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Synthesis, characterization and evaluation of anti-epileptic activity of four new 2-pyrazoline derivatives compounds

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Abstract – Two varieties of acetophenones were condensed with two varieties of aromatic benzaldehydes dto get four chalcone derivatives. They undergo condensation followed by cyclisation with isoniazid to get the final four 2-pyrazoline derivatives. The synthesized compounds were characterized by IR, ^{1}H-NMR and mass spectral studies. The synthesized compounds were evaluated for their antiepileptic activity by two methods. The compounds exhibited good antiepileptic activity when compared to the standards.

Keywords – 2-pyrazoline derivatives, ^{1}H-NMR, synthesis, anti-epileptic activity

INTRODUCTION:

Heterocyclic compounds have gained much importance in medicinal chemistry due to its presence in large number of pharmacologically active moieties. Among the five membered heterocyclic containing two hetero atoms in its ring structure, pyrazole is one of the most important one as large variety of biological activities have been reported for various pyrazole derivatives. Pyrazoline is dihydropyrazole, a five membered heterocyclic compound containing two nitrogen atoms in adjacent positions and possessing only one endocyclic double bond. Among all the pyrazolines, 2-pyrazoline has gained attraction and is frequently studied one [1].

Conventional method of synthesis of pyrazolines involves the base-catalyzed condensation of aromatic ketones to give α, β- unsaturated ketones (also called as chalcones), which undergo subsequent cyclization with hydrazine and hydrazine derivatives yielding 2-pyrazoline and 2-pyrazoline derivatives. In this method, hydrazones are formed as intermediates that can subsequently cyclized to 2-pyrazolines in presence of a suitable catalyst such as NaOH [**2]** or acetic acid [3].

2-Pyrazolines are very much promising when the biological activities of pyrazolines are taken into consideration. The literature survey reveals that 2-pyrazoline derivatives are reported to possess wide range biological activities like antimicrobial[4], antimycobacterial [5], antiamoebic [6], anti-inflammatory [7,8], analgesic [9,10], anticonvulsant [11,12], antidepressant [13,14], MAO-B inhibitory [15], anticancer [16], acyl-CoA inhibitory [17], hypotensive [18], androgen receptor modulatory [19], neuroprotective [20], antiviral [21], ACE inhibitory [22], amine oxidase inhibitory [23] ,antioxidant [24].

Many pyrazole derivatives has already found their application as NSAIDs clinically [5] such as Antipyrine or phenazone (analgesic and antipyretic), Metamizole or Dipyrone (analgesic and antipyretic), Aminopyrine or aminophenazone (anti-inflammatory, antipyretic and analgesic), Phenylbutazone or bute (Anti-inflammatory, antipyretic mainly used in Osteoarthritis, Rheumatoid arthritis, Spondylitis, Reiter's disease), Sulfinpyrazone (Chronic gout), Oxyphenbutazone (antipyretic, analgesic, anti-inflammatory, mild uricosuric).

Based on the literature review it was planned to synthesize four 2-pyrazoline derivatives and to evaluate their anti-epileptic activity.

MATERIALS AND METHODS

Drugs , Chemicals and Instrumentations

All reagents were of analytical/laboratory grade obtained from SD Fine Chemicals, Merck India, and Chem Sure. The melting points of the synthesized compounds were determined by open capillary tube method and are uncorrected. The ^{1}H-NMR spectra were recorded at 400 MHz at BRUKER NMR spectrophotometer in DMSO and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. The IR spectra were recorded on Shimadzu FTIR 8400S using potassium bromide pellet technique. The completions of the reaction were monitored by Thin Layer Chromatographic technique (TLC) on pre-coated silica gel (HF254-200 mesh)

aluminium plates from E-merk using ethyl acetate: n-hexane (4:1) as the mobile phase. Detection of the spots was done under UV chamber. Before using in experiment animals got clearance from CPCSEA. Registration No. 177/99/CPCSEA.

Synthesis:

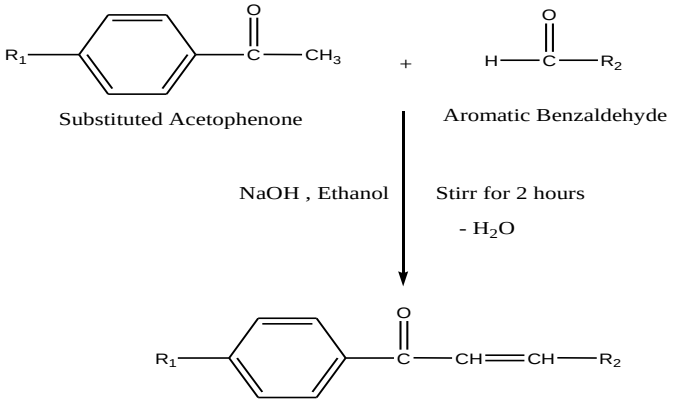
Scheme 1: Preparation of Chalcones (A_1-A_6) [27]

A mixture of acetophenone (0.01mol) and aryl aldehyde (0.01 mol) was stirred in ethanol (30ml) and then an aqueous solution of KOH (40%, 15ml) added to it. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with HCl. The solid separated was filtered and crystallized from ethanol.

Scheme 2: Preparation of Pyrazoline derivatives [28]

To a solution of compounds A_1-A_6 (0.01mol) in absolute ethanol (30 ml), hydrazine derivatives (0.01mol) and few drops of glacial acetic acid were added. The reaction mixtures were refluxed for 8 hr. The excess of solvent was distilled off and crude products were poured into ice water. The separated solids were filtered and recrystallised from ethanol.

Scheme 1:

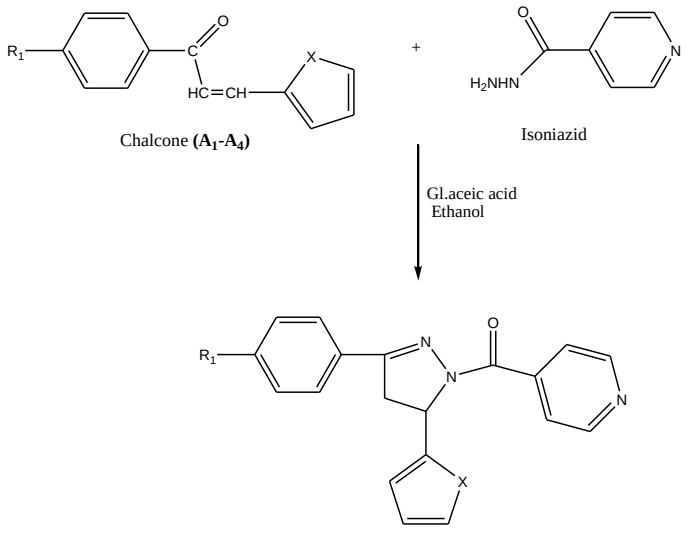


Chalcone **(A_1-A_4)**

R_1 = -Cl , -OH

R_2 = 2-furyl , 2-thienyl

Scheme 2:



TITLE COMPOUNDS (M_1-M_4)

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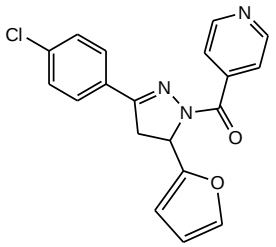
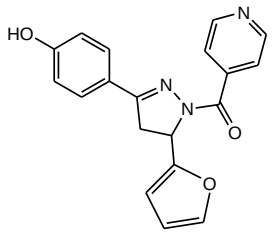
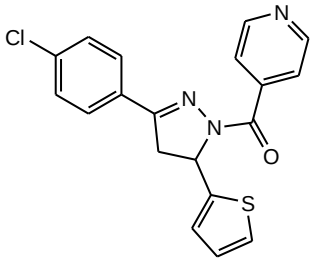
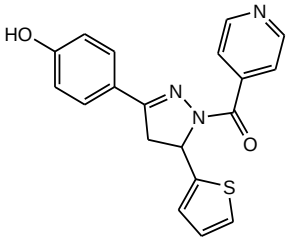
Compounds	Structure and IUPAC Name
M1	 (3-(4-chlorophenyl)- 4,5-dihydro-5-(furan-2-yl)pyrazol-1-yl)(pyridin-4-yl)methanone
M2	 (3-(4-hydroxyphenyl)- 4,5-dihydro-5-(furan-2-yl)pyrazol-1-yl)(pyridin-4-yl)methanone
M3	 (3-(4-chlorophenyl)-4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)(pyridin-4- yl)methanone
M4	 (3-(4-hydroxyphenyl)- 4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)(pyridine-4-yl)methanone

Compound M1: (3-(4-chlorophenyl)- 4,5-dihydro-5-(furan-2-yl)pyrazol-1-yl)(pyridin-4-yl)methanone, Mf-$C_{19}H_{14}ClN_3O_2$, Mp-102-104^{0}C, R_f- 0.57, yield- 65.5%, IR (KBr) ν_{max} (cm^{-1}) :
3141.84 (C-H aromatic str. furan), 3101.30 (C-H aromatic str. substituted benzene), 1651.09 (C=O str.), 1605.93 (C=N str. Pyrazoline), 1587.06 (C=C str.), 702.30 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 6.75-8.31(m,6H, ArH), 5.23-5.29(dd,1H,CH Pyrazoline), 3.43-3.49(dd,1H, CH_2 Pyrazoline), 3.17-3.22(dd,1H, CH_2 Pyrazoline); MS(m/e): 351.8(M^+), 353.8(M+2)

Compound M2: (3-(4-hydroxyphenyl)- 4,5-dihydro-5-(furan-2-yl)pyrazol-1-yl)(pyridin-4-yl)methanone. Mf-$C_{19}H_{15}N_3O_3$, Mp-128-130^{0}C, R_f- 0.52, yield- 47.74%, IR (KBr) ν_{max} (cm^{-1}) :
3611.37 (O-H aromatic str.), 3141.82 (C-H aromatic str. furan), 3101.65 (C-H aromatic str. substituted benzene), 1659.58 (C=O str.), 1612.05 (C=N str. Pyrazoline), 1594.06 (C=C str.); ^{1}H NMR (DMSO) δ ppm: 9.88(s,1H,ArOH), 6.93-8.30(m,6H, ArH), 5.20-5.25(dd,1H,CH Pyrazoline), 3.49-3.55(dd,1H, CH_2 Pyrazoline), 3.16-3.21(dd,1H, CH_2 Pyrazoline); MS(m/e): 333.3(M^+)

Compound M3: (3-(4-chlorophenyl)-4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)(pyridin-4- yl)methanone Mf- $C_{19}H_{14}ClN_3OS$, Mp-124-126^{0}C, R_f- 0.60, yield-57.85%, IR (KBr) ν_{max} (cm^{-1}) :3128.91 (C-H aromatic str. thiophene), 3091.68(C-H aromatic str. substituted benzene), 1657.70 (C=O str.), 1610.17 (C=N str. Pyrazoline), 1599.12 (C=C str.), 705.03 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 6.94-8.38(m,6H, ArH), 5.42-5.47(dd,1H,CH Pyrazoline), 3.33-3.39(dd,1H, CH_2 Pyrazoline), 3.09-3.16(dd,1H, CH_2 Pyrazoline); MS(m/e): 367.8(M^+), 369.8(M+2)

Compound M4: (3-(4-hydroxyphenyl)- 4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)(pyridine-4-yl)methanone. Mf- $C_{19}H_{15}N_3O_2S$, Mp-163-165^{0}C , R_f- 0.48, yield-66.33; IR (KBr) ν_{max} (cm^{-1}) : 3621.43 (O-H aromatic str.), 3127.14 (C-H aromatic str. thiophene), 3089.12 (C-H aromatic str. substituted benzene), 1640.57 (C=O str.), 1604.57 (C=N str. Pyrazoline), 1600.47 (C=C str.)^{1}H NMR (DMSO) δ ppm: 9.98(s,1H,ArOH), 7.12-8.28(m,6H,ArH), 5.38-5.43(dd,1H,CHPyrazoline), 3.58-3.63(dd,1H, CH_2 Pyrazoline), 3.17-3.22(dd,1H,CH_2 Pyrazoline); MS(m/e): 349.4(M^+)

The synthesized compounds were characterized by IR, NMR and MASS spectroscopic studies. The acute oral toxicity studies were performed according to the OECD guideline 423 and found to be LD^{50} as 2500 mg/kg. and selected as dose 100 mg/kg body weight.

Evaluation of Anti-epileptic Activity

Maximal Electroshock (MES) test

Electrical stimulation was applied using ear electrodes. The electrodes were moistened with saline before application. All animals were stimulated with 150mA for 0.2 seconds, with constant voltage stimulators of 250 V. [28]. Duration of tonic hind extension (THE) was recorded for antiepileptic activity. The Morality of the no. of animals was also recorded. Percentage protection was calculated.

Experimental design:

Animals: Rats (Either Sex), Weight: (120-150 gm), Total Groups=6, n=6, (n means No. of animals in each group). The animals were divided into six groups. Each group consists of six animals.

Group I: Control (0.5% CMC, p.o) + after 90 min. MES.

Group II: T1 compound (100 mg/kg, p.o) + after 90 min. MES.

Group III: T2 compound (100 mg/kg , p.o) + after 90 min. MES.

Group IV: T3 compound (100 mg/kg , p.o) + after 90 min. MES.

Group V: T4 compound (100 mg/kg , p.o) + after 90 min. MES.

Group VI: Phenytoin (30 mg/kg, p.o)+ after 90 min. MES.

After 90 min of the administration of drugs MES is given to all groups of and animals. Suppression of tonic hind limb extension was taken as a measure of efficacy in this test.

Pentylene tetrazole (PTZ) induced convulsion

PTZ 70mg/kg I.P was administered to rats. The parameter noted was duration of convulsions [28].

Animals: Rats (Either Sex), Weight: (120-150 gm), Total Groups=6, n=6, (n means No. of animals in each group) The animals were divided into six groups. Each group consists of six animals.

Group I: Control (0.5% CMC, p.o) + after 90 min PTZ.

Group II: T1 compound (100 mg/kg, p.o) + after 90 min. PTZ.

Group III: T2 compound (100 mg/kg , p.o) + after 90 min. PTZ.

Group IV: T3 compound (100 mg/kg , p.o) + after 90 min. PTZ.

Group V: T4 compound (100 mg/kg , p.o) + after 90 min. PTZ.

Group VI: Phenytoin (30 mg/kg, p.o)+ after 90 min. PTZ.

After 90 min of the administration of the Test and standard drugs, PTZ (70mg/kg , i.p) is administered. Abolition of the convulsions was taken as a measure of efficacy in this test.

RESULTS AND DISCUSSIONS:

Result of Anti-epileptic Activity on MES Model in rats

There was significant effect of percentage protection in MES model in drug treated animals with T1,T2, T3, and T4 compounds. The group-II (T1-100mg) produce percentage protection and onset of tonic hind limb extension statistically significant($p<0.01$) hind limb and duration of tonic hind limb extension shows significant($p<0.001$) and group-III (T2-100mg) produce percentage protection and statistically significant($p<0.001$) hind limb and duration of tonic hind limb extension shows significant($p<0.001$)and group-IV (T3-100mg) produce percentage protection and but was not statistically significant and duration of tonic hind limb extension shows significant($p<0.001$)and group-V (T4-100mg) produce percentage protection and but was not statistically significant and duration of tonic hind limb extension shows significant($p<0.001$) which is comparable to standard drug phenytoin(group-VI) ($p<0.001$).

Table-1: Results of Antiepileptic activity for test compounds on MES Model in rats

Group	Treatment	Dose	Onset of Tonic Hind Limb extension (Sec)	Duration of Tonic Hind Limb extension (sec)	N	Recovery/Death	% Protection
I	Control	10 ml/kg	2.483 ± 0.283	307.1±6.174	6	0/6	0
II	T1	100 mg/kg	4.117± 0.389**	11.47±0.659***	6	4/2	66.6
III	T2	100 mg/kg	7.90± 0.388***	4.517±0.725***	6	6/0	100
IV	T3	100 mg/kg	3.400± 0.302^{ns}	11.31±0.868***	6	2/4	33.3
V	T4	100 mg/kg	2.633± 0.217 ^{ns}	14.57±1.169***	6	2/4	33.3
VI	Phenytoin	25mg/kg	00.0± 0.00***	0.0±0.0***	6	6/0	100

(Values are in Mean ± S.E.M (n=6); ^{ns} -Non Significant, *p<0.05, **p<0.01, ***p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.)

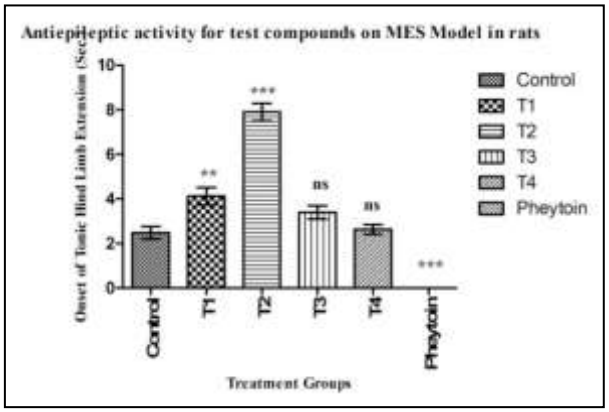


Fig-1 (a): Antiepileptic activity for test compounds on onset of Tonic Hind Limb Extension in MES Model in rats

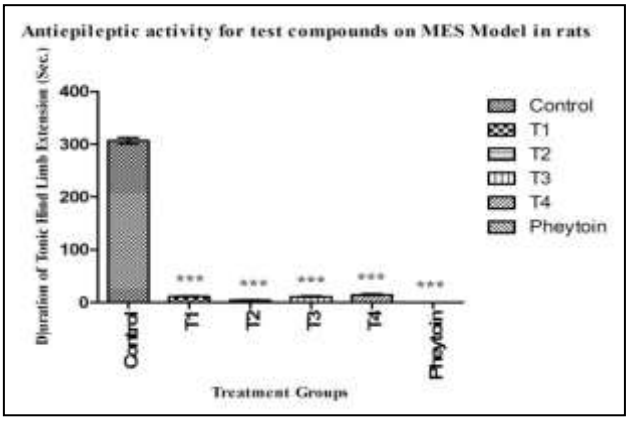


Fig-1(b):Antiepileptic activity for test compounds on Duration of Tonic Hind Limb Extension in MES Model in rats

Result of Anti-epileptic Activity on PTZ Model in rats

There was significant effect of percentage inhibition in PTZ model in drug treated animals with T1,T2, T3, and T4 compounds. The group-II (T1-100mg) produce percentage inhibition and onset of jerky movements are statistically significant($p<0.01$) and duration of jerky movements shows significant($p<0.001$) and group-III (T2-100mg) produce percentage inhibition and statistically significant($p<0.001$) onset and duration of jerky movements extension shows significant($p<0.001$) and group-IV (T3-100mg) produce percentage inhibition and but was not statistically significant and duration of tonic hind limb extension shows significant($p<0.001$)and group-V (T4-100mg) produce percentage inhibition and but was not statistically significant and duration of jerky movements shows significant($p<0.05$) which is comparable to standard drug phenytoin(group-VI) ($p<0.001$).

Table-2: Results of Antiepileptic activity for test compounds on PTZ Model in rats

Group	Treatment	Dose	Onset of Jerky Movements (min)	Duration Jerky Movements (Min)	%inhibition of Jerky Movements
I	Control	10 ml/kg	2.850±0.408	42.20±2.043	0
II	T1	100 mg/kg	7.483±0.575***	21.47±1.281***	96.55
III	T2	100 mg/kg	11.60±0.648***	16.98±0.847***	**66.56**
IV	T3	100 mg/kg	5.400±0.863*	23.17±1.201***	59.67
V	T4	100 mg/kg	4.900±0.432 ^{ns}	35.92±1.745*	45.56
VI	Phenytoin	25mg/kg(oral)	14.35±0.753***	10.62±0.904***	100

(Values are in Mean ± S.E.M (n=6); ^{ns} -Non Significant, *p<0.05, **p<0.01, ***p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.)

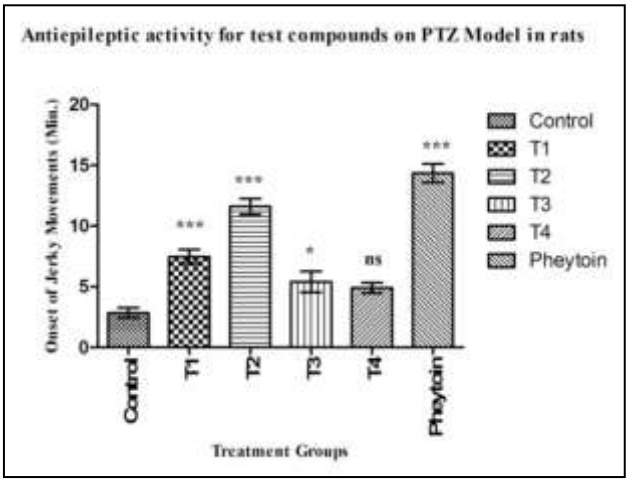


Fig-2(a): Antiepileptic activity for test compounds on Onset of Jerky Movements Extension in PTZ Model in rats

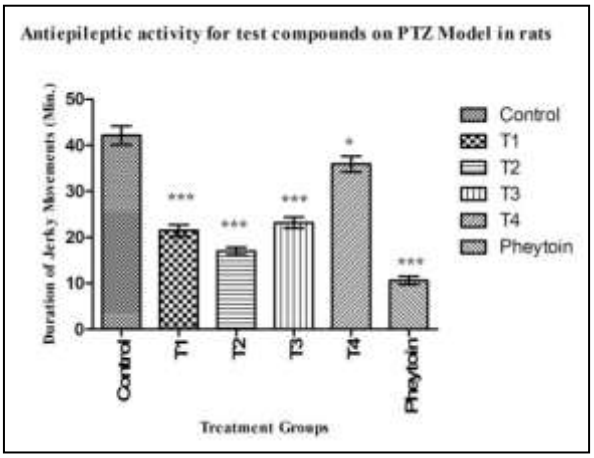


Fig-2(b): Antiepileptic activity for test compounds on Duration of Jerky Movements Extension in PTZ Model in Rats

CONCLUSION

In conclusion it is clear that 2- pyrazoline derivatives have Anti-epileptic activity. It was found that T1 and T2 .The 2-furyl derivatives names of T2 (5-(furan-2-yl)-4,5-dihydro-3-(4-hydroxyphenyl)pyrazol-1-yl)(pyridin-4-yl)methanone and T1(3-(4-chlorophenyl)-5-(furan-2-yl)-4,5-dihydropyrazol-1-yl)(pyridin-4-yl) methanone has prominent anti-epileptic activity on hydroxy-2 and furyl have the most potential anti-epileptic activity.

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