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Anti-depressant activity of Pyrimidine derivatives in mice

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Abstract

Plan: *Depression is a common chronic recurrent syndrome, characterised by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation. The present study refers to the anti-depressant activity of pyrimidine derivatives.*
Methodology: *Anti-depressant activity was evaluated by using forced swim test and tail suspension test in mice. The behavioural parameter observed in this test was immobility period, which is indicative of anti-depressant effect of the compounds.*
Outcome: *Test compounds 1b and 1c (15mg & 30mg) were effective and significant reduction in immobility period was observed when compared to standard drug Fluoxetine (20mg/kg) without any accompanying changes in the ambulation in open field test. These results indicate that the pyrimidine derivatives 1b and 1c are potential compounds for use in the designing of new candidates for the treatment of depression.*
Key words: *Anti-depressant activity, Pyrimidine derivatives, Forced swim test, Tail suspension test.*

1. Introduction

The World Health Organisation (2010) estimates that 151 million people suffer from depression and around 844 thousand people die by suicide every year. Globally mental health conditions account for 13% of the total burden of disease and 31% of all years lived with disability. More than 80% of the global burden of disease due to mental health conditions can be found in low- and middle-income countries[1].Clinically used antidepressants have several limitations and side effects which demand continuous development of novel, efficient, and safe drugs for the treatment of depression.

Depression is a common chronic recurrent syndrome, characterised by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation[2]. In spite of the availability of anti-depressant drugs like tricyclic anti-depressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective nor adrenaline reuptake inhibitors (SNRIs), depression continues to be a major medical problem[2]. Insomnia and loss of libido with SSRIs and tolerance and physical dependence with tricyclic anti-depressants are very common adverse effects[3,4].

The development of synthetic heterocyclic compounds as antidepressants progressed considerably during the past decade[5].

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Pyrimidine derivatives possess a broad spectrum of activities anti-epileptic[6], analgesic, anti-inflammatory, anti-pyretic [7, 8, 9], antimicrobial, anti-tubercular[10], anti-histaminic[11], anti-convulsant[12], anti-cancer[13].The primary aim of the present study was to investigate the possible anti-depressant-like effect of the pyrimidine derivatives in the forced swim test, tail suspension test with a simultaneous study of their effect on the locomotor activity.

2. Materials and methods

2.1 Animals:

Male Swiss albino mice weighing 25-30g were selected for the present study. The animals were purchased from Teena Bio labs Pvt Ltd. (177/99/CPSCEA), Hyderabad and allowed to acclimatize for one week. They were maintained under standard environmental conditions (25 ± 2^0C and a relative humidity of 45-55%) and were fed with a standard pellet diet and water *ad libitum*. Food was withdrawn 12h before the experiments. The study was approved by Institutional Animal Ethics Committee (IAEC), Vaagdevi College of Pharmacy, Hanamkonda. CPSCEA guidelines were adhered during the maintenance and experimental work. All efforts were made to minimise animal suffering.

2.2 Drugs and Treatment:

Fluoxetine hydrochloride (Cadila Pharmaceuticals Ltd., Ahmedabad, India) was used as a reference standard for evaluation of anti-depressant activity in both FST and TST. Test compounds were synthesised in our laboratory.

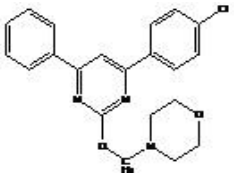
2.3. Scheme of synthesis

Mixed aldol condensation of equimolar quantities of differently substituted acetophenone and benzaldehyde in the presence of sodium hydroxide yielded E-1,3-diaryl-prop-2-en-1-ones (Chalcones).

When E-1,3-diaryl-prop-2-en-1-ones were refluxed with urea in the presence of potassium hydroxide, 4,6-diaryl-3,4-dihyropyrimidine-2(1H)-ones were formed. A series of N-[(4, 6-substituted-diaryl -3, 4-dihydropyrimidin-2-yl-oxy) methyl]-amines **(1a-1c)** have been prepared by the Mannich condensation of the respective 4, 6-diaryl-3,4dihydropyrimidne 2(1H)-ones with different secondary amines (dimethylamine, diethylamine and morpholine).

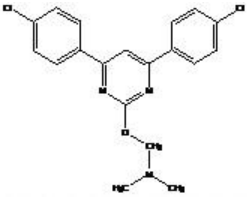
Spectral data of the synthesised compounds was analysed and the spectral data of the compound 1c (4-((4-(4-chlorophenyl)-6-phenylpyrimidine-2-yloxy) methyl) morpholine is represented here. MP 146-149^{o}C; **IR** (KBr) cm^{-1}: 3062(Ar-C-H str), 2860(C-H str), 1660(Ar-C=N str), 1596 and 1475(Ar-C=C str), 1250 and 1090(Ar-O-C str), 750(C-Cl str); **^{1}H NMR** (δ ppm): 2.87(t,4H,2CH_2), 3.54(t,4H,2CH_2), 4.70(s,2H,CH_2), 7.22(s,1H,CH) 7.31-8.10(m,8H,Ar-H); **EI-MS**: 416(M^+); Anal. Calcd. For $C_{21}H_{19}Cl_2N_3O_2$: C, 60.59; H, 4.60; N, 10.09. Found: C, 60.57; H, 4.58; N, 10.07%.

Fluoxetine hydrochloride was dissolved in normal saline solution (0.9% NaCl) and administered at a dose of 20mg/kg. Test compounds were dissolved in saline with 1% tween 80 and administered at an i.p. dose of 15mg/kg and 30mg/kg body weight. Test compounds, vehicle and standard drugs were administered i.p. 30 min before the experiments of FST, TST and locomotor activity



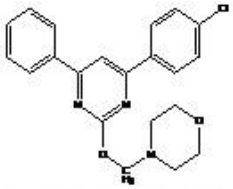
Test compound 1a

(4,6-bis –chlorophenyl)pyrimidin-2-yloxy) N,N-dimethyl methanamine



Test compound 1b

N-(4,6-bis(4-chlorophenyl)pyrimidin-2-yloxy) methyl) –N-ethylethanamine



Test compound 1c

(4-((4-(4-chlorophenyl)-6-phenylpyrimidine-2-yloxy) methyl)

All the solutions were freshly prepared and administered in a volume of 0.1ml per 10g body weight. The doses and the pre-treatment schedules were based on those reported in the literature[5, 14, 15].

2.4 Experimental protocols

Overnight fasted animals were randomly divided into the following groups for experiments. The animals were acclimatized to the laboratory conditions before one hour of the behavioural tests. Group-I (control), received normal saline i.p., Group-II (standard), received Fluoxetine hydrochloride 20mg/kg i.p, Group-III and IV received test compound 1a at doses of 15 and 30 mg/kg i.p respectively, Group- V and VI received test compound 1b at doses of 15 and 30 mg/kg i.p respectively, Group VII and VIII received test compound 1c at doses of 15 and 30 mg/kg i.p respectively. All the groups of animals were taken for Forced swim test and Tail suspension test to know the behavioural activity for screening anti-depressant activity[16] and Locomotor activity test was measured to differentiate between sedative and central nervous system stimulant activity of drugs[17].

Table 1: Comparative profile of Immobility Parameter in Forced Swim Test in mice after treatment with Test compounds

Group	*Control*	*Standard*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
Treatment	*Normal saline*	*Fluoxetine*	*Test 1a*	*Test 1a*	*Test 1b*	*Test 1b*	*Test 1c*	*Test 1c*
Dose(mg/kg)	10	20	15	30	15	30	15	30
Mean number of counts/300s	163.8 ± 6.058	51.8 ± 4.324**	154.6 ±6.40	161.8 ±9.705	76.4 ± 5.41**	33.8 ±6.22**	24.6 ± 4.774**	34.4± 4.669**

Table 2: Comparative profile of Immobility Parameter in Tail Suspension Test in mice after treatment with Test compounds.

Group	*Control*	*Standard*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
Treatment	*Normal saline*	*Fluoxetine*	*Test 1a*	*Test 1a*	*Test 1b*	*Test 1b*	*Test 1c*	*Test 1c*
Dose(mg/kg)	10	20	15	30	15	30	15	30
Mean number of counts/300sec	177.6 ± 9.043	74.8± 6.892**	167.1 ±13.583	170.7 ±8.426	114.3 ±11.575**	85.8± 8.899**	87.1± 9.219**	77.5± 10.653**

Table 3: Locomotor activity: Effect of synthetic pyrimidine derivatives on locomotor activity of mice.

Group	*Control*	*Standard*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
Treatment	Normal saline	Fluoxetine	Test 1a	Test 1a	Test 1b	Test 1b	Test 1c	Test 1c
Dose(mg/kg)	10	20	15	30	15	30	15	30
Mean number of counts/300sec	141.65 ±9.36	146.6 ±10.69	139.34 ±9.72	141.2 ±7.66	133.82 ±15.67	151.48 ±9.58	144.41 ±8.44	141.25 ± 10.18

*For Table 1 & 2: Mean±SD (n=5). *P<0.05, **P<0.01, ***P<0.001. Statistically significant when compared to vehicle treated group analysed by one-way ANOVA followed by Dunnett's test.*

3. Statistical analysis

All the data represent mean±SD (standard deviation). The data obtained in behavioural tests were evaluated by using one way analysis of variance (one-way ANOVA) followed by Dunnett's test for multiple comparisons. Statistical significance was set at $p<0.05$ level.

4. Results

Forced swim test: The possible anti-depressant effect of the test compounds after i.p. administration was studied in the forced swim test. In this test, the test compounds 1b and 1c at 15 and 30mg/kg showed decrease in the immobility times significantly when compared with the control (Table 1). Standard group treated with Fluoxetine hydrochloride has shown a decrease in the immobility time as expected. Tail suspension test: In this test, test compounds 1b and 1c, at 15 and 30mg/kg; have reduced the immobility periods significantly when compared with the control (Table 2). Similarly the animals treated with the Fluoxetine had a reduced immobility time as expected. The results were supportive with that of forced swim test. Locomotor activity: Locomotor activity in mice as measured using photoactometer was found to be similar in all groups (Table 3).

5. Discussion and Conclusion

The incidence of depression in the society is high and the available medication for depression suffers from many side-effects. So there is an immediate need for alternative medication. The development of heterocyclic compounds such as pyrimidine derivatives, pyrazoline derivatives as potent antidepressants is progressing during the past decade. In this study, it was demonstrated that pyrimidine derivatives 1b and 1c were potent as anti-depressants.

Many animal models are available for assessing the stress-precipitated depressive behaviour. The two most common behavioural models for studying the anti-depressant activity are the forced swim test and the tail suspension test. These tests are quite sensitive and relatively specific to all major classes of anti-depressant drugs. In FST, mice were forced to swim in a restricted space from which they cannot escape induced characteristic behaviour of immobility, which reflects a state of despair. In TST, mice when suspended by tail are subjected to unavoidable and inescapable stress display immobility which reflects behavioural despair indicative of depressive disorder in humans.

The results from the forced swim test and tail suspension test indicate the potency of test compounds 1b and 1c as they have shown a decrease in the immobility time. Drugs that alter the general motor activity of the animals may give false positive/negative results. From the photoactometer test for a period of 5min for each mouse, it is demonstrated that the test compounds didnot effect the locomotor activity in mice. And hence, it is probable that the anti-depressant effect of the test compounds is specific and not due to the motor stimulation.

The exact mechanism by which these compounds is not understood however, the activity of the compounds 1b and 1c may be supported due to the presence of bulky groups of ethyl and morpholine in their structure. Test compound 1b might be active due to the presence of electron withdrawing group of Cl^- also. The activity of the compounds might be worth for further investigation and elucidation.

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