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Anticonvulsant screening of three *N*-(2,6-dimethylphenyl)-substituted-benzamides

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Abstract
The *N*-(substituted)-4-aminobenzamides have provided several anticonvulsants that have been extensively investigated. In these series, Ameltolide®, 4-amino-*N*-(2,6-dimethylphenyl) benzamide (LY201116) is the most potent analogue studied to date. This drug is inactivated *in vivo* by metabolic *N*-acetylation, resulting in 4-(acetylamino)-*N*-(2,6-dimethylphenyl)benzamide. Efforts to limit this metabolic inactivation led to the synthesis of two analogues: 3,4-diamino-*N*-(2,6-dimethylphenyl) benzamide and *N*-(2,6-dimethylphenyl) benzamide. The anticonvulsant activities of these two compounds were determined against their ability to inhibit pentylenetetrazole induced seizure in rats after oral administration at three dose levels, i.e. 10mg/Kg, 30mg/Kg, and 100mg/Kg at 30 minutes interval. In addition, the effect of time of oral administration at 30mg/Kg dose level was determined at time intervals of 1h, 2h, and 4h respectively for 3,4-diamino-N-(2,6-dimethylphenyl)benzamide. Our results were compared with that of ameltolide® and carbamazepine.

Keywords: Anticonvulsant, Benzamide, Epilepsy and Seizures

INTRODUCTION

Epilepsy is a neurological disorder associated with excessive temporary neuronal discharge, characterized by discrete recurrent episodes which results in disturbance of movement, sensation, behaviour, perception and consciousness (Guelen and van der Kleijn, 1978). Epilepsy affects about 0.5-1% of the world's population (Daniels and Jorgensen, 1982). There are many drugs used in the management of epilepsy. Among these anticonvulsants armamentarium, the benzamides have shown some promising evidence in anticonvulsant properties.

The benzamides have a unique behavioural profile and they appear to exert their pharmacological activity selectively at the D_2 dopamine receptors. This D_2 receptor subtype is responsible for their therapeutic use as anti-schizophrenic. Clark *et al.*, (1984) demonstrated that *N*-(substituted)-4-aminobenzamides show a high level of protection against maximal electrostatic–induced convulsions in animal models.

The Wittig reaction with ester-exchange was used to synthesis: *N*-(2,6-dimethylphenyl)benzamide, *N*-(2,6-dimethyl phenyl)-4-aminobenzamide (LY201116), ameltolide® and *N*-(2,6-dimethylphenyl)-3,4-diaminobenzamide. Benzoic acid, 4-aminobenzoic acid and 3,4-diaminobenzoic acid, were reacted in parallel with the Wittig

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reaction of triphenylphosphine with carbon tetrachloride. Each of the esters formed were exchanged with 2,6-dimethylaniline to give the corresponding product.

EXPERIMENTAL

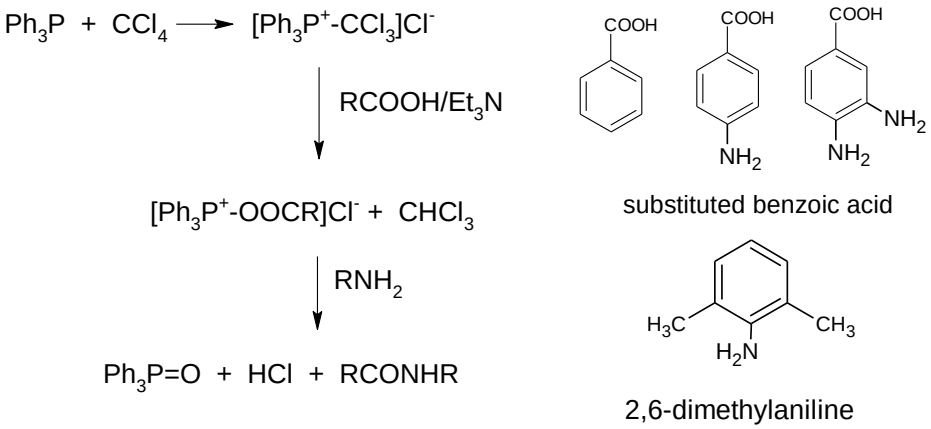
Animals. Mice (25-40 g) were obtained from National Veterinary Research Institute, Vom, Nigeria. The animals were kept in the Animal house of the Department of Pharmacology, University of Jos, Nigeria for 2 days to acclimatize to laboratory condition before the commencement of experiment. They were fed with standard feed and water *ad libitum*.

Synthesis of *N*-(2,6-dimethylphenyl)-3,4-diaminobenzamide. In a typical reaction, a mixture of 2.623g (0.01M) triphenylphosphine, 9.6ml (0.1M) carbon tetrachloride and 30ml of tetrahydrofuran (THF) was refluxed for thirty (30) minutes. The solution was cooled in an ice bath to 5^{o}C and a mixture of 1.5215g (0.01M) 3,4-diaminobenzoic acid and 1.4ml (0.01M) triethylamine was added and allowed to stand at 5^{o}C for ten (10) minutes. 1.212g (0.01M) of 2,6-dimethylaniline was added and the mixture was heated under refluxed for forty-five (45) minutes. The precipitated triphenylphosphite was removed by filtration while the solvents were removed under vacuum. The crude product was purified with preparative TLC on silica, and its melting point was found to be 85 ^{o}C. The CHN analysis agreed within $\pm$ 0.04% range from the expected formula.

Anticonvulsant test. Animals were pretreated with the compounds for 30 minutes and later pentylenetetrazol (PTZ) (85mg/Kg) was administered intra-peritoneally. The animals were observed for 30 minutes. Failure to observe a single episode of clonic spasms for at least 5 seconds duration was considered as protection, and the results expressed as number of animals protected/number of animals tested.

RESULTS AND DISCUSSION

The Wittig reaction with ester-exchange was used to synthesis *N*-(2,6-dimethylphenyl) benzamide, *N*-(2,6-dimethylphenyl)-4-amino-benzamide (ameltolide®) and *N*-(2,6-dimethylphenyl)-3,4-diaminobenzamide. Results of anticonvulsant screening test for the three synthesized compounds are presented in Table 1 and Figures 1 to 3.



Wittig Reaction with Ester-Exchange

Table 1: Anticonvulsant Screening test of *N*-(2,6-dimethylphenyl)substituted-benzamides

Drug	Dose(Rat)	Onset (s)	Episode	Death time (s)	Survived
N-(2,6-dimethylphenyl) benzamide	10mg/Kg	105.0 ± 0.6	1	14400 ± 300	3/ 3
	30mg/Kg	145.5 ± 0.6	3	126 ± 40	1/ 3
	100mg/Kg	99 ± 39	0	14400 ± 300	3/ 3
N-(2,6-dimethyl phenyl) -4-amino benzamide	10mg/Kg	180 ± 20	1	960 ± 180	0 / 3
	30mg/Kg(0.5h)	100 ± 20	2	480 ± 140	0 / 3
	30mg/Kg(1h)	2220 ± 180	2	1020 ± 20	1 / 2
	30mg/Kg(2h)	960 ± 250	1	14400	2 / 2
	30mg/Kg(4h)	480 ± 10	2	870 ± 30	0 / 2
	100mg/Kg	60 ± 40	2	780 ± 260	2 / 3
N-(2,6-dimethyl phenyl) -3,4-diamino benzamide	10mg/Kg	100 ± 20	1	5040 ± 60	1 / 3
	30mg/Kg	60 ± 10	2	5640 ± 540	1 / 3
	30mg/Kg(1h)	120 ± 10	2	8640 ± 1440	1 / 2
	30mg/Kg(2h)	210 ± 30	3	8100 ± *6300*	1 / 2
	30mg/Kg(4h)	270 + 150	3	14400 ± 300	1 / 2
	100mg/Kg	80 ± 20	1	1500 ± 10	1 / 3
Control (-C)	90mg/Kg	120 ± 10	2	1200 ± 500	0 / 2
Control (+C)	10mg/Kg	2280 ± 10	1	1710 ± 630	0 / 2

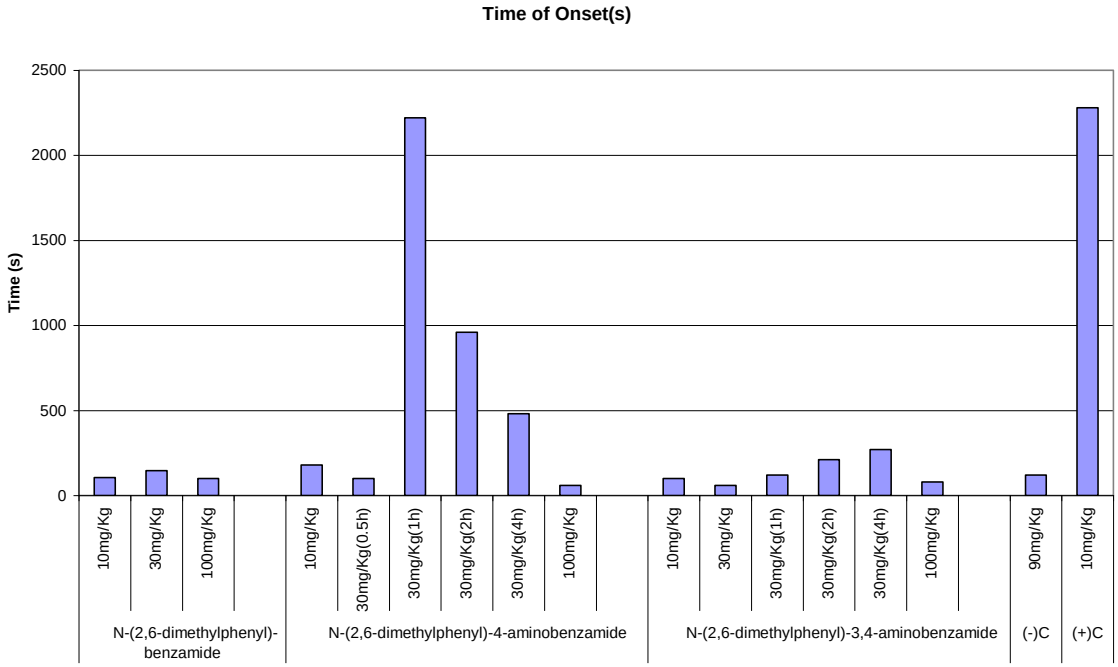


Figure 1: Onset of Seizure(s) of *N*-(2,6-dimethylphenyl)-substituted-benzamides

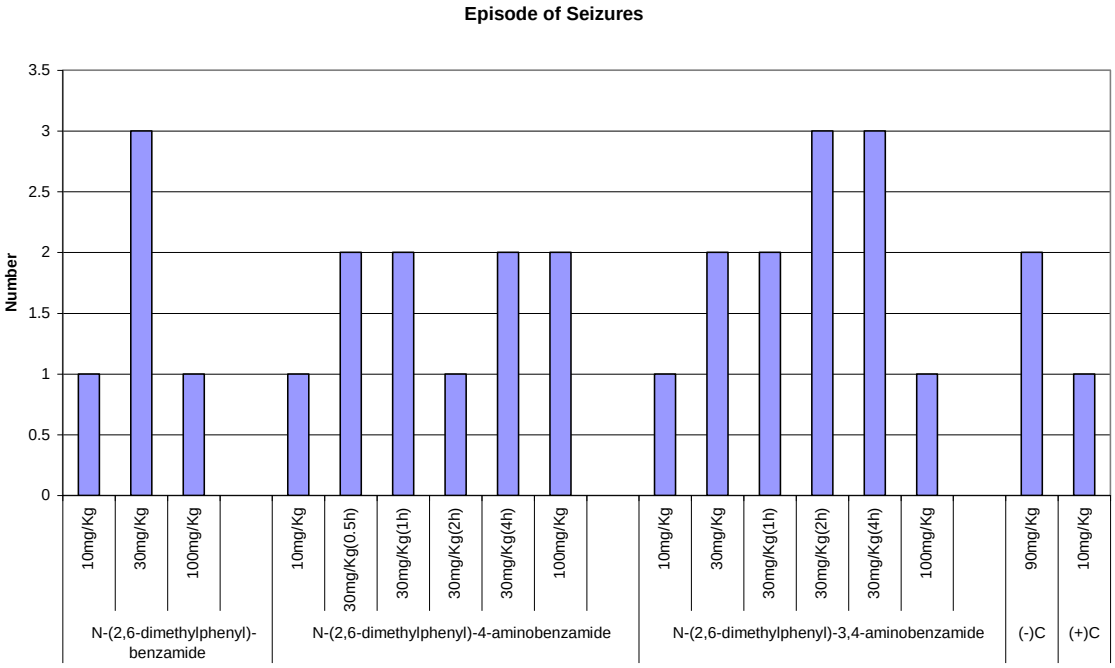


Figure 2: Episode of Seizure of *N*-(2,6-dimethylphenyl)-substituted-benzamides

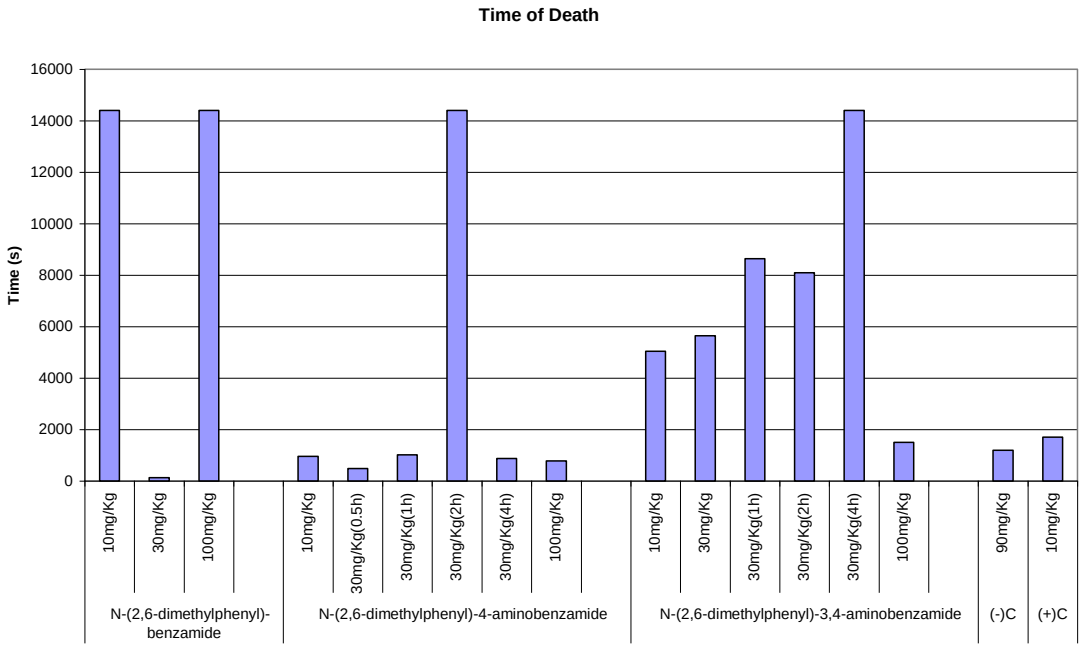


Figure 3: Time of Death for *N*-(2,6-dimethylphenyl)-substituted-benzamides

The anticonvulsant activities of the 4-aminobenzamide is better than that of 3,4-diamino and that of the unsubstituted benzamide, in terms of onset of seizures and number of seizures. But the protection offered by the increased length of time of death by the 3,4-diamino substitution reveal a better promising profile than that offered by the 4-

aminobenzamide. Swinyard *et al.* (1989) demonstrated that some compounds are termed "preventing seizure spread" rather than "raising seizure threshold". In this experiment, the compounds were seen to increase seizure threshold, probably through GABA pathway. Therefore, the observed activity of the three compounds (see Table 1) may not be phenytoin- or carbamazepine-like. Pentylenetetrazole blocks Gamma amino butyric acid (GABA) receptors, thus, interfering with the function of this inhibitory neurotransmitter (Kupferberg, 1989). The anticonvulsant activities of the compounds suggest that they are mediated, at least in part, through a GABA-agonist effect.

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