Anticonvulsant Activity of Some 4-Aminophenylacetamides

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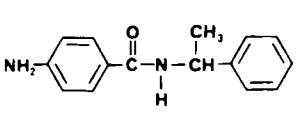
Abstract □ A series of 4-aminophenylacetamides was prepared and evaluated for anticonvulsant activity. These compounds were prepared during studies designed to determine the relationship between benzamide-like compounds and anticonvulsant effects. Unlike benzamides, these phenylacetamides have a methylene group between the aromatic ring and the amide carbonyl. Consequently, formal conjugation is lost, and the number of conformational degrees of freedom has increased. The compounds were tested in mice against seizures induced by electroshock and pentylenetetrazol, and in the rotorod assay for neurologic deficit. The more active and selective anticonvulsants prepared in this study were those having an additional aromatic ring as part of the substituent on the amide nitrogen. Compound **16**, the 4-aminophenylacetamide derived from 2,6-dimethylaniline, was the most potent compound observed (ED_{50} = 50.50 mg/kg against electroshock-induced convulsions and ED_{50} = 93.20 mg/kg against pentylenetetrazol-induced convulsions).

The search for new antiepileptic drugs has not reached the point of focusing entirely on compounds that modify precise neuronal transmissions, but still is based on compounds active in various animal models of epilepsy.[1] The precise mechanisms by which clinically useful antiepileptic drugs exert their anticonvulsant activity are not well understood.[2] However, the role of γ-aminobutyric acid (GABA) mimetics in the treatment of epilepsy is well known.[3]

Recent reports[4-6] from this laboratory have described the anticonvulsant activity of numerous amino-substituted benzamides derived from alkyl- and arylamines. Several of these amides produce a high level of protection against seizures in animal models induced by maximal electroshock (MES). These compounds are less effective against convulsions induced by subcutaneous pentylenetetrazol (scMet). The profile of anticonvulsant activity and toxicity of the more potent analogues resembles those of phenobarbital and phenytoin.

Structurally, some of the simplest compounds possessing anticonvulsant properties are carboxylic acids and their amides.[7] Valproic acid is perhaps the best known example of this class of compounds.[8] Valproic acid amide has been shown[9] to be approximately twice as potent as valproic acid. Various reports[10,11] have described the anticonvulsant effects of substituted cinnamamides and several derivatives of 3-phenyl-2-piperidinone have been shown to possess anti-MES and anti-scMet activity in animal models.[12]

The unique behavioral profile produced in animals by substituted benzamide neuroleptics such as metoclopramide has generated considerable interest in recent years.[13] The benzamide neuroleptics are useful in the treatment of schizophrenia and appear to act selectively on a subpopulation of the D-2 type dopamine receptors.[14] The 4-aminophenylacetamides reported in this paper were prepared in an effort to determine the role of conjugation in the aminobenzoyl moiety on anticonvulsant activity. These compounds represent a continuation of our studies on the relationship between benzamide-like compounds and anticonvulsant effects.



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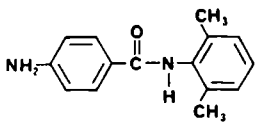

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Experimental Section

All melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. The IR spectra were recorded on a Beckman 4230 IR spectrophotometer and ^1H NMR spectra were recorded on a Varian T-60A spectrometer with Me_4Si as an internal standard. Samples for NMR analysis were dissolved in $CDCl_3$ and, when necessary, Me_2SO-d_6 was added to aid dissolution. Elemental analyses (C, H, N) were performed by Atlantic Microlabs, Inc., (Atlanta, GA); the results obtained were within ±0.4 of the calculated percentages.

4-Nitrophenylacetyl Chloride—To a suspension of 15 g (0.08 mol) of 4-nitrophenylacetic acid in ~100 mL of benzene was added a solution of 40 g (0.33 mol) of thionyl chloride in 100 mL of benzene. The thionyl chloride was added in a dropwise manner with stirring. The resulting mixture was stirred at 65–70 °C until the IR scan showed the complete disappearance of the acid carbonyl. The solvent was removed under reduced pressure to yield a yellowish oil which solidified upon cooling. Recrystallization from petroleum ether:benzene (10:1) yielded large yellow needles; mp 46–47 °C. Typically, the crude acid chloride was used in the subsequent acylation step.

4-Nitrophenylacetamides—To a solution of 200 mL of 20% K_2CO_3 in H_2O and 0.04 mol of amine in 35 mL of tetrahydrofuran was added a 50-mL solution of ~0.08 mol of the crude acid chloride in tetrahydrofuran in a dropwise manner. The resulting mixture was stirred at 50 °C for 12 h, then cooled and extracted with $CHCl_3$ (3 × 75 mL). The combined extracts were dried with $MgSO_4$ and evaporated to yield a brown, crystalline solid.

4-Aminophenylacetamides—A solution of 5.0 g of nitroacetamide in 150 mL of absolute ethanol and 250 mg of 5% palladium on carbon were added to a Parr flask. The vessel was installed on a Parr hydrogenator and the mixture was shaken at 50 psi until no further H_2 uptake could be detected. The mixture was filtered through Celite and the solvent was removed under reduced pressure to yield a yellow solid that was purified by recrystallization.

Pharmacology—Initial anticonvulsant evaluation of these compounds was conducted by using three dose levels (30, 100, and 300 mg/kg) and, in some cases, a fourth (600 mg/kg). All tests were performed on male Carworth Farms number-one mice. Test solutions of all compounds were prepared in polyethylene glycol 400:water (30:70, v/v). Animals were dosed intraperitoneally 30 min prior to testing.

Maximal electroshock seizures (MES) were induced by applying an alternating current of 60 Hz and 50 mA via corneal electrodes. A drop of 0.9% saline was put on the eye prior to application of the electrodes. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test.

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

Neurological deficit was measured in mice 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.

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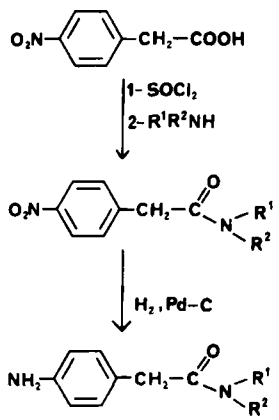
Results and Discussion

A series of 4-aminophenylacetamides was prepared according to the methods outlined in Scheme I. The 4-nitrophenylacetyl chloride was prepared from the acid using excess thionyl chloride in refluxing benzene. The crude acid chloride was allowed to react with the appropriate amine under basic conditions to produce the corresponding 4-nitrophenylacetamide. Catalytic hydrogenation of the aromatic nitro group under low pressure (50 psi H_2) yielded the 4-aminophenylacetamides. The physical properties of these 4-aminophenylacetamides are given in Table I.

Previous studies[4,5] have identified 1 and 2 as potent aminobenzamide anticonvulsants. Structure–activity relationship studies[6] have shown that the 4-aminobenzamides are more potent anticonvulsants than the 3-aminobenzamides, and the 2-aminobenzamides are essentially inactive against MES-induced convulsions. Compound 2, having an ED_{50} of 2.60 mg/kg following ip administration, is the most potent anti-MES anticonvulsant identified in our studies.

The phenylacetamide analogues of 1 and 2 differ from 1 and 2 in having a methylene group between the aromatic ring and the amide carbonyl. Addition of the methylene group increases the distance between the ring and the carbonyl and removes the amide moiety from formal conjugation with the aromatic ring. The electronic differences between benzamides and phenylacetamides can be demonstrated by noting the pK_a differences between *p*-aminobenzoic acid (PABA) and 4-aminophenylacetic acid (PAPA). For PABA, the carboxylic acid pK_a is 4.8 and the aromatic amino group pK_a is 2.51.[15] For PAPA, these values are 5.2 and 3.5, respectively.[15] The extended conjugation in PABA provides greater delocalization of the lone pair of electrons on the ring nitrogen. Thus, as a result of its extended system of conjugation, PABA is less basic than PAPA. The amino group of PAPA is a full order of magnitude more basic than that of PABA.



Scheme I

A more direct index of these electronic differences is a comparison of the carbonyl absorptions in the IR spectra of 12 and its benzamide homologue 1. The difference in carbonyl absorption of 1 (1640 cm^{-1}) and 12 (1655 cm^{-1}) indicates a greater electron density at the carbonyl carbon of 1. In addition to electronic and intramolecular distance variations, the phenylacetamides display more conformational flexibility than the benzamides. These considerations make the addition of a methylene group between the aromatic ring and the amide carbonyl a potentially dramatic modification in effects on possible receptor interactions.

Results of the initial anticonvulsant screening of the 4-aminophenylacetamides are reported in Table I. The preliminary screening was done at doses of the test compounds ranging from 30 to 600 mg/kg, administered ip in mice and evaluated against both MES- and scMet-induced convulsions, and in the rotorod assay for neurologic deficit. The responses were determined 30 min and 4 h after dosing; the time of peak effect was not established. The 4-nitrophenylacetamides were essentially inactive in the anticonvulsant tests even at the high dosing levels. The lack of anticonvulsant activity in the nitro amides is consistent with observations of the nitrobenzamide derivatives.[4,5]

The initial anticonvulsant testing of the alkyl-substituted secondary amides 3–9 showed the higher homologues 8 and 9 to be the most active. The *n*-hexylamide showed activity against both MES and scMet at a dose of 100 mg/kg; however, rotorod toxicity was also evident. In general, rotorod toxicity was present at the same dose level that produced anticonvulsant effects in the alkyl-substituted secondary amides. The two alkyl-substituted tertiary amides 10 and 11 gave a similar activity profile. Compound 11 showed anti-MES activity at 100 and 300 mg/kg, 30 min and 4 h after administration, respectively. Toxicity was also evident at similar doses. Thus, the differences in quantity between effective and toxic doses of the alkyl secondary and tertiary amides appeared very small.

Table I—Physical Properties and Anticonvulsant Screening Data for 4-Aminophenylacetamides[a,b]

Compound	R_1	R_2	mp, °C	Yield,%[c,d]	(scMet)[e]		(MES)[e]		Toxicity[e,f]	
					30 min	4 h	30 min	4 h	30 min	4 h
3	H	CH_3	90–92	53[g]	—[h]	—[h]	—[h]	1	—[h]	—[h]
4	H	C_2H_5	116–118	50	—[h]	—[h]	1	—[h]	—[h]	—[h]
5	H	*n*-C_3H_7	113–115	53	1	1	2	1	2	—[h]
6	H	*n*-C_4H_9	96–97	51	2	—[i]	2	2	2	—[i]
7	H	sec-C_4H_9	132–134	53	1	—[h]	—[h]	—[h]	1	—[h]
8	H	*n*-C_5H_{11}	89–90	55	2	2	2	2	2	—[i]
9	H	*n*-C_6H_{13}	85–87	50	3	—[i]	3	—[i]	3	—[i]
10	CH_3	CH_3	99–100	54	—[h]	—[h]	2	—[i]	—[h]	—[h]
11	*n*-C_3H_7	*n*-C_3H_7	70–72	63[k]	2	—[i]	3	2	2	2
12	H	$CH(CH_3)C_6H_5$	118–120	58	—[i]	—[i]	2	1	1	1
13	H	C_6H_5	138–140	54	2	—[h]	3	1	2	1
14	H	$CH_2C_6H_5$	136–138	60	2	1	2	—[i]	2	—[h]
15	H	$CH_2CH_2C_6H_5$	76–77	60	2	—[h]	2	—[i]	2	—[h]
16	H	2,6-dimethylphenyl	180–181	66	—[i]	—[i]	3	—[i]	2	—[i]

[a] The infrared and nuclear magnetic resonance (^1H) spectra were consistent with structural assignments. [b] The elemental analyses (C, H, and N) for all compounds were within ±0.4 of the calculated percentage. [c] Yields based on the recovery of recrystallized product. [d] Compounds recrystallized from toluene unless otherwise noted. [e] 3 = activity at 100 mg/kg; 2 = activity at 300 mg/kg; 1 = activity at 600 mg/kg. [f] Determined by the rotorod procedure. [g] Recrystallized from toluene:petroleum ether (bp 30–60 °C). [h] No activity at 600 mg/kg. [i] No activity at 300 mg/kg. [j] No activity at 100 mg/kg. [k] Recrystallized from benzene:acetone.

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Table II—Quantitative Anticonvulsant Activity of 4-Aminophenylacetamides

Compound	TD_{50},[c] mg/kg	(MES)[a]		(scMet)[b]	
		ED_{50},[d] mg/kg	PI[e]	ED_{50},[d] mg/kg	PI[e]
1	170.78 (153.02–189.96)[f]	18.02 (13.41–21.43)[f]	9.5	41.72 (38.83–46.00)[f]	4.1
2	15.01 (13.27–16.88)	2.60 (2.18–3.07)	5.77	—	—
12	108.03 (95.8–121.10)	69.46 (60.80–76.50)	1.56	94.79 (83.93–109.71)	1.14
14	230.36 (194.00–255.20)	92.08 (80.70–107.12)	2.50	152.41 (111.20–223.41)	1.51
15	547.69 (366.10–746.90)	82.19 (62.20–96.71)	6.66	157.92 (111.20–223.41)	3.47
16	285.57 (266.70–314.10)	50.50 (43.60–60.20)	5.65	92.03 (71.11–115.93)	3.10
Phenobarbital	69.01 (62.84–72.89)	21.78 (14.99–25.52)	3.17	13.17 (15.87–15.95)	5.24
Phenytoin	65.46 (52.49–72.11)	9.50 (8.13–10.44)	6.89	—	—
Valproic Acid	424.84 (368.91–450.40)	271.66 (246.97–337.89)	1.57	148.59 (122.64–177.02)	2.87

[a] Seizures induced by maximal electroshock. [b] Seizures induced by sc pentylenetetrazol. [c] Toxic dose 50 determined by the rotorod procedure. [d] Effective dose 50. [e] PI = protective index = TD_{50}/ED_{50}. [f] Ninety-five percent confidence limits.

Initial anticonvulsant screening of the amides **12–16**, prepared from aryl- and arylalkylamines, indicated some significant differences between toxic and effective doses. Compound **12**, a direct homologue of **1**, showed activity at 100 mg/kg in the anti-MES screen. Compound **16**, the direct homologue of **2**, showed an activity profile similar to that of **12**. The toxicity of **12** and **16** was observed at 600 and 300 mg/kg, respectively.

From the initial anticonvulsant and toxicity screening results, compounds **12**, **14**, **15**, and **16** were selected for quantitative evaluation. Table II shows the results of this study as well as the values obtained for **1**, **2**, and some standard anticonvulsant drugs. Compound **12** gave an anti-MES value of 69.46 mg/kg and an anti-scMet value of 94.76, yielding protective index (PI = TD_{50}/ED_{50}) values of 1.56 and 1.14, respectively. These PI values are based upon a TD_{50} value of 108.03 mg/kg for **12**, as determined by the rotorod procedure. Comparison of the data for **12** and its benzamide analogue **1** shows much loss of activity in **12** as a result of the presence of the methylene group. Compounds **1** and **12** were screened as the racemic mixture. Compound **14** showed an activity pattern similar to that of **12** with a slightly higher PI in both MES and scMet assays. Compounds **15** and **16** showed higher PI values, which indicated more selective anticonvulsant effects. Compound **15** gave the highest TD_{50} value; however, the slope of the dose–response curve for rotorod toxicity was fairly small as evidenced by the broad confidence interval.

Compound **16**, showing ED_{50} = 50.50 mg/kg and PI = 5.65, is the most potent anti-MES agent in the 4-aminophenylacetamide series. It is the direct homologue of **2**, which appears to be the most potent aminobenzamide in our structure–activity studies. Addition of a methylene group between the amide carbonyl and the aromatic ring of **2** results in a significant loss of activity in **16** (Table II). Although **16** is much less potent than **2**, it is the most active 4-aminophenylacetamide observed in this study. Compound **16** showed activity against scMet-induced convulsions (ED_{50} = 92.03 mg/kg), whereas **2** showed no activity at doses less than its TD_{50} in the assay.

In summary, the additional methylene group in the phenylacetamides relative to the benzamides significantly decreases anticonvulsant activity. The decreased activity is likely the result of different electronic and conformational factors.

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