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SYNTHESIS AND EVALUATION OF SOME ALDEHYDE DERIVATIVES OF 1, 4 DI-HYDRO PYRIDINE AND THEIR BIOLOGICAL EVALUVATION

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Keywords:

1, 4 di hydro pyridine, Anticonvulsant, Analgesic and Antimicrobial activity

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ABSTRACT

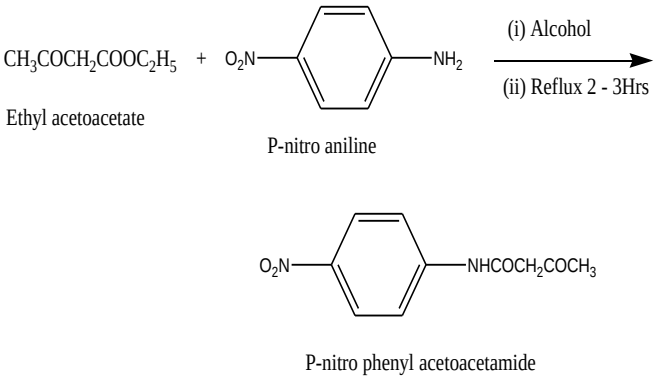
Synthesis of some aldehyde derivatives of 1, 4 di- hydro pyridine has been done. The entire synthesized compounds were characterized by UV, IR and ^{1}HNMR spectroscopy. The anticonvulsant and antimicrobial activity were also evaluated. The present investigation deals with the synthesized compounds possessing good anticonvulsant, analgesic and antimicrobial activity. Synthesized compounds like PNH, PNA, ONF, MND, PAH posses significant anti convulsant activity. However, all the compounds showed analgesic activity less than the reference standard. Anti bacterial activities of the compounds were evaluated against staphylococcus aureus and the zone of inhibition was measured as the parameter of activity. Amikacin, the standard showed a zone of inhibition of 18 mm out of 8 synthesized compounds only one PNN (Ia) compound showed an equal activity when compared with standard. In which (ONF, PAN) showed considerable anti bacterial activity. The remaining four compounds PNM, MNC, MNA, PAK showed moderate antibacterial activity. Anti bacterial activity of the compounds were evaluated against *E. Coli* and the zone of inhibition was measured as the parameter of activity. Amikacin, the standard showed a zone of inhibition of 17 mm. out of 8 synthesized compounds, compound PNM, PAH showed maximum high degree of anti bacterial activity. The remaining compounds PNC, ONF, MNA, PAN showed moderate anti bacterial activity. The anti fungal activities of the compounds were evaluated against Candida albicans using Ketaconazole as the standard compound. The activity of the compounds measured in terms of zone of inhibition ranged between 7 to 15 mm where the upper limit is lower than that of Ketaconazole (15 mm), the compound ONF showed high degree of anti fungal activity.

INTRODUCTION: Literature survey reveals that some aldehyde derivatives of 1, 4 di- hydro pyridine possess broad spectrum biological activities, which include Antibacterial [1, 2, 3, 4], anaphylaxis [5], insecticidal activity [6], Antitumor [7], *in vitro* growth inhibitory activity [8], calcium channel antagonist [9], antifungal [10, 11], anti inflammatory and analgesic activity [12], bronchodilatory activity [13], hypertensive activity [14], hemolytic activity [15], anticonvulsant activity [16], congestive heart failure [17], interferon inducing activity [18], vascular smooth muscle relaxant [19], antiulcer activity [20]. On the basis of our observation the present research work was carried out to synthesize some aldehyde derivatives of 1, 4 dihydro pyridine and to further evaluate antimicrobial and anticonvulsant and activity.

MATERIALS AND METHODS: All the chemicals are analytical grade and were purified by the established methods. Melting points and were determined by open capillary tubes method purity and homogeneity of the compounds was routinely determined by thin layer chromatography on glass plates using silica gel G as absorbent and solvent system. Benzene: Ethylacetate: Methanol (8.5:1.4:0.1). Spots were visualized by iodine vapor by irradiation with UV light. ^{1}HNMRspectra was recorded on Bruker Ultra shield (300MHZ) spectrometer using DMSO (TMS as internal standard). The anti microbial activities of the synthesized compounds were evaluated on *S. aureus* and *E. coli*. The anti convulsant activity was determined and phenytoin used as standard.

SCHEME- 1:

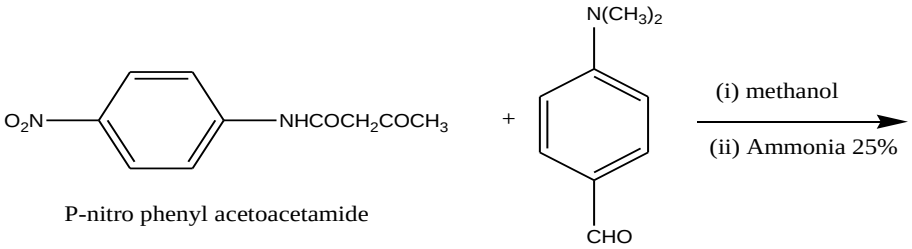
Synthesis of P-nitro phenyl aceto actamide: Ethyl acetoacetate (0.05mole, 6.6ml) and p-nitro aniline (0.05mole, 6.913gm) were taken into a dry round bottom flask and dissolved in alcohol (20ml) and refluxed for about 2-3 hours. The reaction mixture was cooled. The solid that separated out was filtered, washed with cold water and dried. The crude solid of anilide was purified by recrystallization from ethanol.



Synthesis of P-nitro phenyl aceto actamide

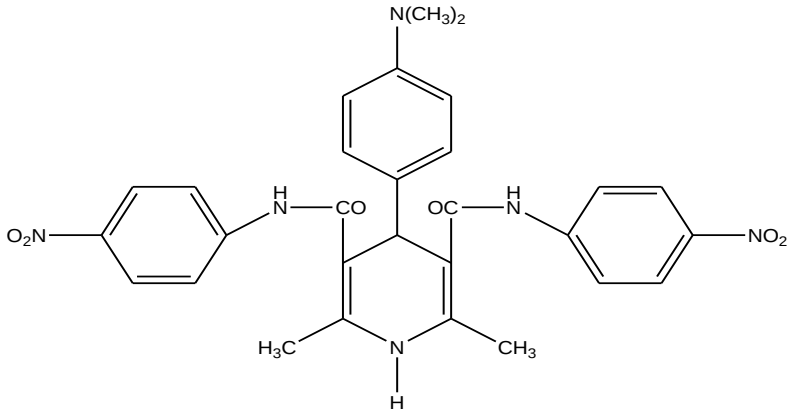
a) Synthesis of 4- (4 - nitro phenyl) - 1, 4- dihydro - 2, 6- dimethyl N^3, N^5, bis (4 - nitro phenyl) pyridine - 3, 5- dicarboxamide (PNN): P-nitro phenyl aceto acetamide (0.01mole, 2.2 gm) was dissolved in methanol and appropriate 4-nitro benzaldehyde (0.05, 7.5 gm) was added followed by the addition of excess of ammonia (25%). The reaction mixture was mechanically stirred for 10min and then heated on water bath under reflux for 10-12 Hrs. Methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with small portions of methanol and dried. It was purified by recrystallization from ethanol.

b) Synthesis of 4- (4- dimethyl amino phenyl) - 1, 4- dihydro- 2, 6- dimethyl N^3, N^5, bis (4- nitro phenyl) pyridine - 3, 5 - dicarboxamide (PND): P-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol and 4- chloro benzaldehyde (0.05 mole, 7.05 gm) was added followed by the addition of excess of ammonia (25%). The reaction mixture was mechanically stirred for 10 min and then heated on water bath under reflux for 10-12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol and dried. It was purified by recrystallization from ethanol.



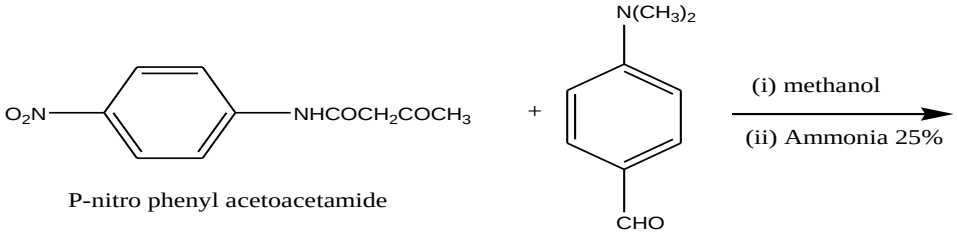
P-nitro phenyl acetoacetamide

Di methyl amino benzaldehyde



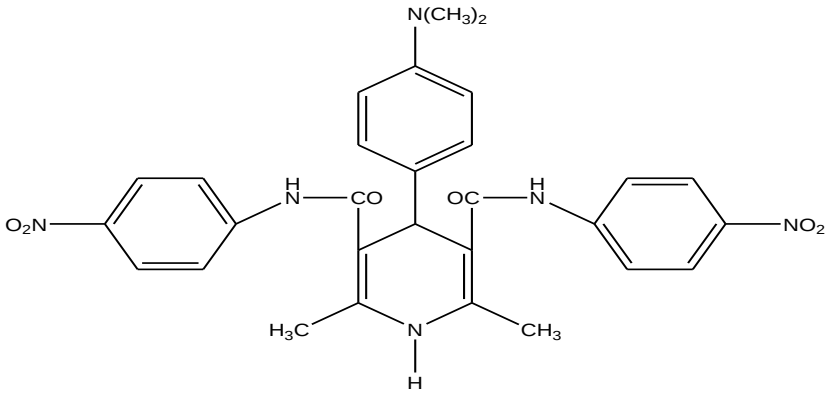
4-(4-dimethyl amino phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-nitro phenyl) pyridine -3,5-dicarboxamide (PND)

a) PNN



P-nitro phenyl acetoacetamide

Di methyl amino benzaldehyde

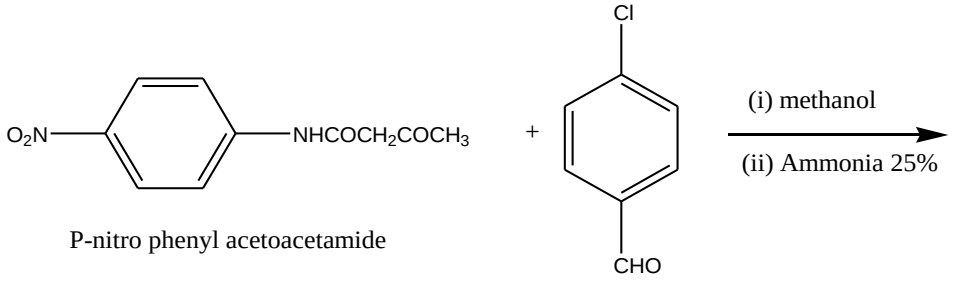


4-(4-dimethyl amino phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-nitro phenyl) pyridine -3,5-dicarboxamide (PND)

b) PND

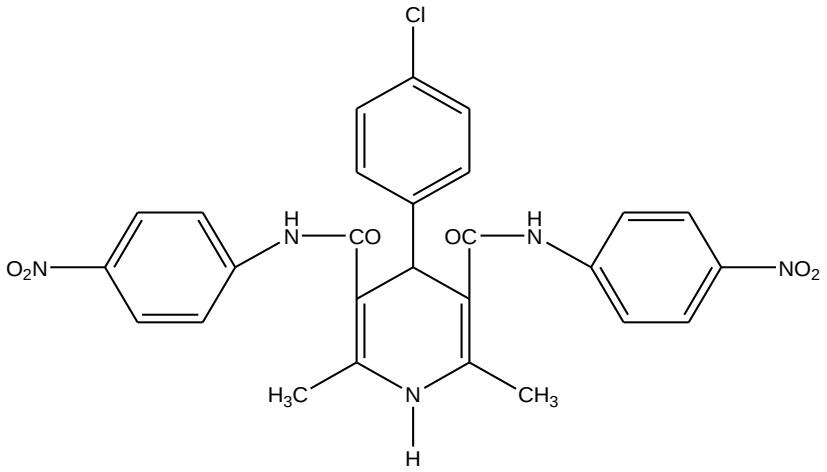
c) Synthesis of 4- (4- chloro phenyl) -1, 4- dihydro- 2, 6- dimethyl N^3, N^5, bis (4- nitro phenyl) pyridine - 3, 5-dicarboxamide (PNC): P-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol and 4 chloro benzaldehyde (0.05 mole, 7.05 gm) was added followed by the addition of excess of ammonia (25%). The reaction mixture was mechanically stirred for 10 min and then heated on water bath under reflux for 10 – 12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol and dried. It was purified by recrystallization from ethanol.

d) Synthesis of 4- (4- hydroxy phenyl) -1, 4- dihydro- 2, 6- dimethyl N^3, N^5, bis (4- nitro phenyl) pyridine - 3, 5-dicarboxamide (PNH): P-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol and P- Hydroxy benzaldehyde (0.05 mole, 6.106 gm) was added followed by the addition of excess of ammonia (25%) q s. The reaction mixture was mechanically stirred for 10 min and then heated on water bath under reflux for 10 – 12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol and dried. It was purified by recrystallization from ethanol.



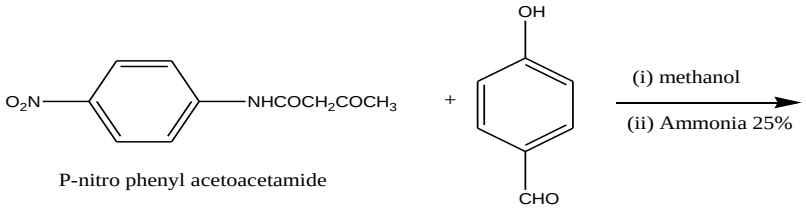
P-nitro phenyl acetoacetamide

P- Chloro benzaldehyde



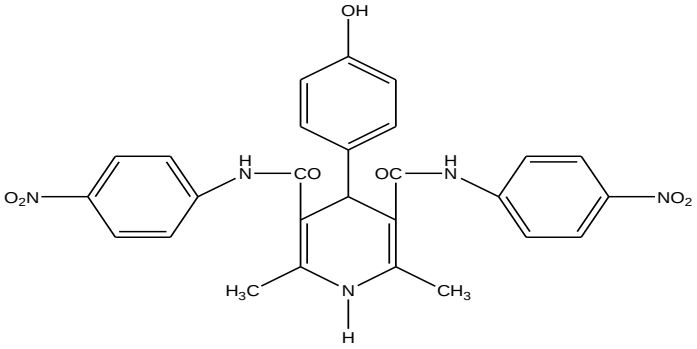
4-(4-chloro phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-nitro phenyl) pyridine -3,5-dicarboxamide (PNC)

c) PNC



P-nitro phenyl acetoacetamide

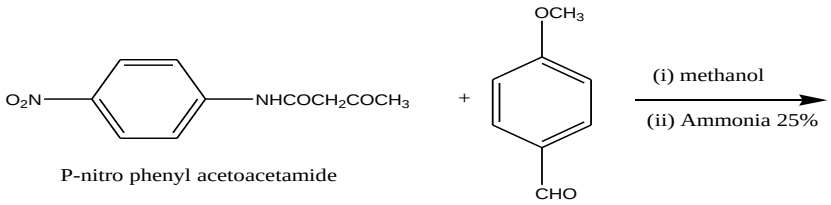
P- Hydroxy benzaldehyde



4-(4-hydroxy phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-nitro phenyl) pyridine -3,5-dicarboxamide (PNH)

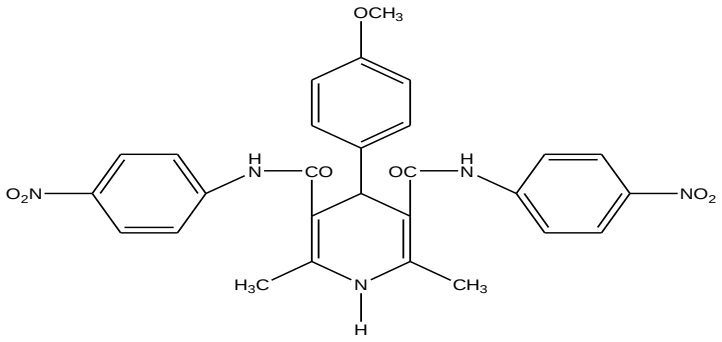
d) PNH

e) Synthesis of 4- (4- methoxy phenyl) -1, 4-dihydro-2, 6- dimethyl N^3, N^5, bis (4- nitro phenyl) pyridine -3, 5- dicarboxamide (PNA): P-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol and P- methoxy benzaldehyde (0.05 mole, 6.05 gm) was added followed by the addition of excess of ammonia (25%) (20 ml). The reaction mixture was mechanically stirred for 10 min and then heated on water bath under reflux for 10 – 12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol and dried. It was purified by recrystallization from ethanol.



P-nitro phenyl acetoacetamide

P- Methoxy benzaldehyde

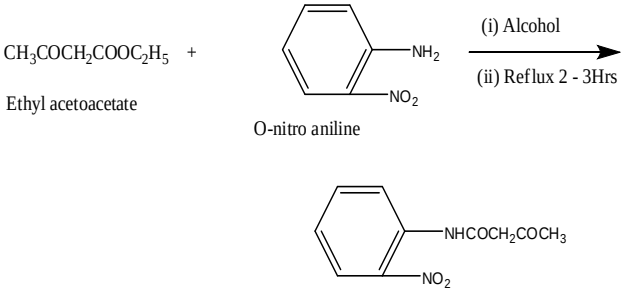


4-(4-methoxy phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-nitro phenyl) pyridine -3,5-dicarboxamide (PNA)

e) PNA

SCHEME – II:

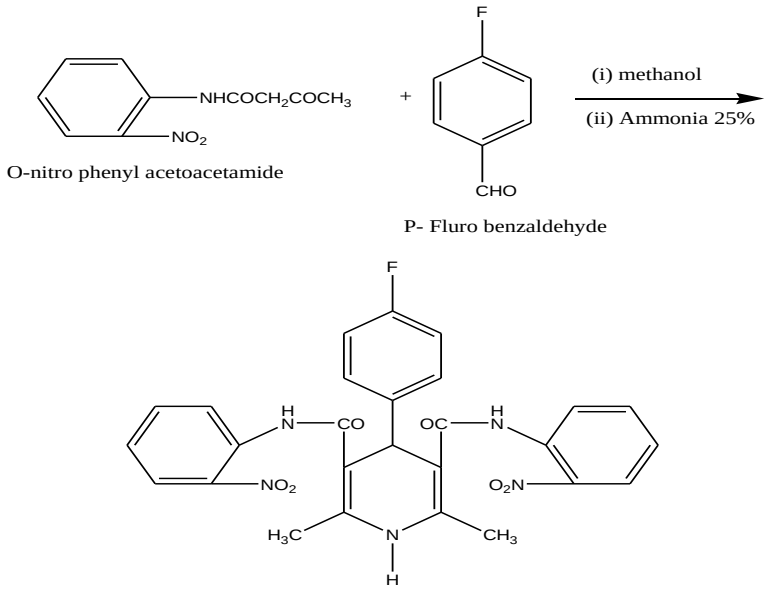
Synthesis of O-nitro phenyl aceto acetamide: Ethyl aceto acetate (0.05 mole, 6.5 ml) and O-nitro aniline (0.05 mole, 6.93 gm) were taken in the round bottom flask and dissolved in alcohol (quantity sufficient to obtain a complete solution) and reflux for about 2-3 hrs. The reaction mixture was cooled at -5° C. the solid that separated out was purified by recrystallization from ethanol to give yellowish crystalline compound.



Synthesis of O-nitro phenyl aceto acetamide

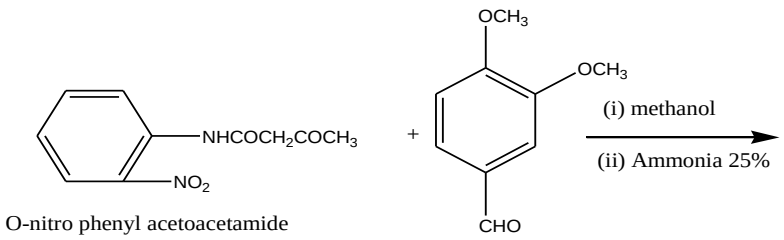
a) Synthesis of 4- (4- fluro phenyl) -1, 4- dihydro-2, 6- dimethyl N^3, N^5, bis (2- nitro phenyl) pyridine -3, 5- dicarboxamide (ONF): O- nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol (quantity sufficient) and P-fluro benzaldehyde was added followed by the addition of excess of ammonia (25%) q. s. The reaction mixture was mechanically stirred for 10min and then heated on water bath under reflux for 10 – 12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol. It was purified by recrystallization from ethanol.

b) Synthesis of 4- (3, 4- dimethoxy phenyl) -1, 4- dihydro-2, 6- dimethyl N^3, N^5, bis (2- nitro phenyl) pyridine -3, 5- dicarboxamide (OND): O- nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol (quantity sufficient) and 3,4-dimethoxy benzaldehyde (0.05 mole, 8.3 gm) was added followed by the addition of excess of ammonia (25%) q. s. The reaction mixture was mechanically stirred for 10- 15 min and then heated on water bath under reflux for 10 – 12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with small portions of methanol and dried. It was purified by recrystallization from ethanol.



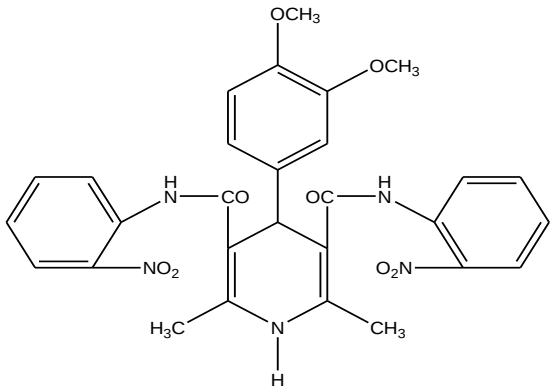
4-(4-fluro phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (2-nitro phenyl) pyridine -3,5-dicarboxamide (ONF)

a) ONF



O-nitro phenyl acetoacetamide

3,4-Dimethoxy benzaldehyde

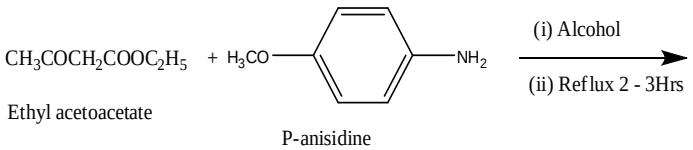


4-(3,4-dimethoxy phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (2-nitro phenyl) pyridine -3,5-dicarboxamide (OND)

b) OND

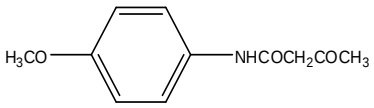
SCHEME – III:

Synthesis of P-methoxy phenyl aceto acetamide: Ethyl aceto acetate (0.05 mole, 6.5 ml) and P-methoxy aniline (0.05 mole, 6.08 gm) were taken in the round bottom flask and dissolved in alcohol (quantity sufficient) and refluxed for about 3 Hrs. the reaction mixture was cooled. The solid that separated out was filtered and dried. The crude solid of anilide was purified by recrystallization twice from ethanol.



Ethyl acetoacetate

P-anisidine



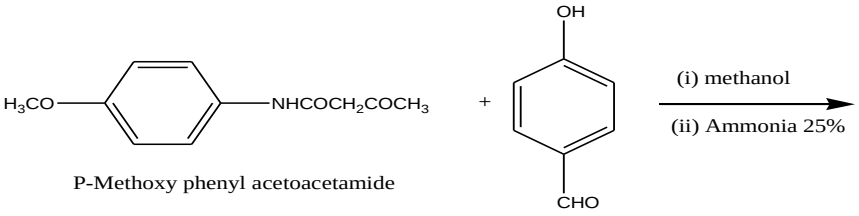
P-methoxy phenyl acetoacetamide

Synthesis of P-methoxy phenyl aceto acetamide

a) Synthesis of 4- (4- hydroxy phenyl) -1, 4-dihydro- 2, 6-dimethyl N^3, N^5, bis (4 -methoxy phenyl) pyridine -3, 5- dicarboxamide (PAH): P-methoxy phenyl aceto acetamide (0.01 mole, 2.83 gm) was dissolved in methanol and 4-hydroxy benzaldehyde (0.05 mole, 6.1 gm) was added followed by the addition of Excess of ammonia (25 %) q. s. The reaction mixture was mechanically stirred for 10 min and then heated on water bath under reflux for 10 Hrs. Methanol was removed from the reaction mixture under reduced pressure and cooled. The product thus separated, was filtered and washed with small portions of methanol and dried. It was purified by recrystallization from ethanol.

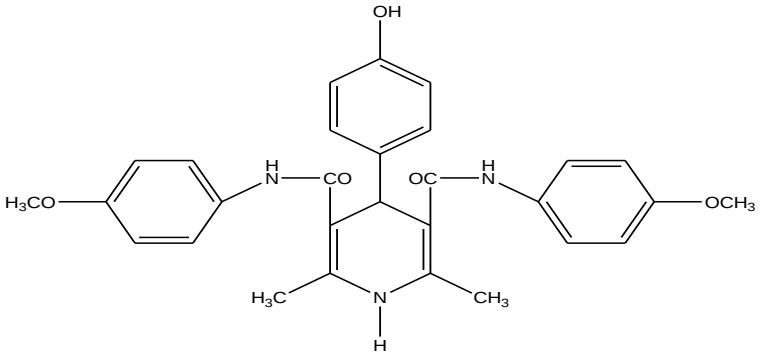
b) Synthesis of 4- (4- nitro phenyl) - 1, 4- dihydro- 2, 6 - dimethyl N^3, N^5, bis (4- methoxy phenyl) pyridine - 3, 5- dicarboxamide (PAN): P-methoxy phenyl aceto acetamide (0.01 mole, 1.24 gm) was dissolved in methanol and 4-nitro benzaldehyde

(0.05 mole, 7.5 gm) was added followed by the addition of excess of ammonia (25 %) q. s. The reaction mixture was mechanically stirred for 10 min and then heated on hot water bath under reflux for 10 - 12 Hrs. Methanol was removed from the reaction mixture under reduced pressure and cooled. The product thus separated, was filtered and washed with small portions of methanol and dried. It was purified by recrystallization from ethanol.



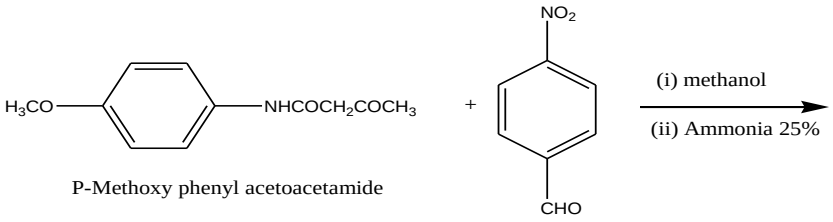
P-Methoxy phenyl acetoacetamide

P- Hydroxy benzaldehyde



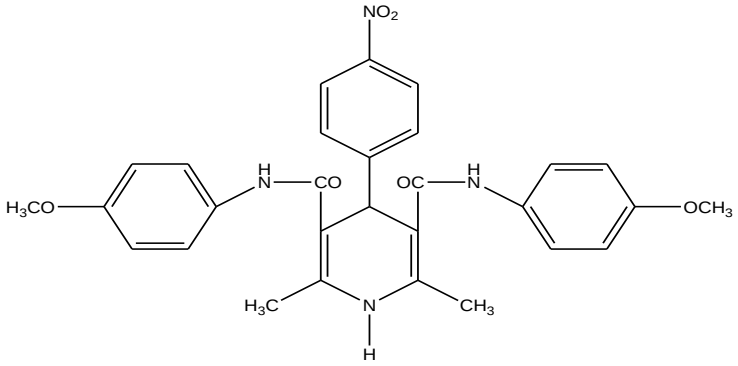
4-(4-hydroxy phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-methoxy phenyl) pyridine -3,5-dicarboxamide (PAH)

a) PAH



P-Methoxy phenyl acetoacetamide

P- Nitro benzaldehyde

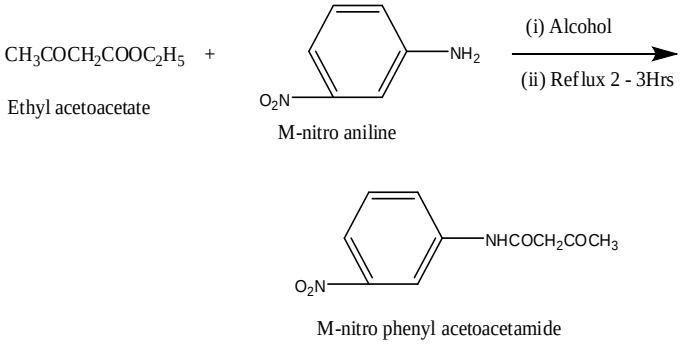


4-(4-nitro phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (4-methoxy phenyl) pyridine -3,5-dicarboxamide (PAN)

b) PAN

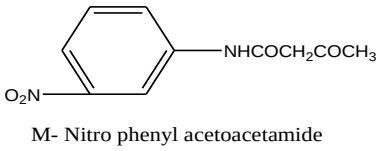
SCHEME IV:

Synthesis of M- nitro phenyl aceto acetamide: Ethyl aceto acetate (0.05 mole, 6.5 ml) and M- nitro aniline (0.05 mole, 6.913 gm) were taken into a dry round bottom flask and dissolved in alcohol and refluxed for about 2-3 Hrs. the reaction mixture was cooled. The solid that separated out was filtered washed with cold water and dried. The crude solid of anilide was purified by recrystallization from ethanol.



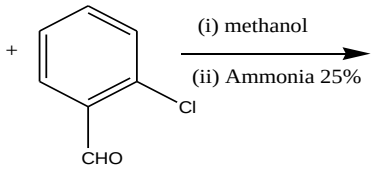
Synthesis of M- nitro phenyl aceto acetamide

a) Synthesis of 4- (2- chloro phenyl) -1, 4- dihydro- 2, 6-dimethyl N^3, N^5, bis (m- nitro phenyl) pyridine -3, 5-dicarboxamide (MNC): M-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol (quantity sufficient) and O- Chloro benzaldehyde (0.05 mole, 6.19 gm) was added followed by the addition of Excess of ammonia (25%) q. s. The reaction mixture was mechanically stirred for 10 min, and then heated on water bath under reflux for 10-12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol. It was purified by recrystallization from ethanol.

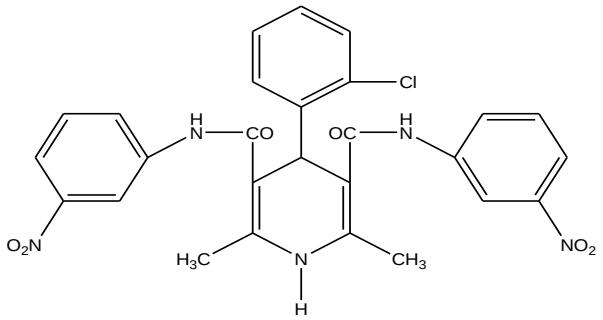


M- Nitro phenyl acetoacetamide

b) Synthesis of 4- (4- methoxy phenyl) - 1, 4- dihydro- 2, 6- dimethyl N^3, N^5, bis (m- nitro phenyl) pyridine - 3, 5-dicarboxamide (MNA): M-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol (quantity sufficient) and P- methoxy benzaldehyde (Anisaldehyde) (0.05 mole, 6.05 ml) was added followed by the addition of Excess of ammonia (25%) (quantity sufficient). The reaction mixture was mechanically stirred for 10 min, and then heated on water bath under reflux for 10 – 12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol. It was purified by recrystallization from ethanol.

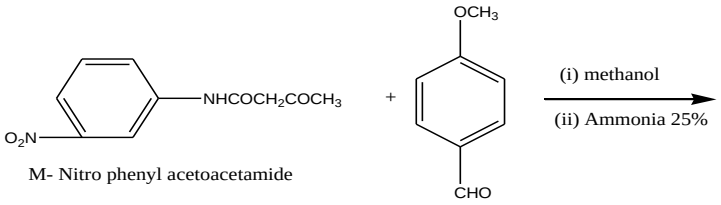


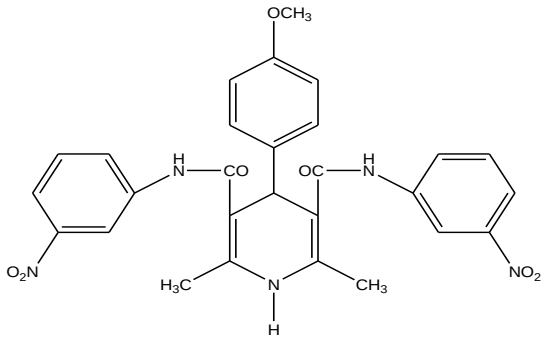
O- Chloro benzaldehyde



4-(2-chloro phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (m-nitro phenyl) pyridine -3,5-dicarboxamide (MNC)

a) MNC

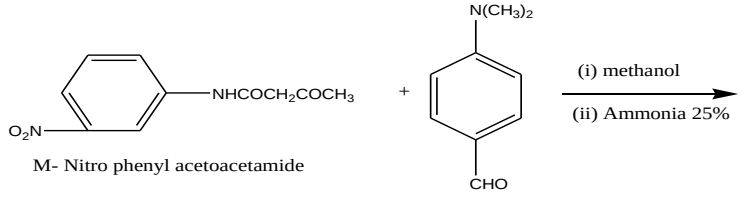


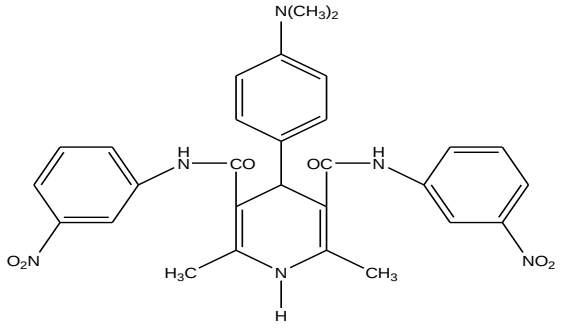


4-(4-methoxy phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (m-nitro phenyl) pyridine -3,5-dicarboxamide (MNA)

b) MNA

c) Synthesis of 4- (4- dimethyl amino phenyl) -1, 4-dihydro-2, 6- dimethyl N^3, N^5, bis (m- nitro phenyl) pyridine - 3, 5-dicarboxamide (MND): M-nitro phenyl aceto acetamide (0.01 mole, 2.2 gm) was dissolved in methanol (quantity sufficient) and P-dimethyl amino benzaldehyde (0.05 mole, 4.78 gm) was added followed by the addition of Excess of ammonia (25%) q. s. The reaction mixture was mechanically stirred for 10 min, and then heated on water bath under reflux for 10-12 Hrs. methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol. It was purified by recrystallization from ethanol.





4-(4-dimethyl amino phenyl) -1,4-dihydro-2,6-dimethyl N3,N5, bis (m-nitro phenyl) pyridine -3,5-dicarboxamide (MND)

c) MND

Spectral analysis [21,22]:

Compound PNN:
IR Spectral data: 3392, 3105 N-H stretching, 2928 C-H stretching, 1603 N=N stretching, 1103-1006 C-O stretching, 1526 C-NO_2 Stretching, 14201420.

NMR Spectral data: 2.337 Ar-CH_3, 7.474 Ar-H, 2.263 Ar-C, 4.411 CH-NO_2, 8.050 N=O.

Compound PND:
IR Spectral data: 3449 N-H Stretching, 2918 Aliphatic C-H Stretching, 1623 C=C Stretching, 1406 C-N Stretching, 1565 C-NO_2 Stretching.

Compound PNC:
IR Spectral data: 3448.1 N-H Stretching, 2913 C-H Stretching, 814 C-Cl Stretching, 1066 C-O stretching.

Compound PNH:
IR Spectral data: 3481 N-H Stretching, 3361, 3215 O-H Stretching, 1669 C=O (amide), 1597 Ar-NO_2 Stretching, 1632 C=C Stretching.

NMR Spectral data: 9.859 Ar-OH, 2.337 Ar-CH_3, 4.449 CH-NO_2, 8.059 Ar-CN, 8.063 Ar-NH.

Compound PNA:
IR Spectral data: 3429 N-H Stretching, 2275 C-H Stretching, 1602 C=C Stretching,1565 Ar-NO_2 Stretching, 1109 C-O Stretching.

Compound ONF:
IR Spectral data: 3430 N-H Stretching, 2372 C-H Stretching, 1628 C=C Stretching, 1684 C=O (amide), 1518 Ar-NO_2 Stretching, 1351 C-F Stretching.

NMR Spectral data: 7.954 Ar-H, 6.967 Ar-F, 7.967 CH-NO, 1.261 CH-CH_3, 8.036 Ar-NH.

Compound OND:
IR Spectral data: 3429 N-H Stretching, 2841 C-H Stretching, 1628 C=C Stretching, 1587 C-NO_2 (aromatic), 1680 C=O (amide), 1021 C-O Stretching.

Compound PAH:
IR Spectral data: 3449 N-H Stretching, 2918 C-H Stretching, 1606 C=C Stretching.

NMR Spectral data: 7.263 Ar-CH_3, 5.186 Ar-OH, 7.872 Ar-H, 3.833 CH-OH, 8.064 Ar-NH.

Compound PAN:
IR Spectral data: 3074 C-H Stretching, 1604 C-NO_2 Stretching, 1686 C=O (amide) Stretching.

Compound MNC:
IR Spectral data: 3427 N-H Stretching, 1620 C=C Stretching, 1525 Ar-NO_2 group, 862 C-Cl Stretching, 2490 C-H Stretching, 1079 C-O Stretching.

Compound MNA:
IR Spectral data: 3429 N-H Stretching, 2373 C-H Stretching,1595 C=C Stretching,1520 Ar-NO_2 Stretching,1160 C-O Stretching.

NMR Spectral data: 3.892 Ar-H, 3.995 CH-NO_2, 3.752 CH-CH_3, 6.952 CH-CO, 8.049 Ar-CN, 8.010 Ar-NH.

Compound MND:
IR Spectral data: 3477, 3349 N-H Stretching, 2370 C-H Stretching, 1628 C=C Stretching, 1569 Ar-NO_2 Stretching, 1429 C-N (amide), 1099 C-O Stretching.

Pharmacological studies:

Supra Maximal Electrical Method [23, 24, 25]: The electro shock assay in rat is used primarily as a indication for compounds which are effective in grandmal epilepsy. Male albino wistar rats are stimulated through are stimulated through pinna electrodes (150 mA- alternating current, 0.2 sec-stimulus duration). The resultant seizure in normal rats shows a tonic phase of limb flexion around 2 seconds, followed by full tonic extension phase around 10 -13 seconds and a few clonic jerks there after the number of post- tonic as physical death are noted.

Procedure: Healthy albino wistar rats weighing from 200 – 250 g were selected. They were kept in separate cages, fed with balanced diet, water and *ad libitum*. Then the animals were divided into 12 groups each groups containing six animals. The first groups of animals were served as control, which received 0.5 ml DMSO. Second group served as standard which received phenytoin sodium (25 mh/kg).Third group treated with PNH compound (10 mg/kg).Fourth group treated with PNA compound (10 mg/kg). Fifth group treated with ONF compound (10 mg/kg). Sixth group treated with MNA compound (10 mg/kg). Seventh group treated with PAH compound (10 mg/kg).All the test compounds were dissolved in solvent like DMSO and administered through intra-peritoneal route.The evaluation was started 30 mins after administration of test compounds. Pinna electrodes with the intensity of 150 mA current were used to deliver the stimuli.Inhibition of seizure relative to the control was calculated and the data shown on the **Table 1**.

Table 1: ANTI CONVULSANT ACTIVITY OF VARIOUS SYNTHESIZED DRUGS BY SUPRA MAXIMAL ELECTRICAL SHOCK METHOD

Treatment	Body Wt.	Drug	Dose	Duration of extension phase in seconds	% inhibition of extension phase
Group I	180 – 200 gm	Normal saline	10 ml / kg	13.52 ± 0.12	--
Group II	180 – 200 gm	Phenytoin sodium	25 ml / kg	2.2 ± 0.18	83.72 %
Group III	180 – 200 gm	PNH	10 ml / kg	4.30 ± 0.28	68.19 %
Group IV	180 – 200 gm	PNA	10 ml / kg	4.12 ± .0.42	69.52 %
Group V	180 – 200 gm	ONF	10 ml / kg	4.68 ± 0.52	65.38 %
Group VI	180 – 200 gm	MMD	10 ml / kg	4.02 ± 0.36	70.26 %
Group VII	180 – 200 gm	PAH	10 ml / kg	4.50 ±. 0.30	66.71 %

Values are expressed as Mean ± SEM; values are find out by using One way ANOVA followed by Newman Keul's multiple range test

Analgesic activity [26, 27]: The analgesic activities of various synthesized compounds were screened by employing tail flick method. Rats of either sex weighing between 150 – 200 gm were taken in 15 groups of each 6 animals. Diclofenac potassium 10 mg/kg was used as a standard drug for comparison of analgesic activity. Tail flick response was evoked by placing rat tail over a wire heated electrically. The intensity of heat was adjusted so that the baseline flick latency averaged 3-4 seconds in all the animals. Cut off period of 15 seconds was observed to prevent the damage to the tail. Data are expressed as mean ± S.E.M., one way ANOVA followed by Newman's Keul's multiple range tests was applied to determine the significance of the difference between the control group and mice treated with the test compounds. The differences in results were considered significant at $P < 0.01$. (**Table 2**)**.**

TABLE 2: EFFECT OF ANALGESIC ACTIVITIES OF SYNTHESIZED COMPOUNDS IN RATS

Treatment Group	Dose	Time in Minutes					
		0 Min	30 Mins	60 Mins	90 Mins	120 Mins	180 Mins
Control	10 ml / kg Normal saline	2.4 ± 0.19	2.1 ± 0.21	2.02 ± 0.14	2.3 ±0.16	1.8 ± 0.22	2.6 ± 0.21
Standard	10 ml / kg Diclofenac sodium	2.42 ± 0.20	3.9 ± 0.19	5.96 ± 0.22	6.1 ± 0.26	5.9 ± 0.21	5.2 ± 0.16
I	Compd. I PNH	2.26 ± 0.22	3.7 ± 0.21	5.2 ± 0.19	4.9 ± 0.11	4.42 ± 0.14	4.6 ± 0.16
II	Compd. II PNA	2.11 ± 0.17	3.92 ± 0.14	5.81 ± 0.19	5.36 ± 0.14	5.7 ± 0.21	5.12 ± 0.14
III	Compd III ONF	2.29 ± 0.28	3.84 ± 0.20	5.92 ± 0.16	5.64 ± 0.19	6.02 ± 0.14	5.01 ± 0.17
IV	Compd. IV MNA	2.16 ± 0.23	3.6 ± 0.19	6.1 ± 0.10	5.54 ± 0.19	5.6 ± 0.21	4.82 ± 0.12
V	Compd. V PAH	2.31 ± 0.12	3.74 ± 0.16	5.9 ± 0.21	5.41 ± 0.14	5.02 ± 0.24	4.64 ± 0.18

Values are expressed as mean ± SEM; significant at $P < 0.01$.

Antibacterial activity [28,29]: The sterilized Muller Hinton Agar Media was heated on a water bath to melt the media. When the media was lukewarm, the organism were inoculated separately and poured aseptically in to sterile Petri dishes and allowed to solidify.The standard drug Amikacin disc was placed on the media and the whatmann no.2 filter disc (5mm diameter) were cut and filled in to vials plugged with cotton. These vials were kept in hot air oven at 160°C for 30 minutes for sterilization.

Then it was soaked in synthesized compounds separately and evaporated to dryness and then kept on the media (5 mm height). One more disc immersed in Dimethyl sulfoxide and kept on the media as control. It was kept in the refrigerator for one hr to facilitate uniform diffusion of the drug and later kept in the incubator for a period of 24 hrs at 37°C. Observations were made for the zone of inhibition around the synthesized compounds and compared with that of standard. (**Table 3**).

Table 3: ANTI BACTERIAL ACTIVITY OF VARIOUS SYNTHESIZED COMPOUNDS AGAINST G (+) VE AND G (-) VE ORGANISMS

Synthesized compounds	Zone of Inhibition against	
Code	Gram +ve S.aureus in mm (%)	Gram -ve E.Coli in mm (%)
PNN	15	8
PNC	Nil	6
PNM	7	11
ONF	11	7
MNC	8	8
MNA	7	7
PAN	12	8
PPH	9	10
Control	9	8
Standard	18	17

Standard: Amikacin;
Control : DMSO

Antifungal activity[30, 31]: For the screening of anti fungal activity disc diffusion method was used. Sabouraud Dextrose agar plates were prepared aseptically to get a thickness of 5-6 mm. the plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37° C just before inoculation. From the solid culture the clinical sample of *Candida albicans* were inoculated in to Sabouraud Dextrose agar plates by using sterile inoculation loop and agar plates were incubated for about 24 Hrs at 37° C, which showed sufficient growth of fungi. The temperature of the medium should not exceed about 50° C when the organisms were inoculated. The standard drug Ketaconazole (10µg/disc) was placed on the media. The sterile Whatmann No.2 filter paper disc (5 mm diameter) was soaked in synthesized compounds (20µg/disc) separately and evaporated to dryness and then kept on the media. One more disc immersed in dimethyl sulphoxide and kept on the media as control. The Petri dishes were incubated at 37° C for 24 Hrs, after placing them in the refrigerator for one hour to facilitate uniform diffusion. Observations were made for the zone of inhibition around the synthesized compounds and with that of standard (**Table 4**).

Table 4: ANTI FUNGAL ACTIVITY OF VARIOUS SYNTHESIZED COMPOUNDS

Synthesized compounds	Zone of Inhibition in mm
Code	Microorganism used *Candida Albicans*
PNN	7
PNC	12
PNM	14
ONF	17
MNC	RESISTANCE
MNA	10
PAN	11
PPH	14
Control	10
Standard	15

Standard: Ketoconozole
Control : DMSO

RESULTS:

Anti Convulsant activity: Synthesized compounds like PNH, PNA, ONF, MND, PAH posses significant Anti convulsant activity. All compounds were reducing the duration of extensor phase significantly.

Analgesic activity: Analgesic activity of the compounds by Eddy's hot plate method was evaluated against Diclofenac Sodium as the standard the tail flick response was considered as the end point to remove the animals from the source of the pain stimulus. The basal reaction time (latency between the application of the stimulus and the response) of the control animals ranged between (2.2 ± 0.33 - 3.3 ±0.33) after treatment of the animals the basal reaction time was prolonged (statistically significant increase) by the compounds (PNH, PNA, ONF, MND and PAH) which reached maximum levels at the 2ndhr. However all the compounds showed analgesic activity less than the reference standard?

Anti Microbial studies:

Anti Bacterial activity: Anti bacterial activities of the compounds were evaluated against *staphylococcus aureus* and the zone of inhibition was measured as the parameter of activity. Amikacin, the standard showed a zone of inhibition of 18 mm out of 8 synthesized compounds only one PNN (Ia) compound showed an equal activity when compared with standard. In which (ONF, PAN) showed considerable anti bacterial activity. The remaining four compounds PNM, MNC, MNA, PAK showed moderate antibacterial activity. Anti bacterial activity of the compounds was evaluated against *E. Coli* and the zone of inhibition was measured as the parameter of activity. Amikacin, the standard showed a zone of inhibition of 17 mm. out of 8 synthesized compounds, compound PNM, PAH showed maximum high degree of anti bacterial

activity. The remaining compounds PNC, ONF, MNA, PAN showed moderate anti bacterial activity.

Anti Fungal activity: The anti fungal activities of the compounds were evaluated against *Candida albicans* using Ketaconazole as the standard compound. The activity of the compounds measured in terms of zone of inhibition ranged between 7 to 15 mm where the upper limit is lower than that of Ketaconazole (15 mm), the compound ONF showed high degree of anti fungal activity.

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