© Pharmacology & Toxicology 1998, 82, 47–50.
Printed in Denmark. All rights reserved

Pharmacology & Toxicology
ISSN 0901-9928

Anticonvulsant Profile of 4-Amino-(2-methyl-4-aminophenyl)benzamide in Mice and Rats

Martial R. Kanyonyo, Jacques H. Poupaert and Didier M. Lambert

Laboratory of Pharmaceutical Chemistry, Department of Pharmaceutical Sciences, Catholic University of Louvain, B-1200 Bruxelles, Belgium

(Received May 12, 1997; Accepted August 11, 1997)

Abstract: An original ameltolide analogue 4-amino-(2-methyl-4-aminophenyl)benzamide, in which a second amino group has been introduced, was synthesized and evaluated for anticonvulsant activity. After intraperitoneal administration to mice, 4-amino-(2-methyl-4-aminophenyl)benzamide was found active in the maximal electroshock seizure test and against the tonic seizures elicited either by bicuculline or 3-mercaptopropionic acid. 4-amino-(2-methyl-4-aminophenyl)benzamide (4A-2M4A-PB) gave anti maximal electroshock seizures ED_{50} of 63 μmol/kg (15.4 mg/kg) and a TD_{50} of 676 μmol/kg (163 mg/kg), yielding a *PI* of 10.7; the potency is similar to that of the 4-amino-(2-methyl-3-aminophenyl)phthalimide (4-A-2M3A-PP), superior to that of 4-amino-(2,6-dimethylphenyl)phthalimide (4A-2,6-DMPP), close to that of phenytoin and carbamazepine and inferior to that of ameltolide. 4A-2M4A-PB with an ED_{50} of 41[28-60] μmol/kg (9.9 mg/kg) is as active after oral administration to rats as carbamazepine, more active than ameltolide, 4-A-2M3A-PP and phenytoin and slightly less active than the 4A-2,6-DMPP. The introduction of a second amino group on the substituted phenyl ring does not affect drastically the anticonvulsant potency after intraperitoneal administration to mice; moreover, it seems to enhance the activity after oral administration. 4A-2M4A-PB is a good candidate both for further pharmacokinetic studies and for the study of the precise mechanism of action.

Epilepsy has been defined as a symptom of excessive temporary neuronal discharge, characterized by discrete recurrent episodes in which there is disturbance of movement, sensation, behaviour, perception and/or consciousness (Guelen & vander Kleijn 1978). Valproate, phenytoin, and carbamazepine are still the major drugs used today although phenytoin is much less used in Europe than in the U.S.A. Due to the numerous side effects of these molecules, and the existence of refractory epilepsies, the National Institute of Health (N.I.H.) launched in the late seventies an antiepileptic drug development program offering the possibility for research laboratories to screen any new compound in several convulsant rodents models (Krall *et al.* 1978; Porter *et al.* 1984). Quite a number of new drugs with new mechanism of actions have become already available or await their approval: vigabatrin, gabapentin, lamotrigine, oxcarbazepine, tiagabine and remacemide (Macdonald & Kelly 1995; Meldrum 1996). Because some 20% of all patients remain refractory to the current drugs or suffer unacceptable side effects, there is still a need for new antiepilectic drugs.

In this regard, Clarck *et al.* (1984, 1985, 1986 & 1987) have reported the potent anticonvulsant activity of a series of very simple chemical structures, the aminophenylbenzamides. The most active compound is the 4-amino-(2,6-dimethylphenyl)benzamide, later called ameltolide (fig. 1). Ameltolide is a potent and selective anticonvulsant: it is active in the maximal electroshock seizure model and inactive in the subcutaneous pentylenetetrazole test. The anticonvulsant profile of ameltolide is similar to those of phenytoin and carbamazepine (fig. 1) (Clarck 1990; Leander *et al.* 1988). Previous reports from our laboratory (Lambert *et al.* 1995; Poupaert *et al.* 1995) along with the works of Dr. Vamecq's team (Bailleux et al. 1994a,b & 1995) have described several ameltolide-like compounds. After intraperitoneal administration, none of them were more active than ameltolide, even if for some of them significantly higher protective index (PI), i.e. the ratio TD_{50}/ED_{50}, were found. After oral administration, many compounds were superior to ameltolide. One of the most active compound found in our previous works was the 4-amino-(2,6-dimethylphenyl)phthalimide (4A-2,6DMPP fig. 1, Bailleux *et al.* 1995, Poupaert *et al.* 1995). In this aminophenylphthalimide family, Bailleux *et al.* (1994a) reported an active anticonvulsant compound, the 4-amino-(2-methyl-3-aminophenyl)phthalimide (4A-3M4A-PP), in which a second amino group was introduced on the phenyl ring.

The aim of this study was to synthesize and to compare the anticonvulsant activity of an original aminophenylbenzamide, the 4-amino-(2-methyl-4-aminophenyl)benzamide (4A-2M4A-PB) (fig. 2) to those of ameltolide, 4-amino-(2,6-dimethylphenyl)phthalimide (4A-2,6DM-PP), 4-amino-(2-methyl-3-aminophenyl)phthalimide (4A-2M3A-PP), phenytoin and carbamazepine after intraperitoneal administration to mice or after oral administration to rats.

Author for correspondence: Didier M. Lambert, Department of Pharmaceutical Sciences, Catholic University of Louvain, Avenue E. Mounier 73, UCL-CMFA 7340, B-1200 Bruxelles, Belgium (fax +32 2 7647363).

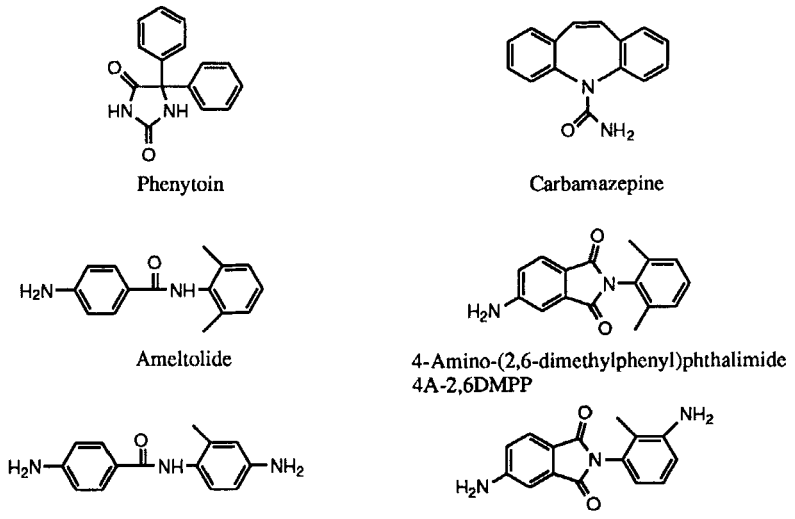

Fig. 1. Structures of phenytoin, carbamazepine, ameltolide, 4-amino-(2,6-dimethylphenyl)phthalimide (4A-2,6DMPP), 4-amino-(2-methyl-4-aminophenyl)benzamide (4A-2M4A-PB) and 4-amino-(2-methyl-3-aminophenyl)phthalimide (4A-2M3A-PP).

Materials and Methods

Chemistry. The phthalimides, i.e. 4-amino-(2,6-dimethylphenyl)phthalimide and the 4-amino-(2-methyl-3-aminophenyl)phthalimide were prepared according to the method of Poupaert *et al.* (1995). Ameltolide and 4-amino-(2-methyl-4-aminophenyl)benzamide were synthesized in our laboratory according to the procedures previously described (Lambert *et al.* 1995).

Briefly, 4-amino-(2-methyl-4-aminophenyl)benzamide was synthesized in two steps. 4-Nitrobenzoyl chloride (5 g, 26.9 mmol, Acros Chimica, Belgium) was added dropwise to 2-methyl-4-nitroaniline (4.9 g, 32.3 mmol, Acros Chimica, Belgium) dissolved in 50 ml of anhydrous pyridine and the reaction mixture was stirred overnight. The resulting precipitate was collected on a Buchner filter and washed with 300 ml 0.1 N HCl and 300 ml of water. Recrystallization from ethanol yields 87% of pure 4-nitro-(2-methyl-4-nitrophenyl)benzamide (4N-2M4N-PB). [m.p. (°C) 238–240, $C_{14}H_{11}N_3O_5$, F.W 301.261, ^{13}C NMR (DMSO-*d6*, δ ppm): 18.08 (CH_3), 121.69, 123.77, 125.61, 126.14, 129.59, 134.35, 139.87, 142.59, 144.74, 149.56 (C AROM.), 164.35 (C=O), $_1H$ NMR (DMSO d6, δ ppm): 1.66 (s, 3H), 6.85-7.56 (m, 7H), IR (KBr, v cm-1): 3280 (CONH), 1770 (C=O)].

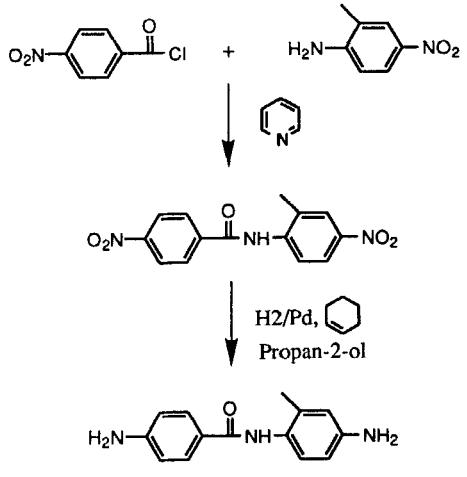

Fig. 2. Synthesis of 4 amino-(2-methyl-4-aminophenyl)benzamide.

4-Amino-(2-methyl-4-aminophenyl)benzamide was obtained by reduction of the nitro derivative after refluxing in a mixture of cyclohexene, propan-2-ol, and Pd/C. After 8 hr, the reaction mixture was filtered and the solvent was removed under reduced pressure. Seventy-five % of the desired product was obtained by recrystallization from ethanol-water mixture (1/1, v:v).

[m.p. (°C) 107-109, $C_{14}H_{15}N_3O$, F.W 241.295, ^{13}C NMR (DMSO-*d6*, δ ppm): 18.26 (CH_3), 111.67, 112.79, 115.48, 121.65, 125.88, 128.07, 129.23, 134.80, 146.73, 151.83 (C AROM.), 165.57 (C=O), $_1H$ NMR (DMSO-*d6*, δ ppm): 1.58 (s, 3H), 6.12-6.88 (m, 7H), IR (KBr, v cm-1): 3285 (CONH), 1770 (C=O)].

Anticonvulsant evaluation. Initial anticonvulsant testing of 4-amino-(2-methyl-4-aminophenyl) benzamide was conducted by the Anticonvulsant Drug Development Programme (ADD, Porter *et al.*, 1984) protocol (6vb 1817) of the Epilepsy Branch of the National Institutes of Health, Bethesda, Maryland, U.S.A). Male albino mice (CF-1 strain, 18–25 g) and male albino rats (Sprague-Dawley, 100 150 g) were used as experimental animals for intraperitoneal and oral administrations respectively. The animals have free access to food (S/L Custom Lab Diet-7) and water, except when they were removed from their cages for the experimental procedures. Drugs were suspended in 0.5% methylcellulose.

Maximal electroshock seizures test. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 50 mA (mice) or 150 mA (rats) delivered for 0.2 sec. through corneal electrodes. A drop of 0.9% sodium chloride was gently instilled in each eyes before the application of electrodes. Abolition of the hind-limb tonic extension component of the seizure was defined as protection in the maximal electroshock seizures test.

Subcutaneous pentylenetetrazol seizure test. The subcutaneous pentylenetetrazol seizure test was conducted by intraperitoneal administration of 85 mg/kg pentylenetetrazole in saline to mice, or 70 mg/kg to rats. Animals were isolated and observed over 30 min. In practice, pentylenetetrazole was dissolved in sufficient 0.9% sodium chloride solution to allow subcutaneous injections to mice and rats of volumes of 0.01 and 0.02 ml/g body weight, respectively. Protection in this test was defined as a failure to observe a single episode of clonic spasms of the tested compound during a 30 min. observation time.

Rotorod test. Neurological deficit was measured by the rotorod test. The dosed animal was placed on a 1-inch diameter knurled plastic

rod rotating at 6 rpm. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min. The median anticonvulsant potency (ED_{50}) and toxicity (TD_{50}) were determined by the graphical method.

Other chemical-induced seizures tests. Male OF1 mice (Iffa-Credo, Les Oncins, France), weighing 18 to 30 g were injected intraperitoneal administration with 30 mg/kg of 4-amino-(2-methyl-4-aminophenyl)benzamide. At the time peak effect 0.25 hr, the convulsant substances, bicuculline (2.7 mg/kg), strychnine (1.2 mg/kg) and 3-mercaptopropionic acid (40 mg/kg, 3-MPA)-were administered subcutaneously to 5 to 8 mice per group. Each animal is then isolated in an individual cage and observed during 30 min. Convulsions (clonic and tonic) were noted.

Results and Discussion

4-Amino-*N*-(2-methyl-4-aminophenyl)benzamide was synthesized in two steps as outlined in the scheme I. It is a position isomer of the open analogue of the 4-amino-*N*-(3-amino-2-methylphenyl)phthalimide (Bailleux *et al.* 1994a&b).

Initial anticonvulsant screening of 4-amino-(2-methyl-4-aminophenyl)benzamide and the intermediate product, 4-nitro-(2-methyl-4-nitrophenyl)benzamide, was conducted in mice and rats according to the protocol of the Antiepileptic Drug Development Program from the N.I.H. (Porter et al. 1984). These compounds were evaluated at two times 0.5 and 4 hr after administration for the anticonvulsant activity in the maximal electroshock and pentylenetetrazole assays respectively, and the neurotoxicity was evaluated using the rotorod test. Results from this phase I protocol indicated after intraperitoneal administration of 30, 100, 300 mg/kg of 4-amino-(2-methyl-4-aminophenyl)benzamide to mice, a 100% protection against maximal electroshock seizures after 0.5 hr but only 0, 100, 100% after 4 hr, respectively. Slight neurotoxicity was observed at 100 mg/kg and more pronounced (as mice were unable to grasp rotorod) at 300 mg/kg after 0.5 hr. The nitro intermediate does not exhibit any significant activity in the maximal electroshock seizures test and in the rotorod assays. It was slightly active in the subcutaneous pentylenetetrazol seizure test as 30 min. after intraperitoneal administration of 300 mg/kg 80% of the mice were protected. Additionally, the oral administration of 30 mg/kg of 4-amino-(2-methyl-4-aminophenyl)benzamide to rats led to a significant protection against maximal electroshock seizures without neurotoxicity (fig. 3). While protection against maximal electroshock seizures was observed in mice and rats, no significant protection against subcutaneous pentylenetetrazol seizure test was shown, as it has been reported in the case of ameltolide. Quantitative anticonvulsant results of 4-amino-(2-methyl-4-aminophenyl)benzamide dosed intraperitoneally in mice are summarized in table 1. 4-Amino-(2-methyl-4-aminophenyl)benzamide gave an anti maximal electroshock seizures ED_{50} of 63 µmol/kg (15.4 mg/kg) and a TD_{50} of 676 µmol/kg (163

Table 1.

Comparative anticonvulsant potency between 4A-2M4A-PB and ameltolide, 4A-2M3A-PP, 4A-2,6DMPP, phenytoin and carbamazepine in mice after intraperitoneal administration.

Compound	Toxicity TD_{50}[a,b]	MES ED_{50}[a,b]	PI[c]	Time of peak (hr)
4A-2M4A-PB	676 [512–926]	63 [50–77]	10.7	0.25
4A-2M3A-PP	942 [640–1292]	67 [61–81]	14.0	0.50
Ameltolide	62 [55–70]	11 [9–13]	5.6	0.25
4A-2,6DMPP	131 [112–153]	50 [42–59]	2.6	0.50
Phenytoin	259 [20–286]	38 [32–41]	6.8	2
Carbamazepine	303 [194–570]	37 [23–60]	8.2	1

MES=Maximal electroshock seizures,

[a] TD_{50} and ED_{50} values are in µmol/kg of drugs delivered intraperitoneally,

[b] Values are TD or ED_{50}; in brackets, the 95% confidence interval.

[c] PI is the protective index defined as the ratio TD_{50}/ED_{50}.

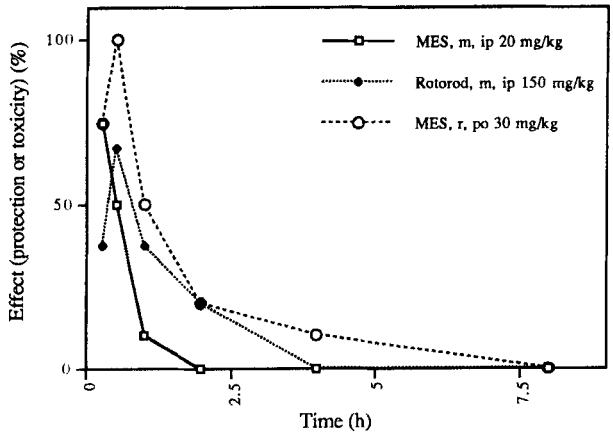

Fig. 3. Time-courses of the effect of 4-amino-(2-methyl-4-aminophenyl)benzamide in the maximal electroshock seizure test (after intraperitoneal administration of 20 mg/kg to mice □; after oral administration of 30 mg/kg to rats ○) and in the rotorod tests (after intraperitoneal administration of 150 mg/kg to mice ◆).

Table 2.

Activity of 4-amino-(2-methyl-4-aminophenyl)benzamide (4A-2M4A-PB) in different chemical-induced seizures after intraperitoneal administration of 30 mg/kg in suspension in 0.5% methylcellulose to mice (n=5–9).

Treatment	Clonic seizures (%)	Tonic seizures (%)
Pentylenetetrazole		
Controls	100	100
4A-2M4A-PB	100	100
3-Mercaptopropionic acid		
Controls	100	100
4A-2M4A-PB	90	40*
Strychnine		
Controls	–	100
4A-2M4A-PB	–	100
Bicuculline		
Controls	–	100
4A-2M4A-PB	–	22**

Different from controls by Mann-Whitney and Chi-squared test *P<0.1 and **P<0.05.

Table 3.

Comparative anticonvulsant potency between 4A-3M4A-PB and ameltolide, 4A-2M3A-PP, 4A-2,6DMPP, phenytoin and carbamazepine after oral administration in rats.

Compound	Toxicity TD_{50}[a,b]	MES ED_{50}[a,b]	PI[c]	Time of peak (hr)
4A-2M4A-PB	>820	41 [28–60]	>20	0.50
4A-2M3A-PP	>1872	75 [62–85]	>25	0.50
Ameltolide	1912 [1547–2284]	135 [122–150]	14.2	1
4A-2,6DMPP	>1688	25 [17–38]	>75	0.25
Phenytoin	>11900	118 [87–154]	>100	0.5
Carbamazepine	3441 [2069–5222]	36 [14–45]	95.6	1

[a] TD_{50} and ED_{50} values are in µmol/kg of drugs delivered orally.
[b] Values are TD or ED_{50}; in brackets, the 95% confidence interval.
[c] PI is the protective index defined as the ratio TD_{50}/ED_{50}.

mg/kg), yielding a protective index of 10.7. So, the potency of 4A-2M4A-PB is similar to that of the phenylphthalimide 4A-2M3A-PP, superior to the 4A-2,6-DMPP and inferior to that of ameltolide. Compared to standard antiepileptic drugs like phenytoin and carbamazepine, the potency of 4A-2M4A-PB is close to those of the two reference compounds. The protective index of this compound is higher than the other compounds tested in this study, except for the 4A-2M3A-PP PI. The addition of a second amino group in the benzamide family does not seem to drastically affect the potency of the compound while it strongly reduces the neurotoxicity. At 30 mg/kg injected intraperitoneally to mice, 4A-2M4A-PB was not active against clonic seizures in the different chemical-induced seizures tests (table 2). However, it slightly protects against tonic seizures in the 3-mercaptopropionic and in the bicuculline tests.

After oral administration to rats, 4A-2M4A-PB exhibits a similar anticonvulsant profile : active in the maximal electroshock seizures and inactive in the subcutaneous pentylenetetrazol seizure test. Quantitative results of 4-amino-(2-methyl-4-aminophenyl)benzamide after oral administration to rats are summarized in table 3. 4A-2M4A-PB with an ED_{50} of 41 [28–60] µmol/kg (9.9 mg/kg) is as active after oral administration to rats as carbamazepine, more active than ameltolide, 4-A-2M3A-PP and phenytoin and slightly less active than the 4A-2,6-DMPP. Despite the difference in species, the fact that the ED_{50}'s of 4A-2M4A-PB after intraperitoneal and oral administrations are close seems to indicate a good bioavailibity. 4A-2M4A-PB is superior to ameltolide on this point of view. Pharmacokinetic studies are planned in order to compare the bioavalibility of these two compounds.

Up to now, the mechanism of action of 4A-2M4A-PB and of ameltolide and congeners remains unknown. According to their anticonvulsive profile, a direct action on the neurotransmitters GABA and glycine does not seem to be implicated. Further studies are clearly necessary to better characterize these compounds.

The introduction of a second amino group on the substituted phenyl ring does not affect drastically the anticonvulsant potency after intraperitoneal administration to mice; moreover, it seems to enhance the activity after oral administration, probably due to pharmacokinetic factors. Amino position isomers are now synthesized in order to define the best orientation for an optimal anticonvulsant activity.

Acknowledgements

The authors deeply appreciate the assistance of the Antiepileptic Drug Development Program, Epilepsy branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, Maryland, USA and in particular, the kind help of Dr. James P. Stables. M. K. is grateful to the International Cooperation Service of the Catholic University of Louvain for the award of a fellowship.

References

Bailleux, V., L. Vallée, J. P. Nuyts & J. Vamecq: Anticonvulsant activity of some 4-amino-*N*-phenylphthalimides and *N*-(3-amino-2-methyl phenyl)phthalimides. *Biomed. Pharmacother.* 1994a, **48**, 95–101.

Bailleux, V., L. Vallée, J. P. Nuyts & J. Vamecq: Synthesis and anticonvulsant activity of some phenylphthalimides. *Biomed. Pharmacother.* 1994b, **42**, 1817–1821.

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

Clark, C. R., M. J. M. Wells, R. T. Samson, G. N. Norris, R. C. Dockens & W. R. Ravis: Anticonvulsant activity of some 4-aminobenzamides. *J. Med. Chem.* 1984, **27**,779–782.

Clark, C. R., C. M. Lin & R. T. Samson: Anticonvulsant activity of some 2-and 3-aminobenzanilinides. *J. Med. Chem.* 1985, **29**, 1534–1537.

Clark, C. R., R. T. Samson, C. M. Lin & G. N. Norris: Anticonvulsant activity of some 4-aminobenzanilinides. *J. Med. Chem.* 1986, **28**, 1259–1262.

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

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*, 1990, **29**, 198–203.

Guelen, P. J. M & E. vander Kleijn: *Rational anti-epileptic drug therapy.* Elsevier, Amsterdam, 1978, p. 1.

Krall, R. L., J. K. Penry, B. G. White, H. J. Kupferberg & E. A. Swinyard: Antiepileptic drug development: II anticonvulsant drug screening. *Epilepsia* 1978, **19**, 409–428.

Lambert, D. M., G. Hamoir, E. Hermans & J. H. Poupaert: Synthesis and anticonvulsant activity of 4-amino-*N*-(2-ethylphenyl)benzamide and 4-amino-*N*-(2,6-diethylphenyl)benzamide, two new ameltolide analogues. *Pharm. Sci.* 1995, **1**, 181–184.

Leander, J. D., R. R. Lawson & D. W. Robertson: Anticonvulsant effects of a novel aminobenzamide (LY201116) in mice. *Neuropharmacology* 1988, **27**, 623–628.

Macdonald, R. L. & K. M. Kelly: Antiepileptic drug mechanisms of action. *Epilepsia* 1995, **36**, S2–S12.

Meldrum, B. S.: Update on the mechanism of action of antiepileptic drugs. *Epilepsia* 1996, **37**, S4–S11.

Porter, R. J., J. J. Cereghino, G. D. Gladding, B. J. Hessie, H. J. Kupferberg, B. Scoville & B. G. White: Antiepileptic drug development programm. *Cleve. Clin. Q.* 1984, **51**, 293–305.

Poupaert, J. H., G. Hamoir, P. Barbeaux, D. M. Lambert & J. P. Hénichart: Anticonvulsant activity of some *N*-phenylphthalimide derivatives in rats and mice. *J. Pharm. Pharmacol.* 1995, **47**, 89–91.