Anticonvulsant Activity of Some 4-Methoxy- and 4-Chlorobenzanilides

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Abstract □ A series of mono-, di-, and trimethylated derivatives of 4-chloro- and 4-methoxybenzanilide was synthesized and evaluated for anticonvulsant activity. This series was prepared in the course of studies designed to examine the relationship between anticonvulsant effects and benzamide structure. The compounds were tested in mice against seizures induced by maximal electroshock (MES) and pentylenetetrazole (scMet), as well as with the rotorod assay for neurologic deficit. In mice dosed intraperitoneally, 4-methoxy-2, 6-dimethylbenzanilide (**4**) showed a median anticonvulsant potency (ED_{50}) of 18.58 mg/kg in the MES test and a median toxicity (TD_{50}) of 133.72 mg/kg in the rotorod toxicity assay, yielding a protective index (PI = TD_{50}/ED_{50}) of 7.2. In mice dosed orally with **4**, the anti-MES ED_{50} was 27.40 mg/kg and the TD_{50} dose was determined to be 342.58 mg/kg, resulting in a protective index of 12.5

Epilepsy has been defined as a symptom of excessive temporary neuronal discharge, characterized by discrete recurrent episodes in which there is a disturbance of movement, sensation, behavior, perception, and/or consciousness.[1] The prevalence of epilepsy is 0.3 to 0.6% of the population,[2] and it is generally recognized that only ~60% of patients are completely controlled with presently available medications.[3] Many of the currently available prototype anticonvulsant drugs are derived from the barbiturate hydantoin or structurally similar heterocyclic ring systems. These structurally similar compounds often share similar anticonvulsant spectra of action and side effects. These and other therapeutic factors point to the need for new anticonvulsant agents.

A series of prior reports from this laboratory[4–8] and others[8–10] has described the anticonvulsant activity of numerous aminobenzamides of alkyl- and arylamines. Many of these amides show a high degree of protection against maximal electroshock (MES)-induced convulsions[4–7] in animal models. The compounds are less effective in the subcutaneous pentylenetetrazole (scMet)[4–7] model of induced convulsions. The initial studies in this series of amides have shown the 4-aminobenzamides to be more effective anticonvulsants than the 3-amino derivatives, while the monosubstituted 2-aminobenzamides are essentially inactive in most anticonvulsant tests. Maximum anticonvulsant activity in the 4-aminobenzamides is found in those compounds derived from aryl- or arylalkylamines. Recent studies[11] on a series of 4-aminophenylacetamides have shown significant loss in anticonvulsant activity from this insertion of a methylene moiety between the aromatic ring and the amide carbonyl group of the aminobenzamide. The 4-methoxy- and 4-chlorobenzanilides reported in this paper were prepared as a continuation of our studies on the SAR of anticonvulsant benzamides.

Experimental Section

Melting points were determined in open glass capillaries using a Thomas-Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in chloroform solutions in matched sodium chloride cells. All ^1H NMR spectra were measured in $CDCl_3$ on a Varion T-60A spectrometer with an internal standard of tetramethylsilane. Elemental analyses, (C,H,N) were performed by Atlantic Microlab (Norcross, GA) and the results obtained were within ±0.4 of the calculated percentage. The following experimental procedure is representative of the general procedure used to synthesize all the compounds. Experimental data for these are provided in Table I.

Synthesis of 4-Substituted Benzanilides—A solution of the appropriate aniline (0.03–0.05 mol) in 25 mL of tetrahydrofuran was added to 100 mL of 20% (w/v) aqueous potassium carbonate in a 500-mL three-necked flask equipped with a reflux condenser, addition funnel, heating mantle, and magnetic stirrer. A solution of 4-methoxybenzoylchloride (twofold molar excess) in 25 mL of tetrahydrofuran was added in a dropwise manner, and the resulting mixture was refluxed for 12 h. The solution was then cooled to room temperature and extracted with chloroform (3 × 75 mL). The extracts were combined, dried over magnesium sulfate, and evaporated. The resulting residues were then purified by recrystallization in toluene.

Pharmacology—Initial anticonvulsant evaluation of these compounds was conducted using three dose levels of 30, 100, and 300 mg/Kg. All tests were performed using male Carworth Farms number-one mice. Test solutions of all compounds were prepared in 30% polyethylene glycol 400, and animals were dosed intraperitoneally 30 min prior to testing.

Maximal electroshock seizures (MES) were produced with a 60-cycle alternating current of 50-mA intensity, delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of electrodes. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test.

The subcutaneous pentylenetetrazole (scMet) seizure threshold test was conducted by administration of 85 mg/kg of pentylenetetrazole as a 0.5% solution in the posterior midline. Protection in this test was defined as a failure to observe a single episode of clonic spasms of at least 5-s duration during a 30-min period following administration of the test compound.

Neurological deficit was measured in mice by the use of the rotorod test. The dosed animal was placed on a 1-inch diameter knurled plastic rod rotating at 6 rpm. Neurological 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.

Results and Discussion

A series of 4-chloro- and 4-methoxybenzanilides was prepared according to well-established synthetic procedures. The various substituted anilines were treated with 4-chlorobenzoylchloride or 4-anisoylchloride under basic conditions to yield the desired anilides. The compounds were purified by recrystallization and the physical properties are reported in Table I. These 4-substituted benzanilides were prepared as a part of our continuing structure–activity relationship studies in benzamides. In previous studies[5,6] on a large series of *N*-substituted benzamides, the 4-aminobenzoyl moiety produced maximum anticonvulsant activity. The 4-amino substitution pattern was more potent than either the 3- or 2-amino substitution in the benzamides. The anticonvulsant potency was further heightened by aryl group substitution on

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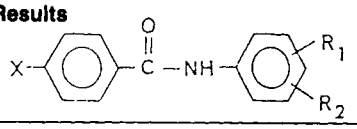
Table I—Anticonvulsant and Toxicity Screening Results



Compound	R_1	R_2	X	mp, °C	MES[a]		scMet[a]		Tox[a,b]	
					30 min	4 h	30 min	4 h	30 min	4 h
1	$2CH_3$	H	OCH_3	165–66	0	0	0	0	0	0
2	$3CH_3$	H	OCH_3	90–92	0	0	0	0	0	1
3	$4CH_3$	H	OCH_3	145–48	0	0	0	0	0	1
4	$2CH_3$	$6CH_3$	OCH_3	157–58	3	2	3	0	0	0
5	$2CH_3$	$5CH_3$	OCH_3	134–36	1	0	0	0	0	0
6	$2CH_3$	$4CH_3$	OCH_3	136–38	0	1	0	0	0	0
7	$2CH_3$	$3CH_3$	OCH_3	175–78	0	1	0	0	1	0
8	$3CH_3$	$4CH_3$	OCH_3	99–102	1	1	2	0	1	0
9	$3CH_3$	$5CH_3$	OCH_3	112–115	0	0	0	0	1	0
10	2Cl	$6CH_3$	OCH_3	158–69	3	1	0	0	1	0
11	$2,4,6\text{-}CH_3$	—	OCH_3	173–75	1	0	0	0	0	0
12	$2CH_3$	H	Cl	148–52	0	0	0	0	0	1
13	$3CH_3$	H	Cl	122–25	0	0	0	0	0	0
14	$4CH_3$	H	Cl	189–90	0	0	0	1	0	0
15	$2CH_3$	$6CH_3$	Cl	174–76	2	0	0	1	0	0
16	$2CH_3$	$5CH_3$	Cl	152–56	0	0	0	0	1	2
17	$2CH_3$	$4CH_3$	Cl	160–63	0	1	0	0	0	1
18	$2CH_3$	$3CH_3$	Cl	174–77	0	0	0	1	1	2
19	$3CH_3$	$4CH_3$	Cl	130–34	0	0	0	0	0	0
20	$3CH_3$	$5CH_3$	Cl	130–33	0	0	0	0	0	0
21	2Cl	$6CH_3$	Cl	174–78	3	2	1	0	0	0
22	$2,4,6\text{-}CH_3$	—	Cl	213–15	0	1	0	0	2	2

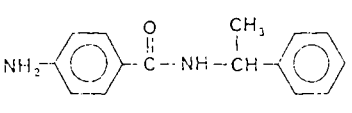
[a] 3 = activity at 30 mg/kg; 2 = activity at 100 mg/kg; 1 = activity at 300 mg/kg; and 0 = no activity at 300 mg/kg. [b] Determined by the rotorod procedure.

the amide nitrogen. The role of the aromatic amino group in the anticonvulsant activity of the benzamides may be to serve as either a donor or acceptor of a hydrogen bond upon interaction with the biophase. The 4-chloro- and 4-methoxybenzamides in this study were prepared to examine the role of hydrogen bond acceptor moieties at the 4-position of the benzamides.

The results of the initial anticonvulsant and toxicity screening of the 4-methoxy- and 4-chlorobenzamides are reported in Table I. The time of peak anticonvulsant activity was not established in these screening tests and toxicities were only estimated. The monomethylated 4-methoxybenzanilides, **1–3**, were inactive in both seizure tests at all dose levels and only **2** and **3** showed rotorod toxicity at 4 h in one of two animals dosed at 300 mg/kg. The 4-methoxyamides of the dimethylanilines, **4–9**, showed varying levels of activity in the anticonvulsant and toxicity tests. Compound **4**, the 2,6-dimethyl derivative, showed both anti-MES and scMet activity 30 min after administration at the lowest dosing level (30 mg/kg). The anti-MES activity was observed in two of three animals at 4 h after the administration of the 100-mg/kg dose, and rotorod toxicity was absent in all four animals dosed at 300 mg/kg. Compounds **5–8** showed some slight anticonvulsant activity at the higher dosing levels; however, the activity was not significant enough to warrant quantitation. The 3,5-dimethyl derivative **9** was inactive in all tests at the highest doses tested. The 2,4,6-trimethyl derivative was also essentially inactive. The isomeric 2-chloro-6-methyl analogue **10** showed anti-MES activity 30 min after administration of 30 mg/kg and rotorod toxicity at a dose of 300 mg/kg. From these initial screening results, **4** and **10** emerged as those showing the most potential as anticonvulsants.

In the 4-chlorobenzanilide series, the screening results again indicated that the same aniline substitution pattern was important for anticonvulsant activity. The monomethylaniline derivatives were essentially inactive in all screens at all dosing levels. The 2,6-dimethylaniline derivative **15** showed anti-MES activity at a dose of 100 mg/kg 30 min after ip dosing and no toxicity at a dose of 300 mg/kg. The other isomeric dimethyl derivatives showed little activity above the baseline levels. The 2-chloro-6-methylbenzanilide showed significant anti-MES activity at the 30-mg/kg dosing level 30 min after administration. The 100-mg/kg dose of **21** maintained anti-MES activity 4 h following administration, while no toxic symptoms were observed at the highest dose tested (300 mg/kg). From these initial screening results, the 2,6-dimethyl- and the 2-chloro-6-methylbenzanilide, **15** and **21**, again emerged as those showing the most potential as anticonvulsants in the 4-chlorobenzanilide series.

The quantitative anticonvulsant and toxicity data for selected 4-chloro- and 4-methoxybenzanilides are given in Table II along with data for some standard antiepileptic drugs. The ED_{50} values were determined against MES-induced convulsion and the TD_{50} values were measured by the rotorod procedure following ip administration of the test compound in mice. None of the 4-substituted benzamides was sufficiently active to obtain ED_{50} values against scMet-induced convulsions. The 4-chlorobenzanilides were generally less toxic and less active than the 4-methoxy derivatives. The most active 4-chlorobenzanilide, **15**, has an anti-MES ED_{50} of 48.39 mg/kg and a TD_{50} of 294.36 mg/kg, producing a good PI value ($PI = TD_{50}/ED_{50}$) of 6.1. Compound **21** was less active, having an ED_{50} of 104.04 mg/kg and, although an exact TD_{50} was not determined, rotorod toxicity was observed in two of eight animals dosed at 225 mg/kg. The 4-methoxybenzanilides **4** and **10** displayed similar anti-MES



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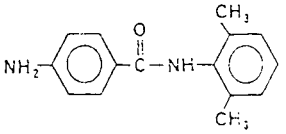

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Table II—Quantitative Anticonvulsant Data for Selected 4-Substituted Benzanilides in Mice

Compound	TD_{50}[a,b]	MES	
		ED_{50}[b]	PI[c]
4	133.72 (122.64–142.01)[d]	18.58 (14.29–21.91)[d]	7.2
10	123.65 (107.49–139.30)	14.35 (11.88–16.76)	8.6
15	294.36 (205.72–385.39)	48.39 (37.93–64.74)	6.1
21	Not determined	104.04 (98.67–111.71)	—
23	170.78 (153.02–189.96)	18.02 (13.41–21.43)	9.5
24	15.01 (13.27–16.88)	2.60 (2.18–3.07)	5.8
Phenobarbital	69.01 (62.84–72.89)	21.78 (14.99–25.52)	3.2
Phenytoin	65.46 (52.49–72.11)	9.50 (8.13–10.44)	6.9
Valproic acid	424.84 (368.91–450.40)	271.66 (246.97–337.89)	1.57

[a] Rotorod procedure. [b] Doses reported in mg/kg. [c] Protective index = TD_{50}/ED_{50}. [d] 95% confidence limits.

activity having ED_{50} values of 18.58 and 14.35 mg/kg, respectively. The 2-chloro-6-methyl derivative **10** showed slightly lower ED_{50} and TD_{50} values, yielding a PI of 8.6 for these tests, while **4**, the 2,6-dimethylbenzanilide, has a higher TD_{50} of 133.72 mg/kg, producing a PI value of 7.2.

The anticonvulsant activity for the two most potent compounds identified in testing in the mouse, **4** and **10**, can be compared with that of some standard antiepileptic drugs in the same tests. The data in Table II show that phenobarbital administered ip in mice produces an anti-MES ED_{50} of 21.78 mg/kg and a TD_{50} of 69.01 mg/kg in the rotorod test (PI = 3.2). Phenytoin is slightly more potent than **4** or **10** with an ED_{50} of 9.5 mg/kg; however, the resulting PI of 6.9 is similar to that of the two 4-methoxybenzanilides. The activity of **4** and **10** should also be compared with the more active anticonvulsant 4-aminobenzamides identified in our previous studies.[4-6] Compounds **23** and **24** have anti-MES ED_{50} values of 18.02 and 2.6 mg/kg, respectively. Thus, **4**, **15**, and **24** allow a direct comparison of the effect of the 4-substituent on anticonvulsant activity. Replacement of the 4-amino group in **24** by either methoxy or chloro substituents results in decreased activity. However these derivatives maintained good activity, with **4** being the more potent on a mg/kg basis. Compound **4** was also evaluated in mice following oral administration. The results indicate that **4** if much less toxic via the oral route, showing a TD_{50} of 342.58 mg/kg. The anti-MES activity is also reduced, producing an ED_{50} of 27.4 mg/kg; however, the resulting PI of 12.5 is quite high, indicating an excellent separation between anticonvulsant and toxic effects in this test.

Compounds **10** and **21** were screened for toxicity and anti-MES activity following oral administration in the rat. Compound **10** gave an anti-MES ED_{50} of 26.08 mg/kg and a time of peak anticonvulsant effect of 1 h. No rotorod toxicity was observed in any animals at doses up to 500 mg/kg with testing done from 0.5 through 24 h. The anti-scMet activity was also absent at all doses up to 500 mg/kg. Compound **21** produced anticonvulsant activity against MES at an ED_{50} of 36.39 mg/kg in the rat following oral administration, with a time of peak effect at 2 h after administration. Again, no activity against scMet-induced convulsions was noted and no rotorod toxicity was observed at doses up to 500 mg/kg, with testing from 0.25 through 24 h.

Although these studies did not produce compounds with anticonvulsant activity equal to or greater that that of **24**, the replacement of the 4-amino group in **24** with hydrogen bond-acceptor groups, such as methoxy and chloro, maintained good anti-MES potencies with excellent protective indices. One of the major problems with the 4-aminobenzamides is the rapid inactivation via metabolic acetylation of the aromatic amino group.[9] Thus, although less potent, a more metabolicly inert 4-substituent may be quite suitable in the development of a potential therapeutic agent from the benzamide class.

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