Muscle Relaxant Property of 1(4-carboxy phenyl)-4,4,6-trimethyl-1H, 4H pyrimidine-2 thiol (a Pyrimidine Derivative) in *Ex vivo* Smooth and Skeletal Muscle Preparations

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ABSTRACT

Background: Pyrimidine and its derivatives demonstrate a wide range of biological and pharmacological activities. **Objective:** To study the smooth muscle and skeletal muscle relaxant activity of a pyrimidine derivative 1 (4-carboxy phenyl)-4,4,6-trimethyl-1H,4H pyrimidine- 2 thiol (4CPTP), the chemical structure of which is similar to phenobarbitone. **Material and Methods:** Spasmolytic activity of 4CPTP was studied using rabbit ileum and guinea pig ileum preparation. Skeletal muscle relaxant activity was assessed on frog rectus abdominis preparation. Mean height of contractions with graded doses of phenobarbitone and 4CPTP along with mean inhibition of contractions were calculated. Statistical analysis was done using chi square test. **Results:** The test compound produced dose dependent decrease in acetylcholine and barium chloride induced contractions of rabbit ileum, histamine induced contractions of guinea pig intestine and acetyl choline induced contractions of frog rectus abdominis. **Conclusion:** 4 CPTP possesses spasmolytic and neuromuscular blocking activity in our *ex vivo* studies.

Key words: Pyrimidines, Phenobarbitone, Spasmolytic, Neuromuscular blocker.

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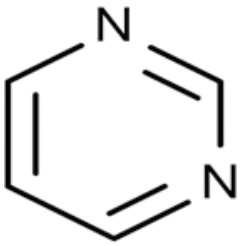
INTRODUCTION

Pyrimidine is a six member cyclic compound containing four carbon and two nitrogen atoms at position 1 and 3.



Structure of pyrimidine

Pyrimidines constitute an important fraction of nucleic acid mainly, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) which participates in the formation of nucleo- proteins in the plants and animal cells and control heredity on molecular level. Pyrimidine and its derivatives also demonstrate a wide range of other biological and pharmacological activities including anticonvulsant, antibacterial, antifungal, antiviral and anticancer properties. [1] Pyrimidines are synthetically versatile substrates. With a slight change in their chemical structure, a large number of synthetic pyrimidine derivatives have been prepared

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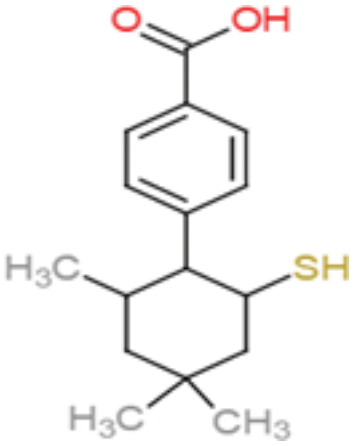
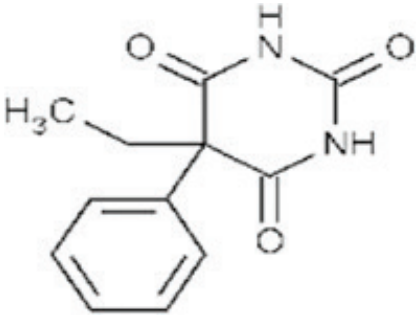
and a number of experimental studies have been carried out to investigate the pharmacological actions of these compounds.[2]

The compound under this study, 1(4-carboxy phenyl)-4,4,6-trimethyl-1H,4H pyrimidine-2 thiol (4CPTP), also belongs to thio pyrimidine series. The chemical structure of the compound is somewhat similar to phenobarbitone. The chemical formula of the compound is 1(4-carboxy phenyl)-4,4,6-trimethyl-1H,4H pyrimidine-2 thiol. Due to its structural resemblance to phenobarbitone (an anti epileptic drug), this compound has been tested for its actions on central nervous system in an earlier study, where it showed dose dependent anti convulsant action, similar to phenobarbitone.[3] In this study, we investigated and compared the effects of 4CPTP and phenobarbitone on smooth muscles and skeletal muscles.



Structure of 4CPTP



Structure of phenobarbitone

MATERIAL AND METHODS

Animals

To study the spasmolytic effect of 4CPTP on smooth muscle preparations, 6 adult rabbits (Oryctologuscuniculus species) of either sex and weight between 1-1.5 kg and 6 adult guinea pigs (Cavitapercellers species) of either sex were used. Effect of 4CPTP on skeletal muscles was studied by using 6 adult frogs (Ranatigrina species) of either sex.

Drugs

4CPTP was synthesised in chemistry department, Punjabi university, Patiala. Phenobarbitone was purchased from May and baker pharmaceuticals Ltd, Bombay. The test compound 4CPTP and standard comparator phenobarbitone was used in graded doses of 25-200 μg/ml of bath concentration for isolated rabbit's ileum preparation. The contractions of the intestine were induced by spasmogens acetyl choline (0.2 μg/ml bath concentration) and barium chloride (50 μg/ml bath concentration) in this experiment. For assessing the effect on guinea pig's ileum, 4CPTP and phenobarbitone in graded doses of 50-400 μg/ml of bath concentration weight were used as test drug and standard comparator respectively. The contractions of the intestine, in this experiment, were induced by spasmogen histamine (0.25 μg/ml bath concentration). In frog's rectus abdominis muscle preparation, graded doses of 25-200 μg/ ml of bath concentration of 4CPTP and phenobarbitone were used. The contractions of the rectus abdominis muscle were induced by acetyl choline (2 μg/ml bath concentration).

Assessment of effect of 4 CPTP on smooth muscles and skeletal muscles

Isolated rabbit's ileum preparation

A healthy adult rabbit was taken, stunned and bled through carotid arteries. A piece of proximal ileum of rabbit's intestine, approximately 5 cm in length was taken out and mounted in Magnus inner bath containing oxygenated ringer locke solution at 37°C. The contractions of the intestine were induced by spasmogens acetyl choline (0.2 μg/ml bath concentration). The effect of graded doses of 4CPTP and phenobarbitone (25-200 μg/ml of bath concentration) was observed alternately against acetylcholine (0.2 μg/ml bath concentration). The drug was allowed to remain in the bath for 60 seconds and then, with the spasmogen, contractions were recorded for 30 seconds. 6 such experiments were set up and their mean values were calculated and analysed statistically. In another set of 6 such similar experiments, the effect of graded doses of 4CPTP and phenobarbitone was observed alternately against barium chloride (50 μg/ml bath concentration). Mean values of the experiments were calculated and analysed statistically.

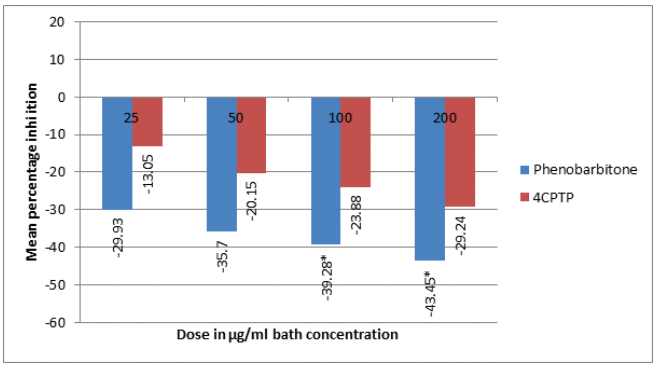
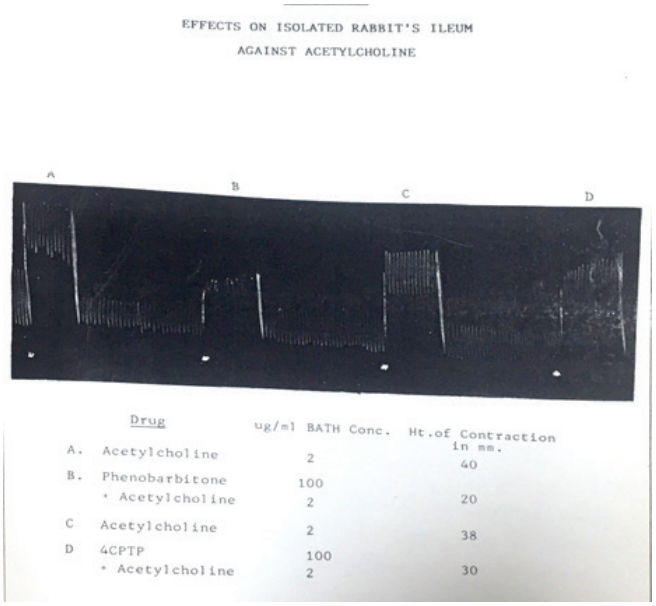


Figure 1: Diagram showing mean percentage inhibition against acetyl choline induced contractions with phenobarbitone and 4CPTP on isolated rabbit's ileum.
*- p value < 0.05.



Graph 1: Graph showing effect of phenobarbitone and 4CPTP on acetyl choline induced contractions on isolated rabbit's ileum.

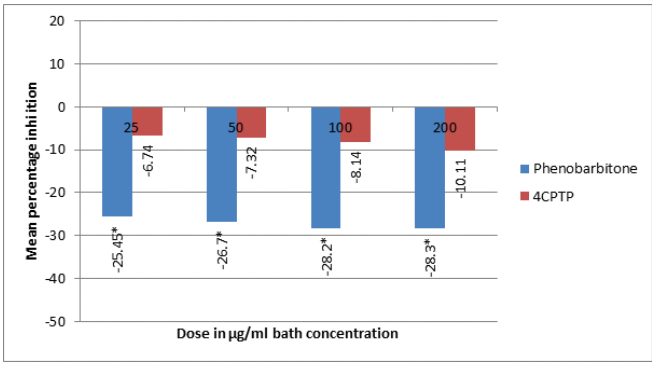
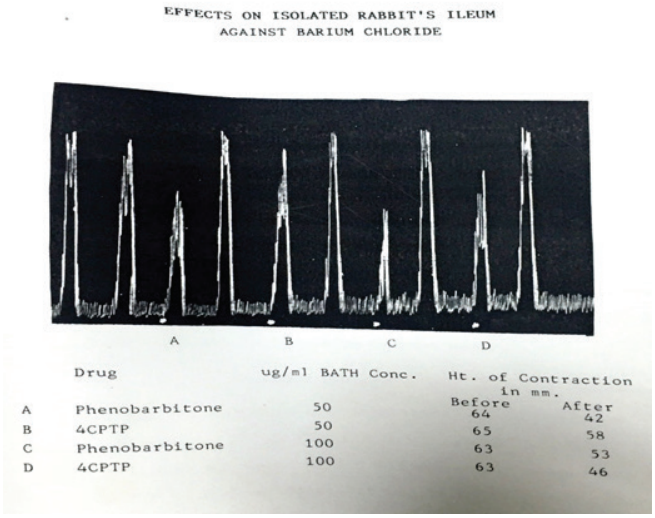


Figure 2: Diagram showing mean percentage inhibition against barium chloride induced contractions with phenobarbitone and 4CPTP on isolated rabbit's ileum.
*- p value<0.05.



Graph 2: Graph showing effect of phenobarbitone and 4CPTP on barium chloride induced contractions on isolated rabbit's ileum.

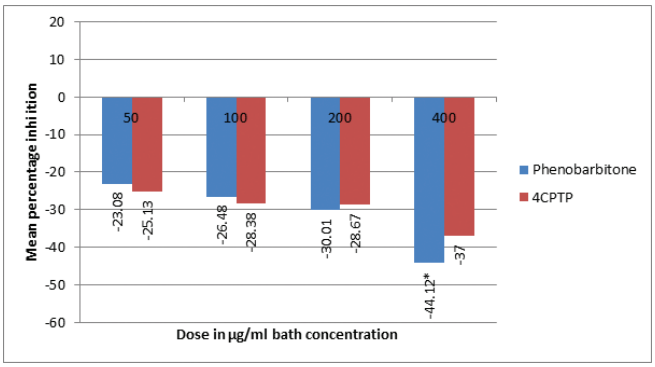
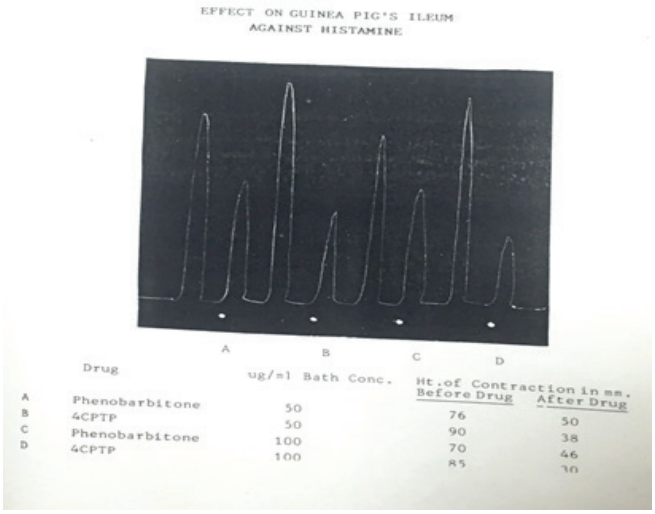


Figure 3: Diagram showing mean percentage inhibition against histamine induced contractions with phenobarbitone and 4CPTP on isolated guinea pig's ileum.
*- p value < 0.05.



Graph 3: Graph showing effect of phenobarbitone and 4CPTP onhistamine induced contractions on isolated rabbit's ileum.

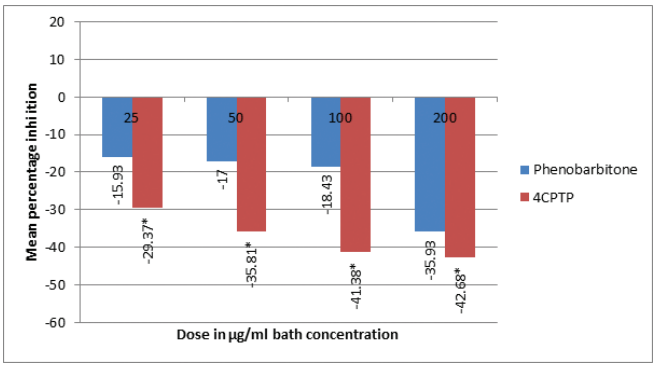
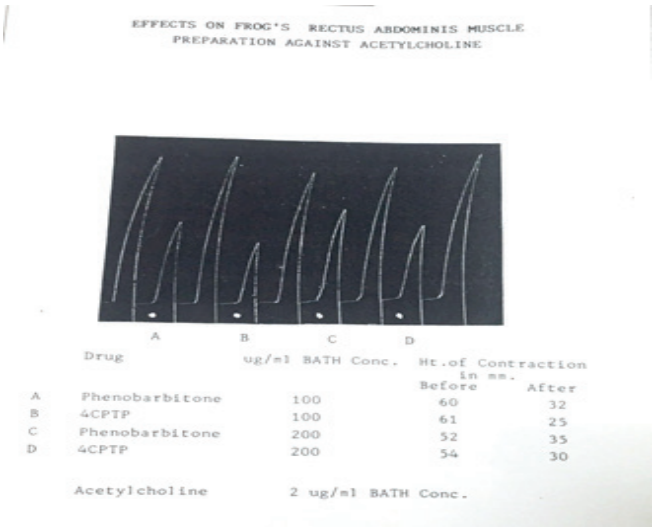


Figure 4: Diagram showing mean percentage inhibition against acetyl choline induced contractions with phenobarbitone and 4CPTP on frog's rectus abdominis muscle preparation.
*- p value<0.05.



Graph 4: Graph showing effect of phenobarbitone and 4CPTP on acetyl choline induced contractions on frog's rectus abdominis.

Isolated guinea pig's ileum preparation

A healthy adult guinea pig was taken after starving overnight and then stunned and bled through carotid arteries. A piece of ileum of guinea pig's intestine, approximately 5 cm in length was taken out and mounted in Magnus inner bath containing oxygenated tyrode's solution at 37°C. The contractions of the intestine, in this experiment, were induced by spasmogen histamine (0.25 µg/ml bath concentration). Then the effect of graded doses of 4CPTP and phenobarbitone (50-400 µg/ml of bath concentration) was observed alternately against histamine (0.25 µg/ml bath concentration). The drug was allowed to remain in the bath for 60 seconds and then, with the spasmogen, contractions were recorded for 30 seconds. 6 such experiments were set up and their mean values were calculated and analysed statistically.

Frog's rectus abdominis muscle preparation

An adult frog was pithed and a strip of the rectus abdominis muscle was dissected out and mounted in Dale's organ bath containing oxygenated frog ringer solution at room temperature. The contractions of the rectus abdominis muscle were induced by acetyl choline (2 µg/ml bath concentration). Then the effect of graded doses of 4CPTP and phenobarbitone (25-200 µg/ml of bath concentration) was observed alternately against acetyl choline (2 µg/ml bath concentration). The drug was allowed to remain in the bath for 60 seconds and then, after adding acetyl choline, contractions were recorded for 30 seconds. 6 such experiments were set up and their mean values were calculated and analysed statistically.

Statistical analysis

Mean height of contractions with graded doses of phenobarbitone and 4CPTP along with mean inhibition of contractions were calculated. Statistical analysis was done using chi square test.

RESULTS

The effect of graded dose of phenobarbitone and 4CPTP on acetylcholine induced contractions in rabbit's ileum has been described in Table 1, Figure 1 and Graph 1. Table 2, Figure 2 and Graph 2 show the effect of graded doses of phenobarbitone and 4CPTP on barium chloride induced contractions in rabbit's ileum. Effect of phenobarbitone and 4CPTP on guinea pig's ileum has been studied by inducing contractions with histamine, as shown in Table 3, Figure 3 and Graph 3. Table 4, Figure 4 and Graph 4 show the effect of graded doses of phenobarbitone and 4CPTP on acetylcholine induced contractions on frog's rectus abdominis preparation.

DISCUSSION

In the present study, 4CPTP was seen to cause decrease in acetyl choline and barium chloride induced contractions of intestinal smooth muscles in rabbit ileum and histamine induced contractions of guinea pig's ileum in a dose dependent manner. However, when used in same doses, spasmolytic activity of 4CPTP was less marked than phenobarbitone. The test compound was also

Table 1: Table showing mean height of contractions (mm) against acetylcholine induced contractions with graded doses of phenobarbitone and 4CPTP on isolated rabbit's ileum.

Dose (µg/ml bath concentration)	Phenobarbitone (mean height of contraction ± S.E.)		4CPTP (mean height of contraction ± S.E.)	
	Before drug	After drug	Before drug	After drug
25	44.33± 4.93	28.50±6.09	44.66±5.12	36.83±6.33
50	43.33±4.45	24.50±5.25	43.33±4.42	30.66±5.05
100	42.33±4.71	29.66±4.41	41.33±4.54	33.00±5.31
200	42.00±4.72	25.50±4.43	41.16±4.75	31.33±4.46

Table 2: Table showing mean height of contractions (mm) against barium chloride induced contractions with graded doses of phenobarbitone and 4CPTP on isolated rabbit's ileum

Dose (µg/ml bath concentration)	Phenobarbitone (mean height of contraction ± S.E.)		4CPTP (mean height of contraction ± S.E.)	
	Before drug	After drug	Before drug	After drug
25	55.00±1.73	41.00±2.62	47.00±2.82	43.83±2.66
50	43.66±1.74	32.00±1.12	38.66±2.17	35.83±2.42
100	47.00±2.67	33.83±2.58	41.00±3.67	37.66±3.67
200	43.50±4.14	31.33±2.96	44.50±2.96	40.00±1.91

Table 3: Table showing mean height of contractions (mm) against histamine induced contractions with graded doses of phenobarbitone and 4CPTP on isolated guinea pig's ileum

Dose (µg/ml bath concentration)	Phenobarbitone (mean height of contraction ± S.E.)		4CPTP (mean height of contraction ± S.E.)	
	Before drug	After drug	Before drug	After drug
50	7.58±1.22	5.58±0.92	7.28±1.15	5.45±0.73
100	7.25±1.26	5.33±1.88	7.75±1.38	5.55±0.92
200	7.23±1.19	5.06±1.01	7.08±1.14	5.5±1.06
400	7.75±1.28	4.33±0.73	7.00±1.21	4.41±0.88

Table 4: Table showing mean height of contractions (mm) against acetylcholine induced contractions with graded doses of phenobarbitone and 4CPTP on frog's rectus abdominis muscle preparation

Dose (µg/ml bath concentration)	Phenobarbitone (mean height of contraction ± S.E.)		4CPTP (mean height of contraction ± S.E.)	
	Before drug	After drug	Before drug	After drug
25	37.66± 6.12	31.66±4.12	33.50±2.80	23.66±1.08
50	27.33±5.81	22.66±4.70	27.00±5.79	17.33±3.87
100	34.33±2.31	28.00±3.88	31.66±2.49	18.83±3.59
200	39.83±7.28	25.50±1.41	35.33±4.24	18.33±3.08

seen to produce statistically significant neuromuscular blocking activity at all the tested doses (25-200 µg/ml bath concentration). The activity was higher than even phenobarbitone when used in same doses. Thus it may be predicted from the study that the test compound may be efficacious as a spasmolytic and skeletal muscle relaxant drug.

To the best of our knowledge, no other similar study could be found which assesses the spasmolytic and neuro muscular blocking activity of 4CPTP in experimental animals. Hence more number of animal studies are required to confirm these findings.

CONCLUSION

The test compound, 4CPTP, a pyrimidine derivative like phenobarbitone is seen to possess spasmolytic and neuromuscular blocking activity in animal models.

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REFERENCES

1. Selvam TP, James CR, Dniandev PV, Valzita SK. A mini review of pyrimidine and fused pyrimidine marketed drugs. Research in Pharmacy. 2012;2(4):1-9.
2. Gupta AK, Sanjay, Kayath HP, Singh A, Sharma G, Mishra KC. Anticonvulsant activity of pyrimidine thiols. Indian Journal of Pharmacology. 1994;26(3):227-8.
3. Verma PK, Bhutani G, Saini R, Rani S. Experimental evaluation of anti convulsant and analgesic activity of a pyrimidine derivative 4CPTP. MedPulse – International Medical Journal. 2015;2(10):652-5.