., Meldrum, B. S., Eds.; Raven Press: New York, 1995; pp 99−110.

(14) Stables, J. P.; Kupferberg, H. J. The NIH Anticonvulsant Drug Development (ADD) Program: Preclinical Anticonvulsant Screening Project. In: *Molecular and Cellular Targets for Antiepileptic drugs*; Avanzini, G., Tanganelli, P., Avoli, M., Eds.; John Libbey & Company Ltd.: London, 1997; pp 191−198. .

(15) Kupferberg, H. K., Stables, J. P. Mechanism of Action Revisited: Drug Discovery, Testing and Clinical Prediction. In *Challenge Epilepsy − New Anticonvulsant Drugs*; Stefan, H., Kramer, G., Mamoli, B., Eds.; Blackwell Science Ltd.: Boston, 1998; pp 7−29.

(16) Bac, P.; Maurois, P.; Dupont, C.; Pages, N.; Stables, J. P.; Gressens, P.; Evrard, Ph.; Vamecq, J. Magnesium deficiency-dependent audiogenic seizures (MDDASs) in adult mice: a nutritional model for discriminatory screening of anticonvulsant drugs and original assessment of neuroprotection properties. *J. Neurosci.* **1998**, *18*, 4363−4373.

(17) Hamil, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. Improved patch clamp technique for high-resolution current recordings from cells and cell free membranes. *Pflugers Arch.* **1981**, *391*, 85−100.

(18) Macdonald, R. L. Antiepileptic drug actions. *Epilepsia* **1989**, *30*, S19−S28.

(19) Leach, M. J.; Lees, G.; Riddall, D. R. Lamotrigine − Mechanisms of Action. In *Antiepileptic Drugs*, 4th ed.; Levy, H. L., Mattson, R. H., Meldrum, B. S., Eds.; Raven Press: New York, 1995; pp 861−869.

(20) Leach, J. P.; Brodie, M. J. Lamotrigine − Clinical Use. In *Antiepileptic Drugs*, 4th ed.; Levy, H. L., Mattson, R. H., Meldrum, B. S., Eds.; Raven Press: New York, 1995; pp 889−895.

(21) Hertz, E.; Yu, A. C. H.; Hertz, L.; Juurlink, B. H. J.; Schousboe, A. Preparation of primary cultures of mouse cortical neurons. In *A Dissection and Tissue Culture Manual of the Nervous System*; Shahar, A., Devillis, J., Vernadakis, A., Haber, B., Eds.; Alan R. Liss, Inc.: New York, 1990; pp 183−186.

(22) Skeen, G. A.; White, H. S.; Twyman, R. E. The dihydropyridine reduces *N*-methyl-D-aspartate (NMDA)-evoked currents of rodent cortical neurons through a direct interaction with the NMDA receptor-associated ion channel. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 30−38.

(23) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculations with computer programs*, 2nd ed.; Raven Press Ltd.: New York, 1986.

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