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In-Vivo Evaluation of Some Novel Chromen- 2 One Based on Pharmacological Activity of Antipsychotic Drug

Rode Mahesh S.[1,2], Suryadevara Vidyadhara[3]

[1]*Research scholar, University college of Pharmaceutical sciences, Acharya Nagarjuna University, Nagarjuna Nagar,Guntur,Andra Pradesh-522510*
[2]*Pharmaceutical Chemistry Department, SVPM's College of Pharmacy, Malegaon (Bk II), Baramati, Dist. Pune, Maharashtra-413115*
[3]*Professor & Principal, Chebrolu Hanumaiah, Institute of Pharmaceutical Sciences,Chandramouli puram, Chowdavaram, Guntur,Andhra Pradesh India-522019*

**Corresponding author's: Rode Mahesh*

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Abstract

Most neuroleptics block the emesis hyper activity and aggression induced by apomorphine and other dopaminergic agonists. In higher doses, most neoroleptics induce characteristic cataleptic immobility that allows the animal to be placed in abnormal postures that persists, the aim of this project work was to carry out preliminary pharmacological screening of 2-[(4-methyl-2-oxo-2H-chromen-7-yl) oxy-(aryl)] acetamide. preliminary pharmacological screening of the synthesized compounds for the antipsychotic activity. The screening was limited to in vivo models only. The synthesized compounds were converted to their hydrochloride salts in order to make them water-soluble. These hydrochloride salts were used for the pharmacological testing purpose. The experiments were performed on Swiss albino mice (male) and the route of administration was intra peritoneal.

Keywords: *Pharmacological, Evaluation, Synthesized compound*

1. Introduction

Pharmacological Actions of Antipsychotic drugs

Central nervous system

In normal individuals

It produces indifference to surroundings, psychomotor slowing, emotional quieting, reduction in initiative and tendency to go off to sleep from which the subject is easily arousable. This has been referred to as the 'neuroleptic syndrome' and is quite different from the sedative action of barbiturates and other similar drugs.

In a psychotic

It reduces agitation and aggressiveness and controls psychotic symptomatology. Thought and behaviour are gradually normalised, anxiety is relieved. Hyperactivity, hallucinations and delusions are suppressed. The effect has been described by a psychotic as a "Chairman taking control of a disturbed meeting." Spontaneous movements are minimised but ataxia or motor incoordination does not occur.

In low doses, operant behavior is reduced but spinal reflexes are unchanged. Exploratory behavior is diminished and responses to a variety of stimuli are fewer, slower and smaller in magnitude, although the ability to discriminate stimuli is retained. Conditioned avoidance behavior is selectively inhibited, whereas unconditioned escape or avoidance responses are not. Behavioral activation stimulated environmentally or pharmacologically is blocked. Feeding is inhibited. Most neuroleptics block the emesis hyper activity and aggression induced by apomorphine and other dopaminergic agonists. In higher doses, most neoroleptics induce characteristic cataleptic immobility that allows the animal to be placed in abnormal postures that persists [1]

Autonomic nervous system

Neuroleptics have varying degrees of alpha-adrenergic blocking activity, which may be

graded as:

Chlorpromazine = Triflupromazine > Flufenazine > Haloperidol > Trifluperazine > Clozapine > Pimozide, i.e., more potent compounds have lower propensity.

Anticholinergic property of neuroleptic is weak and may be graded as:

Thioridazine > Chlorpromazine > Triflupromazine > Trifluperazine = Haloperidol.

The phenothiazines have weak H_1 antihistaminergic activity and anti-5HT actions as well.

Local anaesthetics

Chlorpromazine is a potent local anaesthetic as procaine. However, it is not used for this purpose because of its irritant action. Others have weaker membrane stabilising action.

CVS

Neuroleptics produce hypotension (primarily postural) by a central as well as peripheral action on sympathetic tone. The hypotensive action is more marked after parenteral administration and roughly parallels the alpha-adrenergic blocking potency. This is not prominent in psychotic patients and is accentuated by hypovolemia. Partial tolerance develops after chronic use, tachycardia accompanies hypotension

Motor activity

Neuroleptics reduce certain type of spasticity. Chlorpromazine ceases skeletal muscular relaxation in some types of spastic disorders. In some schizophrenics, neuroleptics can induce catatonic immobility observed in animals.

Seizure threshold (2,3)

Unlike anti-anxiety agents, antipsychotics do not possess anti-convulsant properties and may even exacerbate convulsive disorders. Some neuroleptics such as aliphatic phenothiazines with low potency (especially Chlorpromazine), can lower seizure threshold and induce epileptic seizure discharge. More potent phenothiazines, such as piperazine derivatives, thiaxanthines and molindone, appear to have the least effect on seizure threshold.

2. Materials And Methods

Drugs and chemicals

1) Sterile Water for Injection I.P. (Pyrogen free) used for pharmacological testing was procured from **'Core Healthcare Limited.'**

2) Haloperidol *(Serenace)* used for pharmacological screening was procured from **'RPG Life Sciences'**

3) Apomorphine hydrochloride used for pharmacological testing was procured from **Sigma.**

4) (+) 5-hydroxytryptophan used for pharmacological testing was procured from **Sigma**.

Preparation of solution of test drug [4,5]

The hydrochloride salts of synthesised compounds were dissolved in Water for Injection (WFI).

Preparation of solution of Haloperidol

Haloperidol Injection I.P. (1ml) containing 1 mg of haloperidol was diluted to 10 ml using WFI.

Preparation of solution of Apomorphine Hydrochloride

Apomorphine hydrochloride was dissolved in Normal Saline (1.3 mg/10ml) to which 0.2 mg of ascorbic acid was added as an antioxidant.

Preparation of solution of 5- Hydroxy Tryptophan [6,7]

5-HTP solution was prepared in WFI (8mg/ml). It was stored at 2 to 8 °C.

Preparation of Carbidopa Solution

Carbidopa was added to WFI to which dil. HCl (0.01M) was added drop by drop to make carbidopa soluble. The pH of the solution was checked using pH paper.

The volume of drug administration

The volume of drug administration was based upon the body weight of animal.

Route of Administration [8]

The route of administration of all drugs was intra peritoneal. Only for 5-HTP, the route of administration preferred was intra venous.

Animals

Animals (*Swiss Albino Mice*) were supplied by Serum Institute, Pune (as gratis), and National Toxicological Centre, Pune. Only male mice weighing in the range of 20-25 gm were selected for the purpose of the experiment.

Behavioural Studies [9,10]

Group of six healthy Albino Mice, weighing between 20-25 gm (male mice) were taken for each drug. They were injected with the test compounds (i.p.).

The animals were kept in the cages. The changes in the behaviour were noted down for every interval of 30 min. for four hours and then after 24 hrs, the animals were inspected for any causality.

The behavioural studies were performed at the dose, which was found to have inhibiting action on apomorphine induced climbing behaviour (ED_{min}). The doses tested for each compound having maximum inhibitory action on apomorphine induced climbing behaviour were as under:

a)	4a	60 mg/kg
b)	4b	40 mg/kg
c)	4c	40 mg/kg
d)	4d	40 mg/kg
e)	4e	40 mg/kg

The changes in the behaviour were reported in the terms of degree of changes in behaviour represented by following symbols-

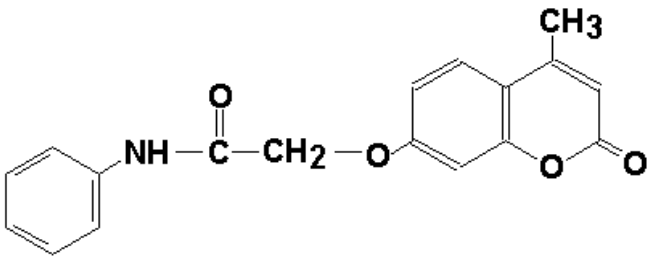
Designation	**Effect**
+	Mild effect
++	Moderate effect
+++	Marked effect

3. Results and Discussion

Preliminary Pharmacological Screening

Behavioural Studies

Effect of 4a (40 mg/kg; i.p) on behavioural parameters of male albino mice



(4a)

Administration of 4a (40mg/kg, i.p.) sedated the animals. The onset of action of sedation was thirty minutes and the duration of action was seen for three hours. The animals showed signs of recovery after three-and-half hours. Presence of righting reflex indicated absence of hypnotic activity.

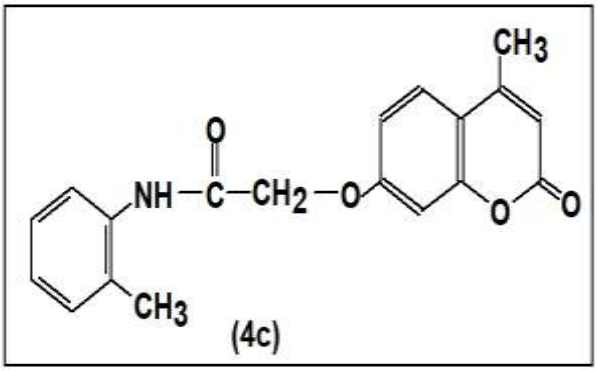
Onset and duration of loss of pinna reflex was also observed to be like that of sedative effect.

The result thus indicated that 4a had mild to moderate sedative action.

Table: Effect of 4a (40 mg/kg; i.p) on behavioural parameters of male albino mice

Sr.No.	Behavioral Parameters	Observations						
		30 min	1hr	2hr	3hr	4hr	hrhr	4hr
1	Hyperactivity	-	-	-	-	-		
2	Jumping		-	-	-	-		
3	Clonic Convulsions	-	-	-	-	-		
4	Tonic Convulsions	-	-	-	-	-		
5	Sedation	+	+	+	++	++		
6	Sleep	-	-	-	-	-		
7	Loss of Traction	-	-	-	-	-		
8	Loss of Pinna Reflex	-	-	+	+	+		
9	Straub Tail	-	-	-	-	-		
10	Catatonia	-	-	-	-			
11	Ataxia	-	-	-	-	-		
12	Loss of Muscle Tone	-	-	-	-	-		

Effect of 4c (40 mg/kg, i.p.) on behavioural parameters of male albino mice-



(4c)

Administration of *4c* (40g/kg, i.p.) sedated the animals. The onset of action was after half hour. The duration of action was also found to be prolonged with sedation lasted for three and half hours and animals showed signs of recovery after two hours. Presence of righting reflex indicated absence of hypnotic activity.

Onset and duration of loss of pinna reflex was also observed to be similar to that of sedative effect.

Table: Effect of *4c* (40 mg/kg, i.p.) on the behavioural parameters of male albino mice

Sr. No.	Behavioural Parameters	Observations				
		30min	1hr	2hr	3hr	4hr
1	Hyperactivity	-	-	-	-	-
2	Jumping	-	-	-	-	-
3	Clonic Convulsions	-	-	-	-	-
4	Tonic Convulsions	-	-	-	-	-
5	Sedation	+	+	++	+	_
6	Sleep	-	-	-	-	-
7	Loss of Traction	-	-	-	-	-
8	Loss of Pinna Reflex	-	-	-	-	-
9	Straub Tail	-	-	-	-	-
10	Catatonia	-	-	-	-	
11	Ataxia	-	-	-	-	-
12	Loss of Muscle Tone	-	-	-	-	-

Inhibition of Apomorphine induced climbing behavior by the synthesized compounds

All the five test drugs were able to inhibit apomorphine induced climbing behaviour. Of them, 4b, 4c, 4d, 4e, were able to inhibit apomorphine induced climbing behaviour. However, 4a was able to cause partial inhibition.

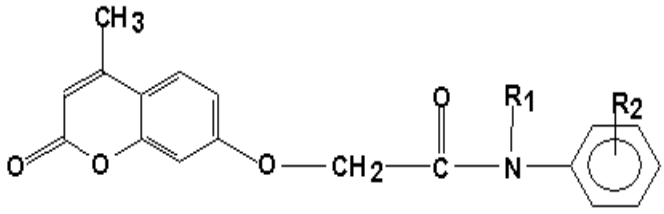


Table: Inhibition of apomorphine induced climbing behaviour by the synthesized compounds

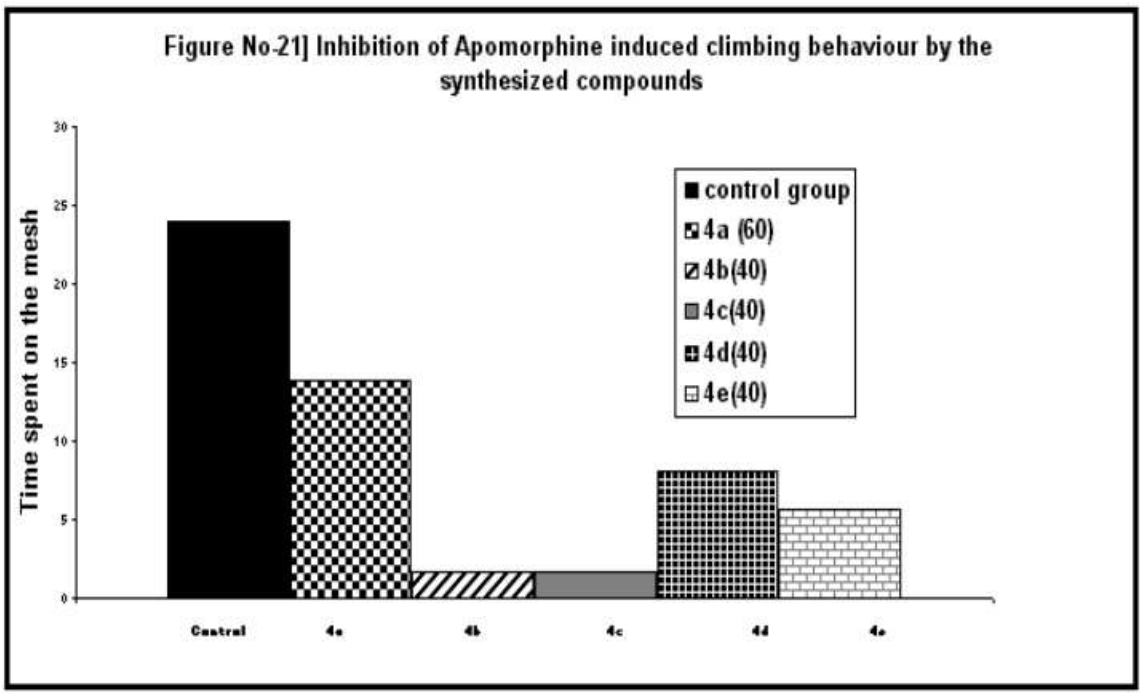
Sr.No.	Drug (mg/kg) ED_{min}	Nature Of		Time spent on mesh (min.)	% Time spent on mesh	% Inhibition
		R1	R2			
1	Control	-	-	23.95 (±) 2.88	79.83	----
2	4a (60)	-H	-H	13.90 (±) 1.724	46.33	41.96
3	4b (40)	-H	4 (-CH_3)	1.653 (±) 0.388	5.5	93.11
4	4C (40)	-H	2 (-CH_3)	1.68 (±) 1.12	5.61	92.97
5	4d (40)	-H	4(-OCH_3)	8.146 (±) 1.724	27.15	65.99
6	4b (40)	-N-CH_3	-H	5.58 (±) 1.65	18.6	76.70

$p<0.05$

n=6

The values in parenthesis indicate standard deviation

ED_{min} -minimum effective dose



Inhibition of 5-HTP (Serotonin) induced Head-Twitches

All of five administered test drugs were able to inhibit 5-HTP induced head-twitches in mice almost completely. These drugs were 4a, 4b, 4c, 4d, 4e at their respective doses of 20,40, 60, 80mg/kg.

However, all the drugs were able to reduce count of 5-HTP induced head-twitches in dose dependent manner.

Thus, it was found that all the five derivatives- 4a, 4b, 4c, 4d, 4e had strong 5-HT antagonizing action evidenced by their ability to totally inhibit 5-HTP induced head-twitches in mice.

Table: Effect of 4a on 5-HTP (Serotonin) induced Head-Twitches

Sr. No.	Drug (mg/kg)	Avg./ Std. Dev (±)	Observation (i.e., count of head-twitches) at the interval of						
			5 min.	15 min.	25 min.	35 min.	45 min.	55 min.	65 min.
1	Control		34.66	48.83	53.5	46.83	33.5	0	0
		(±)	(2.285)	(2.793)	(3.403)	(1.343)	(2.432)	(0)	(0)
2	4a 20		22.33	15.66	14.33	15.33	14.33	12.66	3.66
		(±)	(4.49)	(2.62)	(1.69)	(2.49)	(1.69)	(1.24)	(3.29)
3	4a 40	15	17	15	14.66	16.33	12.66	9	3
		(±)	(6.42)	(4.78)	(4.43)	(4.78)	(4.28)	(3.89)	(2.43)
4	4a 60		14	15	12.66	14.66	7.33	2.33	0.66
		(±)	(2.44)	(2.16)	(1.69)	(2.49)	(2.05)	(1.69)	(0.94)
5	4a 80	13.66	13.66	12	1366	11.66	9.66	9.66	1.33
		(±)	(1.88)	(3.26)	(4.18)	(2.35)	(2.05)	(2.05)	(1.88)

***P<0.05**

n=6

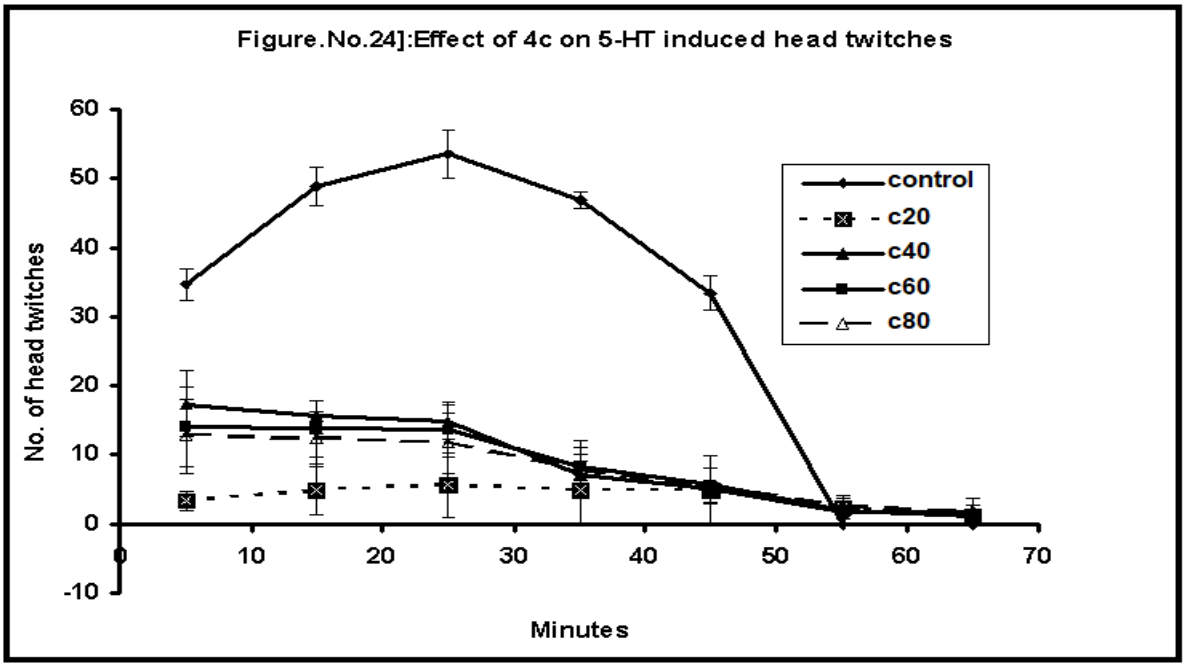
The values in parenthesis indicate standard deviation

Table: Effect of 4c on 5-HTP (Serotonin) induced Head-Twitches

Sr. No.	Drug (mg/kg)	Avg./ Std.Dev (±)	Observation (i.e., count of head-twitches) at the interval of						
			5 min.	15 min.	25 min.	35 min.	45 min.	55 min.	65 min.
1	Control		34.66	48.83	53.5	46.83	33.5	0	0
		(±)	(2.285)	(2.793)	(3.403)	(1.343)	(2.432)	(0)	(0)
2	4c 20		3.34	4.98	5.61	4.98	4.77	2.41	1.16
		(±)	(1.39)	(3.64)	(4.61)	(5.07)	(5.12)	(1.21)	(1.09)
3	4c 40		17.33	15.5	14.83	7.16	5.16	1.66	1.66
		(±)	(4.85)	(2.29)	(2.40)	(1.95)	(1.06)	(1.49)	(1.10)
4	4c 60		14.10	13.70	13.56	8.37	5.65	2.01	1.16
		(±)	(5.78)	(4.19)	(3.96)	(3.82)	(2.55)	(1.32)	(1.09)
5	4c 80		12.74	12.35	11.72	7.91	5.60	2.60	2
		(±)	(5.32)	(4.07)	(4.30)	(3.29)	(2.59)	(1.46)	(1.64)

***P<0.05 n=6**

The values in parenthesis indicate standard deviation



Evaluation of Catalepsy in mice

Comparison of 4a with Haloperidol to evaluate its induction of catalepsy

All administered doses of *4a* induced very weak catalepsy in mice in comparison to haloperidol. The extent of catalepsy induced was however found to be dose dependent. Thus, it was found that *4a* had very weak cataleptic action as compared to haloperidol. Catalepsy was induced by *4a* in mice at all the administered doses. At 20, 40,60 & 80 mg/kg, the extent of catalepsy induced was less as compared to haloperidol.

Table: Comparison of 4a with Haloperidol to evaluate its induction of catalepsy

Sr.No.	Dose mg/kg	Avg./ Std.Dev. (±)	Observations (*in sec*) at the interval of									
			5 min.	10 min.	15 min.	30 min.	60 min.	90 min	120 min.	180 min.	240 min.	300 min.
1	Control		300	300	300	300	300	300	300	300	300	300
		(±)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
2	20		3	2.666	1	3.666	5	3.666	2.333	2.666	1.666	2.666
		(±)	(1)	(2.08)	(1)	(0.57)	(2.64)	(1.52)	(1.52)	(2.08)	(1.52)	(2.30)
3	40		3.333	2	1.666	3.666	2.666	1	3	2.333	4.666	1.333
		(±)	(1.15)	(1)	(0.57)	(0.57)	(0.57)	(1)	(2.64)	(1.15)	(0.57)	(0.57)
4	60		4	4	3.333	3.666	4	6	5.333	5.333	2.333	1
		(±)	(2.64)	(2)	(1.15)	(1.52)	(2.64)	(2)	(1.52)	(2.30)	(0.57)	(1)
5	80		5.666	5.333	1.666	4	4.333	5	7.666	5.333	3.666	1.333
		(±)	(2.08)	(2.30)	(0.57)	(3.46)	(0.57)	(1.73)	(1.15)	(2.30)	(1.52)	(0.57)

*** $p < 0.05$; n = 6**

The values in parenthesis indicate standard deviation

Comparison of 4c with Haloperidol to evaluate its induction of catalepsy

4c in mice at all the administered doses induced lesser degree of Catalepsy. The extent of induction of catalepsy was found to be dose dependent. It was found that 4c induced catalepsy to very lesser degree when compared with haloperidol at doses of 10 & 20mg/kg. Also doses of 40mg/kg and 60mg/kg & 80mg/kg induced catalepsy to lesser degree as haloperidol.

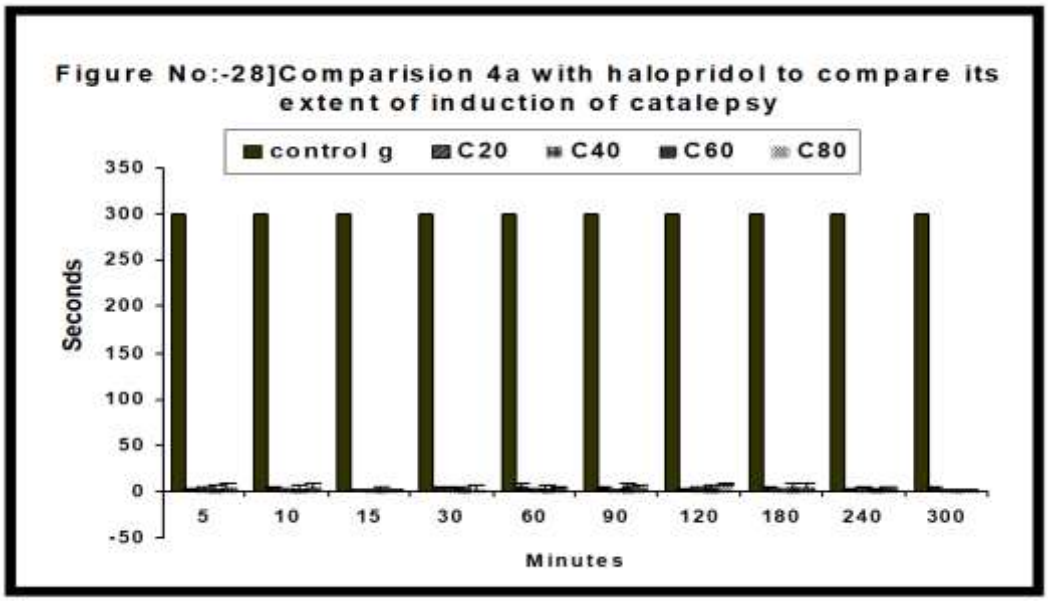
Table: Comparison of 4c with Haloperidol to evaluate its induction of catalepsy

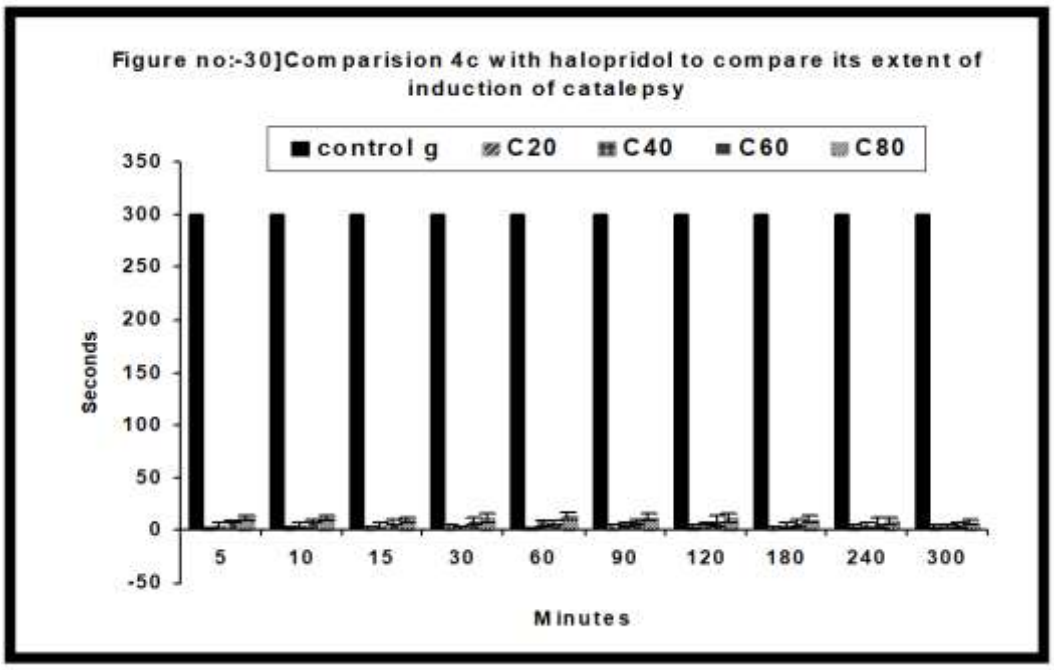
Sr.No.	Dose mg/kg	Avg./ Std.Dev. (±)	Observations (*in sec*) at the interval of									
			5 min.	10 min.	15 min.	30 min.	60 min.	90 min	120 min.	180 min.	240 min.	300 min.
1	Control		300	300	300	300	300	300	300	300	300	300
		(±)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)

2	20		1	2.666	3	4.333	1.333	2.333	2.333	2	1.333	0.333
		(±)	(1)	(0.57)	(1.73)	(1.52)	(0.57)	(1.52)	(2.08)	(2.64)	(2.30)	(0.57)
3	40		3	3	4.333	3.333	5	3.666	2.666	4	1.666	1
		(±)	(1.15)	(2)	(1.527)	(2.88)	(1)	(2.88)	(1.52)	(1)	(0.57)	(1)
4	60		11.33	11.33	11	10.66	14	9.333	7	5.666	4	3
		(±)	(2.08)	(3.05)	(2)	(3.51)	(1)	(3.05)	(1)	(2.08)	(1.73)	(1)
5	80		3.666	11.33	13	13.66	14	12.66	13.66	11	8	4.666
		(±)	(0.57)	(3.05)	(2.645)	(3.05)	(4)	(1.15)	(1.52)	(2)	(1.73)	(1.52)

*** p < 0.05; n = 6**

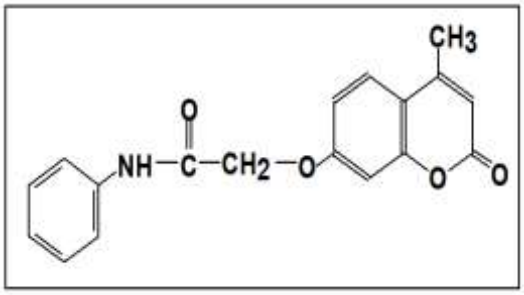
The values in parenthesis indicate standard deviation





Effect on spontaneous motor activity of mice using Actophotometer

Effect of 4a (20, 40,80 mg/kg) on the spontaneous motor activity of mice



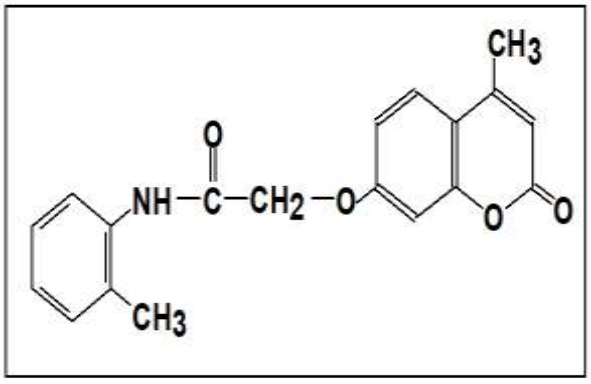
Administration of 4a produced dose dependent inhibitory action on the spontaneous motor activity of mice, as there was reduction in the counts of Actophotometer. The onset of action was found to be half hour 4a reduced the spontaneous motor activity after administration of 20 mg/kg and 40 mg/kg dose. However, at higher doses of 60 mg/kg and 80 mg/kg, reduced the spontaneous motor activity to a greater extent. Thus, 4a was found to have mild to moderate sedation.

Table: Effect of 4a (20, 40, 60,80 mg/kg) on the spontaneous motor activity of albino mice.

Sr.No	Dose (mg/kg)	Avg. counts / *Std. Dev (+)*	Counts			
			30min	**60min**	**90min**	**120min**
1	CONTROL *(WFI)*	(±)	189.66 (± 4.50)	205.66 (± 6)	205.66 (± 5.13)	201.33 (± 2.88)
2	20	(±)	126.33 *(11.01)*	113.66 *(6.02)*	100.33 *(9.29)*	94 *(6.55)*
3	40	(±)	113 *(4.58)*	99.33 *(8.02)*	97.33 *(10.01)*	85.66 *(6.80)*
4	60	(±)	96.33 *(18.00)*	97.33 *(9.29)*	100 *(5)*	88.33 *(4.50)*
5	80	(±)	108.66 *(8.02)*	102.66 *(5.50)*	94.66 *(0.577)*	96.33 *(11.59)*

*** p < 0.05; n = 6 The figure in the parenthesis indicates standard deviation**

Effect of 4c (20, 40,80 mg/kg) on the spontaneous motor activity of mice



Administration of 4c produced dose independent inhibitory action on the spontaneous motor activity of mice, as there was reduction in the counts of Actophotometer. The onset of action was found to be half hour. 4a reduced the spontaneous motor activity after administration of 20 mg/kg and 40 mg/kg dose. However, at higher doses of 60 mg/kg and 80 mg/kg, reduced the spontaneous motor activity to a less extent. Thus, 4c was found to have mild to moderate sedation.

Table: Effect of *4c* (20, 40, 60,80 mg/kg) on the spontaneous motor activity of albino mice

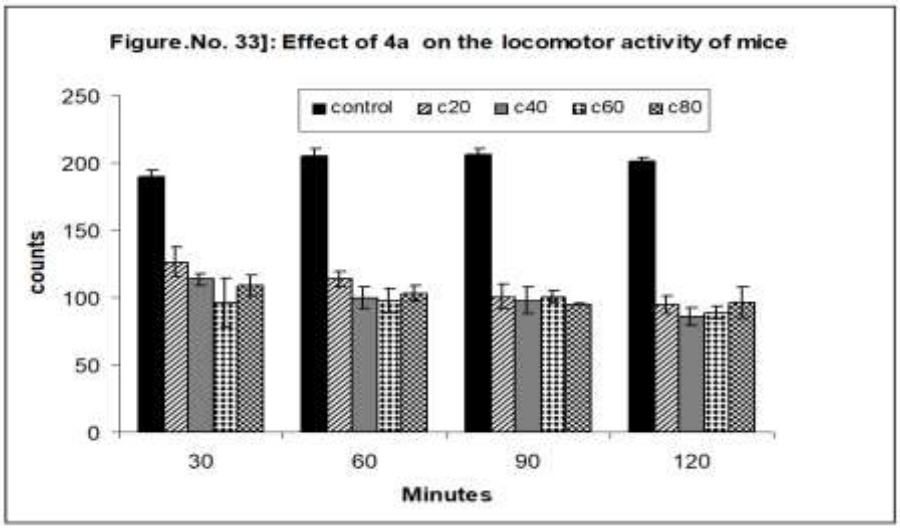
Sr.No	Dose (mg/kg)	Avg. counts *Std.Dev(+)*	Counts			
			30min	**60min**	**90min**	**120min**
1	CONTROL (WFI)	(±)	189.66 (± 4.50)	205.66 (± 6)	205.66 (± 5.13)	201.33 (± 2.88)
2	20	(±)	103.33 (4.72)	105 (11.13)	103 (5.29)	84 (3.60)

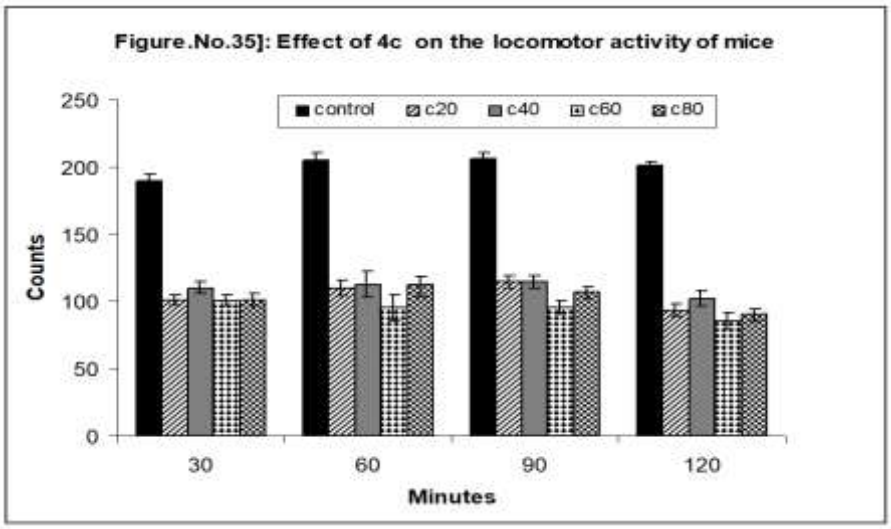
			108.66	110.66	110.33	105.33
3	40	(±)	(5.29)	(5.50)	(6.02)	(8.32)
			105	106	102.66	97
4	60	(±)	(10)	(10.14)	(6.80)	(7.93)
			105	106.66	111.66	88.33
5	80	(±)	(4.35)	(11.06)	(6.11)	(5.50)

***p < 0.05;**

n = 6

The figure in the parenthesis indicates standard deviation





4. Conclusion

The aim of this project work was to carry out preliminary pharmacological screening of 2-[(4-methyl-2-oxo-*2H*-chromen-7-yl) oxy-(aryl)] acetamide. preliminary pharmacological screening of the synthesized compounds for the antipsychotic activity. The screening was limited to in vivo models only. The synthesized compounds were converted to their hydrochloride salts in order to make them water-soluble. These hydrochloride salts were used for the pharmacological testing purpose. The experiments were performed on Swiss albino mice (male) and the route of administration was intra peritoneal. From the above pharmacological results obtained during preliminary screening of **2-[(4-methyl-2-oxo-*2H*-chromen-7-yl) oxy-(aryl)]** acetamide, it was clear that the basic nucleus did possess antipsychotic activity as evident from its ability to inhibit apomorphine induced climbing. Also, it is evident that this molecule blocks both D_2 and 5-HT_2 receptors in the brain, a property shown by all atypical antipsychotics. The hydrochloride salts of these derivatives were used for pharmacological testing.

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