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SYNTHESIS OF SOME- 6- SUBSTITUTED- 6H- PYRROLO [3, 4-D] PYRIDAZINE DERIVATIVES AND THEIR EVALUATION FOR *IN-VIVO* ANTI-CONVULSANT ACTIVITY IN ALBINO RATS

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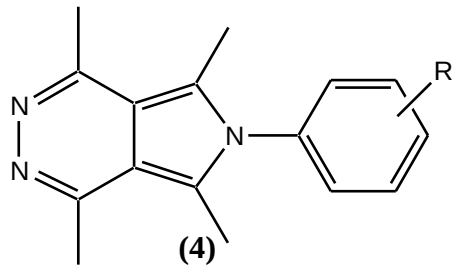
ABSTRACT

Present studies on the synthesis and evaluation of anticonvulsant activity of some 6- Substituted- 6H- Pyrrolo [3, 4-d] Pyridazine derivatives. In the preliminary screening for anticonvulsant activity in albino rats, all the synthesized compounds were found to be active, even more than standard at dose 50mg/kg body weight.

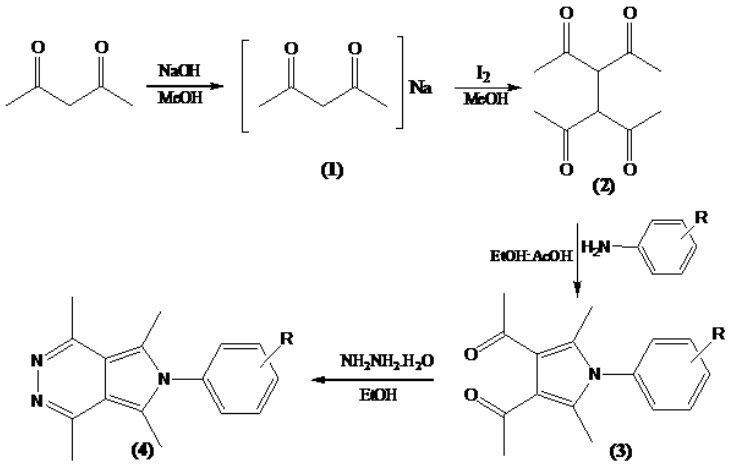
INTRODUCTION: Pyrrole derivatives were reported as anti-inflammatory [1], antifungal [2], anticancer [3], while the Pyridazines are having the number of biological activities, such as anti-inflammatory, anticonvulsant, antimicrobial, antihypertensive, antibacterial, anti-helminetic, CNS stimulant, cardiotonic, antiallergics, antipsychotic, anti-parkinsonian, phosphodiesterase-III inhibitor and in the treatment of infection of hepatitis C virus [4].

In view of potential biological activities of Pyrole and Pyridazine derivatives, promted us to synthesize some innovative and totally new N- substituted Aryl-Pyrolo-Pyridazine derivatives **(4)** and evaluate for their possible anti-convulsant activity.



(4)

Scheme:



Experimental:

Step 1: Synthesis of sodium salt of Acetylacetone (1): A solution was prepared by dissolving sodium hydroxide (0.5 mole) in 25 ml of water. To this solution 100 ml of methanol was added slowly. This solution was slowly added with hand stirring to the acetylacetone (0.5 mole) contained in 250 ml flask. The creamy-white crystalline salt (1) was separated from solution immediately. The flask was stoppered and cooled in a refrigerator for 3 hours. The sodium salt was filtered and washed with two small portion of cold methanol.

After the salt was air-dried, it was dried further in oven at 100°C for 3 hours. Yield was 56-65% and melting point was 151-156^{o}C.

Step 2: Synthesis of Tetraacetylethane (2): Sodium acetylacetonate (1) was ground to a fine powder in a mortar, 24.4 g. (0.2 mole) of the anhydrous material weighed into flask. After that 200 ml of diethyl ether was added, the suspension was stirred vigorously at room temperature with a magnetic stirrer. from a separatory funnel, a solution of 25.4 g. (0.1 mole) of iodine dissolved in 200 ml of diethyl ether was added, dropwise to the stirred mixture. The rate of addition was maintained roughly constant by occasional adjustments of the stopcock, and the total addition was completed in about 2.5 hours.

The reaction mixture was then poured into a large beaker, and the ether was allowed to evaporate overnight at room temperature. To the contents of the flask there is then added 500 ml of water and the mixture was allowed to stand for 2 hours. The remaining solid was collected by filtration, and washed several times with water, and finally dried in a vacuum desiccator. The product was recrystallized from methanol, which produced white colored crystals (2). The melting point was 184-189^{o}C and yield 9-11g (36-45%).

Step 3: Preparation of N-substituted pyrrole (3): The aniline derivatives (0.04 mole) was added to the stirred slurry of tetraacetylethane (0.04 mole, 7.9g) and ethanol**:** acetic acid (3:4). The mixture was refluxed for 12-15 hours. The mixture was cooled to room temperature. For the precipitation of pyrrole derivative (3), water was added to the above mixture with stirring until the complete pyrrole derivative **3a-f** were precipitated out. Products were recrystallized from water. The yield of compounds were 59,77, 68, 67, 71, 69 and Melting points were 128^{o}C, 214, 162^{o}C, 174^{o}C, 168^{o}C, 192^{o}C respectively.

STEP 4: Synthesis of Final Compounds (4): Hydrazine hydrate (80% solution) 1.0 ml was added to the stirred slurry of compound **3** (0.02 mole) and 200 ml ethanol at room temperature. The mixture was stirred for 0.5 hr at room temperature. The mixture was diluted with water to produce precipitate of final compounds **4(a-f).** The synthesized products was recrystallized from the mixture of water and ethanol.

The purity of compounds were established by TLC using 2% silica gel G, ethyl acetate and n-hexane (7:3) as eluents, Iodine chamber and UV light were used for detecting the compounds. MP of the compounds 4a to 4f were 286^{o}C, 306-310^{o}C, 282^{o}C, 298^{o}C, 296-298^{o}C, 302-304^{o}C and yield % were 72%, 71%, 73%, 69%, 77% & 81% respectively. Structures were confirmed on the basis of C, H, N analysis, IR, NMR and Mass Spectroscopy.

Determination of Anti-Convulsant Activity: In the present study the Maximal electroshock (MES) method was used to evaluate the anticonvulsant activity of synthesized compounds (model no. - PP156, Company - INCO, ear electrode, 150 mA current for 0.2 sec). The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC)(Regd No. 1171/C/08/CPCSEA).

The synthesized compounds were evaluated for anticonvulsant activity by assessing the reduction in the degree of electric shock- induced convulsions in the Wistar albino rat compared to standard phenytoin . Healthy Albino rats (Wistar strain) of either sex, weighing 100-160g were selected and provided standard rat feed and water *ad libitum*. Before the experiment, food was withdrawn overnight but adequate water was given to the rats. In this method, synthesized compounds were administered intraperitonially.After an hour of drug administeration the electric shock was given to induce convulsions.

The animals were divided equally into eight groups (Six animal in each). The first group served as control (without anticonvulsant drug administrations) second group served as standard and received phenytoin (25mg/kg). The animals of third, fourth, fifth, sixth seventh and eighth groups were given the synthesized compounds equalvelent to 50mg/kg intraperitonially. After an hour all the animals were given supra-maximal electric shock (60mA for 0.2 sec.) to induce convulsion.

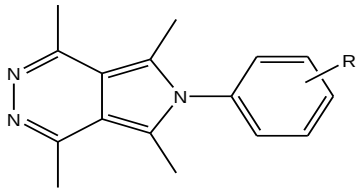
The reduction in the severity in convulsions (abolition or reduction of hind limb extensor phase) were noted [6] and compared the activity of the synthesized compounds to the standard drug phenytoin sodium.

The percentage inhibition of convulsion were determined by the following formula.

Inhibition of convulsion % = [1 - Vt /Vc] 100

Where Vt and Vc are the inhibition of convulsion of the drug treated and control groups respectively.

TABLE 1: ANTICONVULSANT ACTIVITY OF SYNTHESIZED COMPOUNDS



Compound code/R	Molecular Formula/Wt.	Avg. wt. of animal "g"	Dose	Anticonvulsant activity (% inhibition)	R_f value
4a/p- COMe	$C_{18}H_{19}N_3O$ (293)	200 ± 10	50 mg/kg	86.15	0.40
4b/α- Naphthyl	$C_{20}H_{20}N_3$ (301)	175 ± 10	50 mg/kg	33.68	0.55
4c/m- Cl	$C_{16}H_{16}N_3Cl$ (285)	190 ± 10	50 mg/kg	0.99	0.50
4d/o- COOH	$C_{17}H_{17}N_3O_2$ (295)	190 ± 10	50 mg/kg	10	0.56
4e/p- OCH_3	$C_{17}H_{19}N_3O$ (281)	160 ± 10	50 mg/kg	90.13	0.58
4f/p - CH_3	$C_{17}H_{17}N_3$ (265)	220 ± 10	50 mg/kg	0.99	0.60
Control (saline)	--	200 ± 10	2 ml/kg	0.00	--
Phenytoin (Standard)	--	200 ± 10	25 mg/kg	62.38	--

* P<0.01, ANOVA followed by Dunnett's t-test. Mortality (24h) "%" was zero (00)

RESULT AND DISCUSSION: Some novel 6- substituted-6H- pyrrolo [3, 4-d] pyridazine **4** (a-f) were synthesized and evaluated for their anti-convulsant activity by Maximal electroshock (MES) method, all the synthesized compounds were effective as anti-convulsant agents. Compounds **4a** and **4e** were most effective producing 86.15 and 90.13% reduction in convulsions even more than standard.

From the experiment, it was observed that all the synthesized compounds were effective as anti-convulsant agents. Study revealed that o-OCH_3 and P-COMe substituted compounds were more active than p- α- Naphthyl and p-COOH substituted derivtives.

P-COOH substituted derivtive was more active than p-CH_3 and m-Cl substituted derivtives.

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