Research Article

CNS Depressant Activity of Some Novel 4,5-Di substituted Thiophenes Derivatives

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ABSTRACT
A series of new 2-[(substituted benzylidene) imino]-3-(N-Benzyl carboxamido)-4,5-di substituted thiophenes [MSR8a-k, 9a-k & 10a-k] were synthesized in a multi step method of Gewald Reaction and Microwave assistance synthesis. All the derivatives were subjected to CNS depressant activity by pentobarbitone induced sleeping time method and photoactometer method. Almost all the new compounds showed moderate to good results with increase in sleeping time and decrease in locomotor activity respectively.

Keywords: 2-amino-3-(benzyl carboxamido)-4,5-di substituted thiophenes, CNS Depressant activity.

INTRODUCTION

Research and Development of new medicinal compounds comprises of the following major steps, the synthesis of the new chemical entities, characterization using different analytical and spectral analyses; screening of the molecule for pharmacological and biological activity. Published literature and articles are considered the evidence for feasibility of such synthetic procedures and their reported pharmacological activity.

Advance in modern science and technology has contributed to an enormous development in the quality of human life. Though, stress in modern life responsible for the surge in incidence of variety of psychiatric disorders[1,2]. The search for new molecules to combat sickness and to alter mood and consciousness is nearly as basic as the search for food and shelter, so new molecules as potential medicinal agents are discovered. Researchers have reviewed the progress on the thiophene template from time to time. Jatava V et al. published a review on diverse biological activities of thiophenes included with its CNS activity[3].

Various thiophenes have been reported for their biological and pharmacological activities such as antimicrobial[4,10], antifungal[5], anti tubercular[6], anti tumor[7], antimycobacterial[8],anti proliferative[9], antibacterial[11,12], antiviral, anti protozoal, herbicidal, anti ulcer, CNS depressant & anticonvulsant[3], analgesic, anti inflammatory activites[13] and so on

Taking into account these findings and in view of their increasing importance in pharmaceutical and biological field, it was considered of interest to synthesize some new chemical entities incorporating the molecular frame work and to evaluate their biological activities like CNS Depressant activity.

EXPERIMENTAL

MATERIALS AND METHODS

Animals

Albino mice of either sex were randomly taken (20-35 g) for evalution of CNS activity. Animals were divided in to three group each contain six animals in each group. The animals (five percentage) were maintained under standard laboratory conditions (light period of 12 hrs/day and temperature 27° C ± 4° C), with access to food and water ad libitum. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee
regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals[28]. Institutional animal ethical committee is having the reference no. PESCP/IAEC 116/08. CPCSEA has approved all animal experiments protocol.

Acute Oral Toxicity

Acute oral toxicity of synthesized compounds was carried out by using mice of either sex weighing 20-25 gm were used. All the animals were fasted for 3hrs prior to the experiment. A group containing 3 mice were administered initially a dose of 2000mg/kg body weight by oral route and observed for mortality or any toxic signs for 24hrs, DMSO is used as vehicle. Up and Down procedure OECD guideline no. 425 was adopted for toxicity studies. Based on short-term profile of drug, the dose of the next animals was determined as per OECD guideline 425. The compounds tested in general were well tolerated in doses 2000 mg/kg body weight. Above 2000mg/kg body weight the tested animals showed lethal effect.

CNS Depressant Activity

In the present research work we had selected two modules for carrying out CNS depressant activity of the newly synthesized compounds:

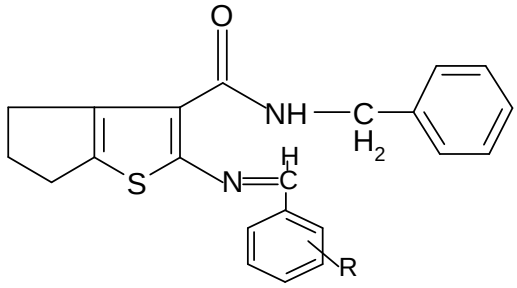
1. CNS depressant activity by pentobarbitone induced sleep[14].
2. CNS depressant activity by actophotometer[15].

General procedure

The general procedure for each module selected is discussed here which we have followed to carry out CNS depressant activity for all the synthesized new compounds.

Determination of pentobarbitone induced sleeping time

CNS depressant activity was performed as describe by Sivaraman and Muralidaran[14]. In this method, mice of either sex were randomly taken and divided into control, standard and different test groups, each group contain six animals. Group I served as control and treated with normal saline (10 ml/kg, i.p.), group II (standard) treated with standard drug chlorpromazine hydrochloride (3mg/kg, i.m.) 15 min before the administration of pentobarbitone (40mg/kg, i.p.). Test groups were treated with the new compounds (100mg/kg, i.p). Pentobarbitone (40mg/kg, i.p.) was administered 30 min later. The onset of sleep and duration of sleep measured for the entire group. Onset of action was recorded by noting the time of loss of reflex for three consecutive trials, duration of sleep was recorded by time difference between loss of righting reflex and recovery time.

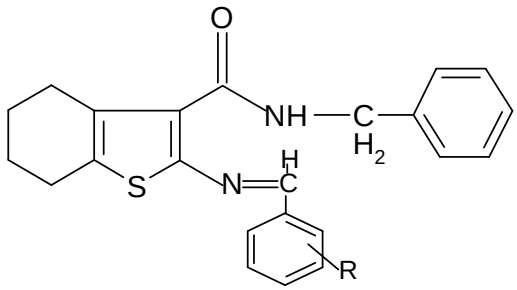


MSR 8a-8k

Table 1: Prolongation of pentobarbital-induced sleeping time by the compound MSR 8a-8k

R	Comp Code	Dose (mg/kg)	Onset of sleep(min)	Duration of Sleep (min)
3',4',5'-trimethoxy	MSR-8a	100	6.1±0.63	74±1.04
3',4'-dimethoxy	MSR-8b	100	4.7±0.48	123±1.89
2'-nitro	MSR-8c	100	6.3±0.45	94.00±1.87
3'-nitro	MSR-8d	100	4.6±0.29	55.50±2.06
2'-chloro	MSR-8e	100	6.5±0.205	101.75±2.135
4'-hydroxy	MSR-8f	100	7.55±0.79	49.22±2.55
4'-hydroxy 3'-methoxy	MSR-8g	100	6.00±0.855	98.25±1.03
2'-hydroxy	MSR-8h	100	7.95±0.48	70.00±1.88
4'-methoxy	MSR-8i	100	5.85±0.41	91.85±1.89
4'-di methyl amino	MSR-8j	100	6.15±0.48	69.15±2.17
4'-chloro	MSR-8k	100	8.02±0.25	30.5±3.07
Standards	Chlorpromazine	1	3.75±0.43	161.00±1.58
	Pentobarbitone	40	5.50±0.29	96.25±0.95

Values are expressed in Mean±SEM, n = 6, p<0.001

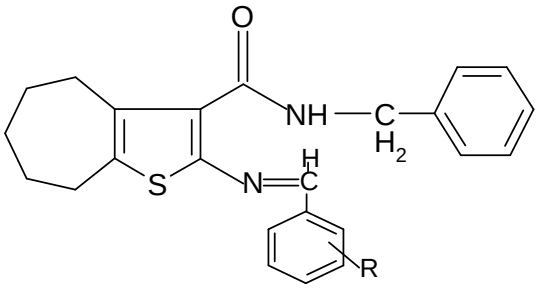


MSR 9a-9k

Table 2: Prolongation of pentobarbital-induced sleeping time by the compound MSR 9a-9k

R	Comp Code	Dose (mg/kg)	Onset of sleep(min)	Duration of Sleep (min)
3',4',5'-trimethoxy	MSR-9a	100	6.0±0.63	76±1.04
3',4'-dimethoxy	MSR-9b	100	4.5±0.48	125±1.89
2'-nitro	MSR-9c	100	6.7±0.45	96.00±1.87
3'-nitro	MSR-9d	100	4.7±0.29	54.54±2.06
2'-chloro	MSR-9e	100	6.1±0.205	105.75±2.135
4'-hydroxy	MSR-9f	100	7.30±0.79	49.34±2.55
4'-hydroxy 3'-methoxy	MSR-9g	100	6.44±0.855	99.25±1.03
2'-hydroxy	MSR-9h	100	7.59±0.48	70.54±1.88
4'-methoxy	MSR-9i	100	5.05±0.41	92.85±1.89
4'-di methyl amino	MSR-9j	100	6.45±0.48	70.22±2.17
4'-chloro	MSR-9k	100	9.02±0.25	32.5±3.07
Standards	Chlorpromazine	1	3.35±0.43	164.00±1.58
	Pentobarbitone	40	5.15±0.29	97.25±0.95

Values are expressed in Mean±SEM, n = 6, p<0.001



MSR 10a-10k

Table 3: Prolongation of pentobarbital-induced sleeping time by the compound MSR 10a-10k

R	Comp Code	Dose (mg/kg)	Onset of sleep(min)	Duration of Sleep (min)
3',4',5'-trimethoxy	MSR-10a	100	6.1±0.63	74±1.04
3',4'-dimethoxy	MSR-10b	100	4.7±0.48	123±1.89
2'-nitro	MSR-10c	100	6.3±0.45	94.00±1.87
3'-nitro	MSR-10d	100	4.6±0.29	55.50±2.06
2'-chloro	MSR-10e	100	6.5±0.205	101.75±2.135
4'-hydroxy	MSR-10f	100	7.55±0.79	49.22±2.55
4'-hydroxy 3'-methoxy	MSR-10g	100	6.00±0.855	98.25±1.03
2'-hydroxy	MSR-10h	100	7.95±0.48	70.00±1.88
4'-methoxy	MSR-10i	100	5.85±0.41	91.85±1.89
4'-di methyl amino	MSR-10j	100	6.15±0.48	69.15±2.17
4'-chloro	MSR-10k	100	8.02±0.25	30.5±3.07
Standards	Chlorpromazine	1	3.75±0.43	161.00±1.58
	Pentobarbitone	40	5.50±0.29	96.25±0.95

Values are expressed in Mean±SEM, n = 6, p<0.001

CNS depressant activity by photoactometer

The CNS depressant activity of the title compounds were evaluated by studying locomotor activity of ice using actophotometer where, in the methodology, Briefly, Albino mice of either sex (20 - 25 g) were randomly divided into control, standard and different test groups, each group contain six animals. The mice were placed individually inside the chamber of photoactometer for 10 min and basal activity score was noted. Group I was treated with vehicle (0.5% sod. CMC) and standard drug diazepam (5 mg/kg, i.p.) administered to group II. The animals of the test group were treated with compounds I a-k (100 mg/kg, i.p.). After 20 min of administration of test compound, the animals were kept into the photoactometer chamber and the counts were noted for 10 min after a 10 min rest in the chamber. The same procedure was repeated after 50 min. Percent decrease in activities were calculated for each group using the formula,

Percent decrease in activity = (1-Wa/Wb) X 100,

Where Wa and Wb are average activity scores after and before administration of test compound respectively and average decrease in activity was calculated for all groups.

Table 4: CNS depressant activities by photoactometer

Compound	Dose Mg/kg	Photoactometer counts			% CNS depressant activity	
		Prior (control) administration of test compound (Wb)	30 min after administration of test compound (Wa)	60 min after administration of test compound (Wa)	After 30 min	After 60 min
Diazepam	5	103±1.67	15±1.21	11±1.17	85.43	89.32
MSR-8[a]	100	117±1.73	60±1.23	42±1.25	48.00	64.59
MSR-8b	100	124±1.75	54±1.40	40±1.35	56.26	67.01
MSR-8c	100	128±1.75	58±1.20	35±1.67	54.07	72.69
MSR-8d	100	103±1.73	67±1.37	51±1.56	34.01	50.00
MSR-8e	100	117±1.75	70±1.42	62±1.28	40.87	47.00
MSR-8f	100	119±1.76	55±1.21	31±1.18	53.18	73.10
MSR-8g	100	113±1.67	43±1.75	22±1.51	61.00	80.00
MSR-8h	100	125±1.56	67±1.19	45±1.23	46.00	64.00
MSR-8i	100	110±1.23	53±1.17	32±1.23	51.00	70.00
MSR-8j	100	123±1.37	63±1.72	52±1.73	48.00	57.20
MSR-8k	100	114±1.72	59±1.35	22±1.23	48.00	80.00
MSR-9[a]	100	117±1.73	60±1.23	42±1.25	48.00	64.59
MSR-9b	100	124±1.75	54±1.40	40±1.35	56.26	67.01
MSR-9c	100	128±1.75	58±1.20	35±1.67	54.07	72.69
MSR-9d	100	103±1.73	67±1.37	51±1.56	34.01	50.00
MSR-9e	100	117±1.75	70±1.42	62±1.28	40.87	47.00
MSR-9f	100	119±1.76	55±1.21	31±1.18	53.18	73.10
MSR-9g	100	113±1.67	43±1.75	22±1.51	61.00	80.00
MSR-9h	100	125±1.56	67±1.19	45±1.23	46.00	64.00
MSR-9i	100	110±1.23	53±1.17	32±1.23	51.00	70.00
MSR-9j	100	123±1.37	63±1.72	52±1.73	48.00	57.20
MSR-9k	100	114±1.72	59±1.35	22±1.23	48.00	80.00
MSR-10a	100	113±1.73	61±1.23	42±1.25	50.00	64.59
MSR-10b	100	124±1.75	54±1.40	40±1.35	56.26	67.01
MSR-10c	100	128±1.75	58±1.20	35±1.67	54.07	72.69
MSR-10d	100	103±1.73	67±1.37	51±1.56	34.01	50.00
MSR-10e	100	111±1.75	72±1.42	58±1.28	42.87	45.00
MSR-10f	100	119±1.76	55±1.21	31±1.18	53.18	73.10
MSR-10g	100	115±1.67	45±1.75	21±1.51	60.00	84.00
MSR-10h	100	125±1.56	67±1.19	45±1.23	46.00	64.00
MSR-10i	100	114±1.23	55±1.17	50±1.23	54.00	73.00
MSR-10j	100	120±1.37	61±1.72	55±1.73	49.00	57.20
MSR-10k	100	111±1.72	57±1.35	24±1.23	46.00	81.00

Values are expressed in Mean±SEM, n = 6, p<0.001

Statistical Analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons test and all the results obtained in the study were compared with the vehicle control group. p values < 0.01 were considered statistically significant.

RESULTS AND DISCUSSION

All the twelve derivatives were subjected to CNS depressant activity by pentobarbitone induced sleeping time (Table-1, 2 & 3) shown increase in sleeping time and photoactometer method (Table 4) shown decrease in locomotor activity.

The result of pentobarbitone induced sleeping time test and CNS depressant activity by photoactometer showed that the newly synthesized derivatives MSR 8a-8k, 9a-9k and MSR 10a-10k have moderate to good CNS depressant activity. Table 1, 2, 3 and 4 showed that compounds having electron donating group like MSR-8a, 8b, 8i, 8j, MSR-9a, 9b, 9i, 9j, MSR-10a, 10b, 10i and MSR-10j produced significant pharmacological action compared to the standard drug.

The other derivatives also showed comparable result to that of the standard drug chlorpromazine. The derivatives having electron donating group such as methoxy showed a comparable increase in sleeping time with chlorpromazine the standard drug used for pentobarbitone induced sleeping time method, and after 60 minutes of administration of these drugs it showed decrease in locomotor activity when compared to standard drug diazepam.

CONCLUSION

A number of substituted thiophenes were synthesized by using different starting material. All the synthesized compounds were analyzed for Melting point and Rf value by TLC. The selected compounds were analyzed by different spectral analysis technique for confirmation of the formation of the new synthetic molecules. The synthesized compounds were screened for CNS depressant activity by using two modules, ie., Pentobarbitone induced sleep and Photoactometer. Among all compounds few compounds have shown comparable potency when compared with the standard drugs used for the test, chlorpromazine and diazepam respectively.

In conclusion, from the CNS depressant acivity results, it can be inferred that electron withdrawing groups on the phenyl and aldehydic phenyl ring of the compounds influenced the CNS depressant activity.

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