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Acute Toxicity Determination Of Pyrrolobenzothiazine And Pyrrolobenzoxazine Ring Systems On Mice

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ABSTRACT

Toxicity tests were carried out on two Bezethiazine and Benzoxazine derivatives. For this experiment, a total of sixty (60) Albino mice with average weight range 18-26g were employed. For each test compound, thirty(30) mice were distributed in five cages and fed *ad libitum* for 14 days for acclimatization. The test compounds were suspended in a non-toxic solvent (Tween 80) and intraperitoneally administered into the mice at doses of 450mg/kg, 500mg/kg, 550mg/kg and 600mg/kg. The results showed that the compound pyrrolobenzothiazine ring system has a LD_{50} value of 500.00mg/kg, while the compound pyrrolobenzoxazine ring system has an LD_{50} value of 517.60mg/kg. These results are indicative of low toxicity values for the two compounds.

Keywords: Toxicity, LD_{50}, Mice, Benzothiazine, Benzoxazine

Bezothiazine and Benzoxazine derivatives have been known to have anti-inflammatory, antiallergic, anthelmintic, antimicrobial, tranquillizing as well as prevention and therapy of respiratory diseases (Shridhar et al 1982, Geunter et al 1986). In order to extend the chemistry and possible use of derivates of these parent compounds, two new related compounds were synthesized and characterized (Okafor and Akpuaka, 1993). The two compounds are 1H, 9H-pyrrolo[3,2-b][1,4] benzothiazin-2(3H)-one (1) and 1H, 9H-pyrrolo[3,2-b][1,4]benzoxazin-2 (3H) –one (2). Since these compounds are possible drugs, it becomes necessary to determine their acute toxicity tests. There are two types of toxicity tests available – the acute toxicity and the long-term toxicity test. The second one refers to the teratogenic and carcinogenic tests. The first one is a simple preliminary assessment of the toxicity of a compound by the determination of the median lethal dose (LD_{50}), which is capable of killing 50% of animals under stated conditions (Goldstein, 1974). This value is important, but it is not a biological constant (Lorke, 1983) since the value can change under different conditions for the same compound. The LD_{50} is a statistical expression of the dose that will kill an animal of average sensitivity to the compound under test. However the data on the percentage of animal dead is quite unreliable at the extremes ie at 0% and 100% where the response is strongly affected by chance factors. For 100% dead, the correction factor is 100 - 25/n and for 0% dead, it is 100 where n is the number of animals in the group. The percentage of animal dead is converted to probit (probability units) (Akpuaka, 1990). By plotting percentage or 'Probit' of response against $10g_{10}$ dose, a straight line is obtained. From the straight line, the dose corresponding to 50% of animals dead or probit 5 gives the LD_{50}.

MATERIAL AND METHODS

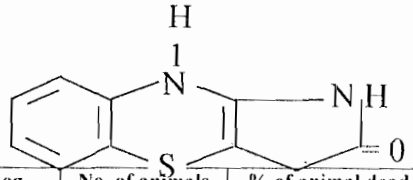
For the test compound (1), a total of thirty albino mice ranging in weight 18 – 26g were used. The mice were obtained from the animal house of Pharmacology Department of the University of Nigeria, Nsukka. The mice were weighed and separated into five groups of six mice and put in different cages. They were fed *ad libitum* with feed and water for fourteen (14) days in order to acclamatize them to the environment. A calculated dose of a suspension of the test compound suspended in tween 80 (solvent) was injected intraperitoneally ranging in values 400mg/kg, 450mg/kg, 500mg/kg, 550mg/kg and 600mg/kg to the mice in the five cages according to their individual body weights. The number of animals dead was observed between 24 – 72 hours. The percentage of the animal dead was calculated, corrected and

converted to 'probit' (probability units). A graph of probit against Log_{10} dose was plotted and from there the LD_{50} was calculated.

RESULTS AND DISCUSSION

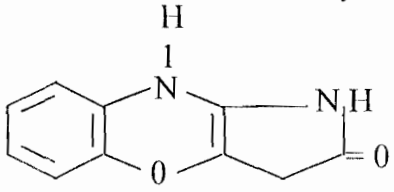
The number of animals dead after 72 hours was recorded for each test compound and this was converted to percentage. Table I and Fig. I show the results for 1H, 9H-pyrrolo[3, 2-b][1, 4] benzothiazin-2 (3H) one (1). For this compound, the LD_{50} is 500mg/kg.

Table I: LD_{50} Determination for 1H, 9H-pyrrolo [3, 2-b] [1,4] benzothiazin-2(3H) one



Group	No. in cage	Dose mg/kg	Log_{10} Dose	No. of animals dead	% of animal dead	% correction	Probit corresponding to %
1	6	400	2.60	1	16.67	16.67	4.05
2	6	450	2.65	2	33.33	33.33	4.56
3	6	500	2.69	3	50.00	50.00	5.00
4	6	550	2.74	4	66.67	66.67	5.44
5	6	600	2.78	5	83.33	83.33	5.95

Table 2: Ld_{50} Determination for 1H, 9H,- Pyrrolo[3,2-b][1,4]benzoxazine2(3H)-one (2)



	No. in cage	Dose mg/kg	Log_{10} Dose	No. of animals dead	% of animal dead	% correction	Probit corresponding to %
1	6	400	2.60	0	0	4.16	3.25
2	6	450	2.65	1	16.67	16.67	4.05
3	6	500	2.69	2	33.33	33.33	4.56
4	6	550	2.74	3	50.00	50.00	5.00
5	6	600	2.78	5	83.33	83.33	5.95

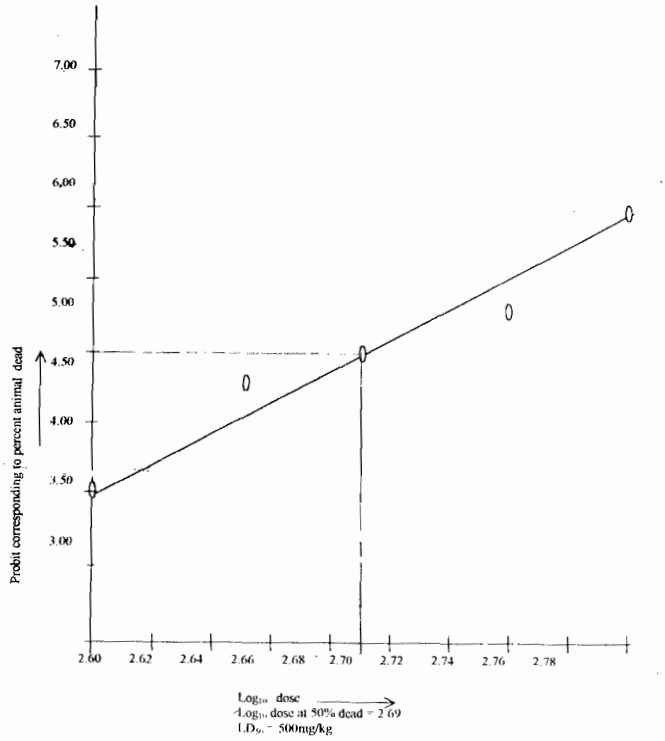


Fig.: 1 Graph of Probit of % animal dead against $\log_{10}$ dose for 1H, 9H pyrrolo[3,2-b][1,4]benzothiazine-2(3H)-one (1)

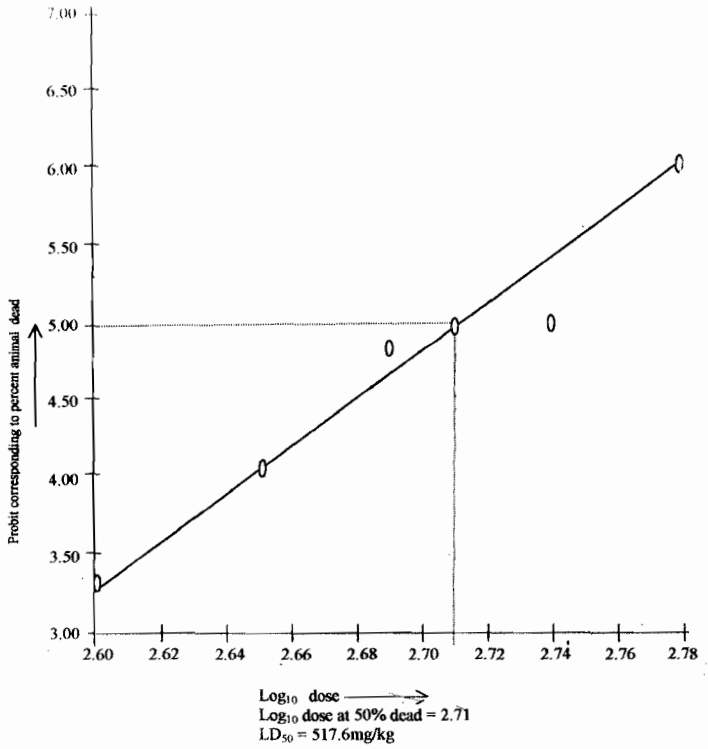


Fig. 2: Graph of Probit of % animal dead against $\log_{10}$ dose for 1H, 9H pyrrolo[3,2-b][1,4]benzoxazine-2(3H)-one (2)

Table 2 and fig. 2 show the results for the 1H,9H–pyrrolo [3,2-b][1,4] benzoxazine-2-(3H)-one (2). The LD_{50} is 517.6mg/kg.

From the results it can be seen that compound 2 is relatively less toxic than compound 1. It appears that replacing sulphur atom with oxygen in these structures reduces toxicity. This is explained by the fact that the sulphur atom readily donates electron to the biological centres in the mice and so compound 1 is better absorbed in the mice than the oxygen analogue and so will be more toxic (Lower LD_{50} value) than compound 2.

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