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ORIGINAL ARTICLE

Evaluation of Anxiolytic and Anti-depressant Effects of a Novel Piperazine Derivative, 2-[4-(4-Nitrophenyl) piperazin-1-yl]-N-Benzylacetamide in Mice

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ABSTRACT
This study focuses on the effects of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide in the case of anxiety and depression in mice. The Anxiolytic and anti-depressant actions are evaluated using various models like the open field, elevated plus maze, light-dark box, TST, and FST. These tests are performed on mice in the laboratory. Two doses of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide were evaluated with a control group and standard group (Diazepam), and it has been observed that 30mg/kg 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamideis more effective in case of anxiety and depression as compared to 15mg/kg on mice. These observations confirm the anxiolytic and anti-depressant action of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide.
Key Words: *Anxiety, Depression, Anti-depressant, 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide*

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INTRODUCTION

Anxiety is one of the most significant psychiatric diseases. The available medications will lower anxiety but also create many harmful adverse actions on various biological actions, which include the risk to cognitive functions. [1]

Depression is a primary psychiatric condition that creates morbidity. There is a significant link between oxidative stress and other mood conditions. [2]. Various medications are available nowadays to treat depression, but they create various other harmful effects. So, we have worked on a molecule that shows therapeutic effects in these mental conditions with lower side effects.

This research mainly focused on the active derivative of phenyl piperazine and its role in CNS activity, such as anxiolytic action and anti-depressant activity—also well as the importance of phenyl piperazine derivative over other treatment options like benzodiazepine diazepam. The piperazine molecules constitute a huge chemical group; some can cross BBB due to their shorter size and lipophilic properties. These derivatives with piperazine moiety play a significant role in various CNS disorders like Schizophrenia, Alzheimer's, depression, and many others [3-6]. The molecule used in this article is 2-[4(4-nitrophenyl) piperazine-1-yl]-N-benzylacetamide.

MATERIAL AND METHODS

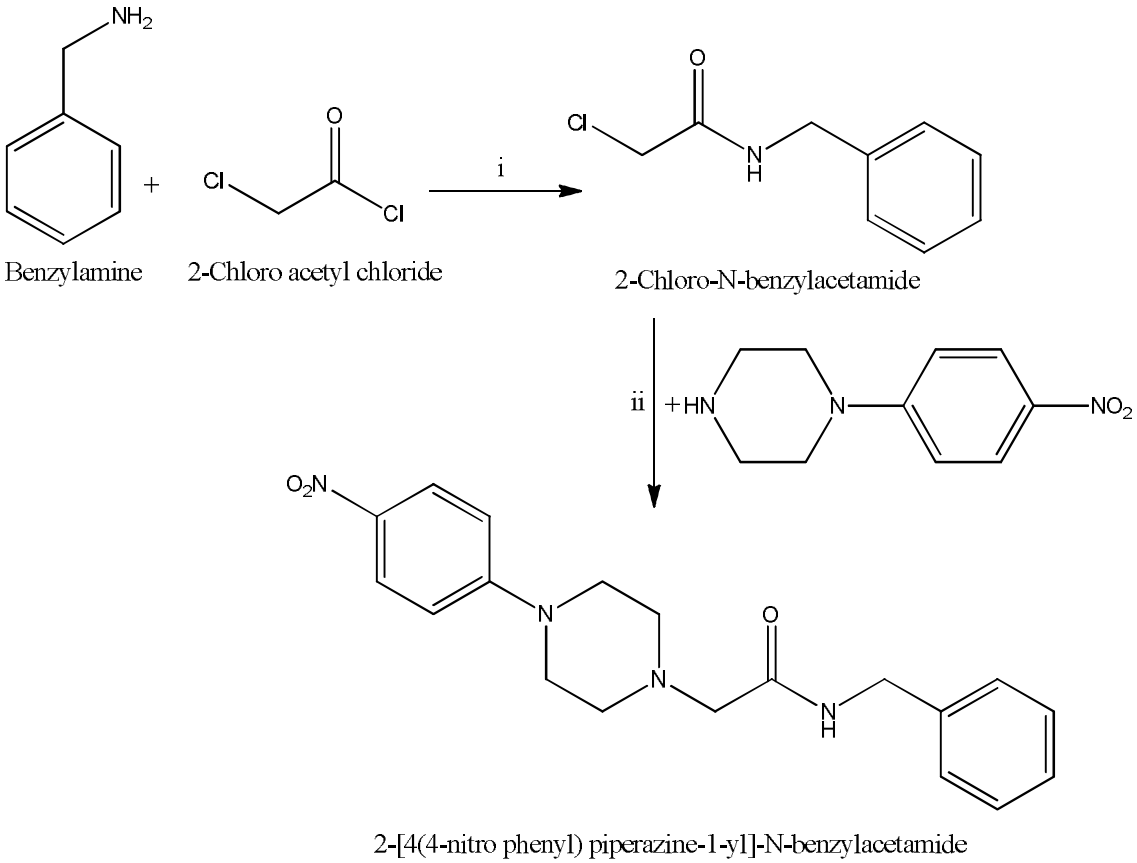
Synthesis of the compound

Synthesis of 2-Chloro-N-benzyl acetamide

4.37 ml of benzylamine in 150 ml of 2N NaOH was treated with 3.18 ml of 2-chloro acetyl chloride in 100 ml dichloromethane at the temperature of 0 °C for about 1 hour to obtain 2-chloro-N-benzylacetamide.

Synthesis of 2-[4(4-nitrophenyl) piperazine-1-yl]-N-benzyl acetamide

0.91 gm of 2-Chloro-N-benzylacetamide was dissolved in acetonitrile (100 ml) in a 250 ml round bottom flask. 0.69 gm of anhydrous potassium carbonate, a catalytic amount of KI, and 1.03 gm of 1-(4-nitrophenyl)piperazine were transferred to the above solution. Then, the mixture was allowed to reflux on a magnetic stirrer for about 12 hours. The final product was collected and washed, as mentioned.[3]



Scheme 1 Synthesis of the target compound. Reagents and conditions: (i) NaOH, dichloromethane (ii) acetonitrile, K_2CO_3, KI

Pharmacological Evaluation

Drugs

Diazepam 2mg/kg as a standard drug for anti-anxiety and anti-depressant activity.2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide 15mg/kg and 30mg/kg as a test drug one and test drug 2 in experimental models, Normal saline (0.9% NaCl).

Animals

Adult male Swiss mice were taken, and weighing around 25-30 grams were selected for the experiment. A group of 6 mice were taken per cage. They all were housed in the animal house with a controlled temperature (25±1 °C), well-ventilated room, proper lighting (light/dark cycle of 12 hours, light on at 7 am), regularly clean environment and adequate food and drink intake. They were kept in the lab one hour before the experiment for the adaptation process. The experiment should be done between 9:00 am to 3:00 pm. The experiment was performed by OECD 423. Animal ethics committee IAEC approves the protocol.

Acute Toxicity Test

Table 1. Animals Required for the Acute Toxicity Study

MODEL	ANIMALS
Acute Oral Toxicity Test	3

Three experimental female & non-pregnant mice were taken for this test. They have given test drug by oral route (5, 50 and 300mg/kg) to check the toxicity and determine the correct dosage for the experiment. After the treatment with the test drug, the animals were observed for any toxic effect, change

in behaviour, mortality, or any other unwanted action. All the animals were observed for 14 days. During the test, the weight of mice, food, drinking intake, and CNS condition were observed and noted daily. LD50 was calculated after the test [7].

Model Design and Dose Schedule

Albino mice were used in this investigation and were separated into four categories. Category 1 was designated as the standard group and fed the 0.9% NaCl; the Standard group received Diazepam 2mg/kg was considered Category 2; test drugs were given 15 mg/kg treated as Category 3; in Category 4, mice received 30 mg/kg. Each category has 6 animals (mice). Readings were taken on different apparatuses of both models.

Anxiety model preparations

Animals open field method

This test determines the mice's fugitive behaviour and finds anxiolytic and anxiogenic-like compounds. This test is commonly used as an anxiolytic model in mice. The different mice were taken for the open field method (n=6). The first group of mice was treated with normal saline. Then, one group with the standard drug Diazepam (2mg/kg) and the other two groups were treated with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide at doses of 15mg/kg and 30mg/kg. After one hour of treatment, the mice were kept in the middle of the apparatus. It contains a circular arena (36cm × 20cm), the lower area contains 8 square fields of the same area. This method recorded and evaluated several squares crossed by the mice, including rearing action, immobility, crossings (no.), and Time spent by the mice at the centre. This reading was taken in 5 mins session [8, 9].

Elevated Plus Maze

Fig: 1 Elevated Plus Maze

It is used to determine the anti-anxiety activity of the molecules. The experiment was done by giving Normal saline to group one, standard drug Diazepam 2mg/kg to group 2 and 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide 15mg/kg and 30mg/kg to group 3 and 4. The apparatus contains two open arms (30×5cm) and two closed arms. All the arms of the apparatus were connected with a central area of dimension 5×5cm. After one hour of treatment, experimental animals were placed individually in the centre area. After this procedure, observe for five minutes [9, 10]. All actions of mice, like entry in closed arms, entry in open arms, and Time spent in open or closed arms, were noted.

Light Dark Box Test

This model is mainly used to determine the anxiolytic activity of the compound in the Animal model. The test is done by giving Normal saline to one group of animals, the standard drug Diazepam 2mg/kg to the second group, and 2-[4(4-nitro phenyl)piperazine-1-yl]-N-benzylacetamide 15mg/kg and 30mg/kg to group 3 and 4. After one hour, the mice were kept in the middle of the lightning zone (20×26.5×26cm) facing towards the opening (7×7cm) of the darkening zone area (20×26.5×17.5cm) and noted down the no. Of transitions between both the areas, Time spent in both the areas. The recordings were done for a 5-minute session [9, 11].

Anti- Depression Activity Model

Animals Forced Swim Test

This test was performed to determine the anti-depressant activity of the test drug on mice. The mice were put into the cylindrical glass that contained water (23-25°C) and observed for 6mins. The dimensions of the cylinder were 25cm in height and 10cm in diameter with $10cm^3$ water in it. The immobility of mice was recorded. The mice were taken as motionless if they kept floating in water [12, 13].

Tail Suspension Method

This test was performed on experimental animals to determine the anti-depressant activity of the test drug. The mice were suspended to a flat surface area by a tape (adhesive tape) around one cm above the tip of the tail, about 50cm on the lower surface. The session continued for 6 minutes, and immobility time was recorded. When mice hung without any movement of limbs, then it was recorded as immobile [13-15]

Social Interaction

The test was performed in three connected rectangular chambers (230×400×220 mm in each compartment). Two openings measuring 9 cm^2 provided access to each compartment. The central compartment was the starting point for each test. Wire cages 10 cm in diameter were placed in the middle of each side compartment. The subject mouse was allowed to roam around the three chambers for 5 minutes as a habituation period. Then, the sociability test was done by putting the stranger (stimulus) mouse into one of the two wire cages, and the other wire cage was left empty. The subject was then allowed to explore the three chambers for 10 minutes.

Fig 2: Social Interaction Activity

Statistical analysis

All the readings were demonstrated as mean ± Standard error of the mean (SEM). These values were evaluated by one-way analysis of variance (ANOVA).

RESULTS

Result of Acute Toxicity Test

The animal who was given 300mg/kg doses by oral administration showed some abnormal behaviour and died at 2 hours. The two mice who received 5 and 50mg/kg doses were fine. After viewing all these conditions, it has been noted that toxic effects increased as high doses were given. The LD_{50} min value and max value were 15mg/kg and 30mg/kg. So, the test drugs 15 and 30mg/kg were taken for the experiment to find out the anti-anxiety and anti-depressant action on mice.

Action of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide in experimental models of anxiety

Evaluation of Anxiolytic Activity

Open Field Method

Treatment with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide increased exploration time in the middle of the open area. There was an enormous no. Square crossed, more no. of transition and lesser Time spent in the light area when animals were treated with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide 30mg/kg dose as compared to 15mg/kg. Also, it has been observed that test drugs have good anxiolytic activity when compared with diazepam 2mg/kg.

Table 2: Data for the Open Field Method

Parameters	Control (0.9% NaCl)	Diazepam (2mg/kg)	Test drug 15mg/kg	Test drug 30mg/kg
Square crossed	14.2± 1.2	27.65±1.6*	26.6±2.2	38.98±3.6**
No. of transition	9.2±1.4	23.6±3.2	19±2.5	37.4±3.8***
Time spent in a light area	101.7±9.2	156±6.21*	136±8.3	187±5.3**

Values are determined as mean ± S.E.M. from 6 mice and analyzed by one-way ANOVA followed by Dunnet's t-tests, *P<0.05, **P<0.01, ***P<0.001 compared to normal group animals

Elevated plus maze

There were more % of Time covered in open arms, a lower % of Time covered in closed arms, more number of open arm entries, and a lesser % number of closed arm entries when animals are treated with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide 30mg/kg dose as compared to 15mg/kg. Also, it has been observed that test drugs have good anxiolytic activity when compared with diazepam 2mg/kg.

Table 3: Data for the Elevated Plus maze model

Parameters	Control(0.9% NaCl)	Diazepam (2mg/kg)	Test drug 15mg/kg	Test drug 30mg/kg
% time spent in open-arm	21.720±1.463	57.718±3.706	43.535±2.204	62.885±2.871*
% time spent in closed arm	78.275±3.42 ***	42.273±1.707	56.465±2.804	37.115±2.171
% number of open-arm entries	59.878±1.356*	83.923±3.270**	73.996±2.026	89.243±2.712
% number of closed-arm entries	41.111±3.983	16.077±1.279	26.003±2.026	10.77±1.97*

Values are determined as mean ± S.E.M. from 6 mice and analyzed by one-way ANOVA followed by Dunnet's t-tests, *P<0.05, **P<0.01, ***P<0.001 compared to normal group animals.

Light dark box

There is more frequency of transition, lesser Frequency of line crosses in the light chamber, more Frequency of line crosses in the dark zone& less Time covered in the light zone when animals are treated with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide 30mg/kg dose as compared to 15mg/kg. Also, it has been observed that the test drug has good anxiolytic activity when compared with diazepam 2mg/kg.

Table 4: Data for the light-dark box

Parameters	Control (Saline solution)	Diazepam (2mg/kg)	Test drug (15mg/kg)	Test drug (30mg/kg)
Frequency of Transition	16.9±1.8	6.8±1.2**	9.8±2.4*	7.3±1.9**
Frequency of line crosses in light chamber	54.6±4.9	19.8±1.9*	26.65±2.87*	20.5±2.2**
Frequency of line crosses in dark chamber	54.7±9.6	18.2±2.1*	24.97±4.9	19.8±5.3**
Frequency of rearing in light chamber	15.9±2.8	11.3±1.87*	14.7±2.3	12.9±1.9**
Frequency of rearing in dark chamber	24.87±1.7	7.8±0.9*	15.2±2.8	13.65±2.05**
Time spent in the light chamber	136.567±6.8	218.8±9.7*	192.56±18.5*	211.45±12.6**

Values are determined as mean ± S.E.M. from 6 mice and analyzed by one-way ANOVA followed by Dunnet's t-tests, *P<0.05, **P<0.01, ***P<0.001 compared to normal group animals.

Evaluation of Anti Depressant Activity

Action of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide in experimental models of Depression

Tail suspension method

There was lesser immobility when animals were treated with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide 30mg/kg dose compared to 15mg/kg. Also, it has been observed that test drug have good anti-depressant activity when compared with diazepam 2mg/kg.

Table 5: Data for the Tail suspension method

TREATMENT GROUPS	DURATION OF IMMOBILITY
Control(0.9% NaCl)	202.56±9.2
Diazepam (2mg/kg)	173.56±8.6*
Test drug 15mg/kg	198.56±8.78
Test drug 30mg/kg	169.56±7.34*

Values are determined as mean ± S.E.M. from 6 mice and analyzed by one-way ANOVA followed by Dunnet's t-tests, *P<0.05, **P<0.01, ***P<0.001 compared to normal group animals.

Force Swim test

There was lesser immobility duration when animals were treated with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide 30mg/kg dose compared to 15mg/kg. Also, it has been observed that test drugs have good anti-depressant activity when compared with diazepam 2mg/kg.

Table 6: Data for the Force Swim test

TREATMENT GROUPS	DURATION OF IMMOBILITY
Control (0.9% NaCl)	250.56±7.2
Diazepam (2mg/kg)	167.56±5.6 ***
Test drug15mg/kg	236.56±6.98
Test drug30mg/kg	194.56±6.74

Values are determined as mean ± S.E.M. from 6 mice and analyzed by one-way ANOVA followed by Dunnet's t-tests, *P<0.05, **P<0.01, ***P<0.001 compared to normal group animals.

Social Interaction

Table 7: Data for the social interaction studies

Parameters	Control (Saline solution)	Diazepam (2mg/kg)	Test drug 15mg/kg	Test drug 30mg/kg
Social interaction ratio	1.87±0.3	2.46±0.2*	1.94±0.46	2.5±0.78**
Time in the interaction zone	45±2.98	57.46±2.18*	49.46±3.4	53.46± 3.23*
Time in the corner zone	17.45±1.95	12.3±0.87*	16.34±2.4	13.98±2.07*

Values are determined as mean ± S.E.M. from 6 mice and analyzed by one-way ANOVA followed by Dunnet's t-tests, *P<0.05, **P<0.01, ***P<0.001 as compared to normal group animals

DISCUSSION

The study showed that 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide is a centrally acting drug with phenyl piperazine as a central moiety. It has excellent anxiolytic and anti-depressant activity, lower side effects, and potential activity. The treatment with 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide in open field method on mice showed potential anxiolytic activity as there is an increase in exploration time in the middle of the open area. Also, more squares are crossed, there is no transition, and less Time is spent in light areas. The compound's 30mg/kg dose showed more efficient action than 15mg/kg.

When 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide is given to the mice in an elevated plus maze. It is generally used to identify anxiolytic activity (Han *et al*., 2009). When the drug is given to mice, it showed more no. of entries in the open zone& fewer in the closed zone, which proves its anxiolytic action.

2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide showed more frequency of transition, lesser Frequency of line crosses in the light chamber, more Frequency of line crosses in a dark chamber, and lesser Time covered in the light chamber in a light-dark box model, which is used to find out the anti-anxiety action of a compound.

This compound is also evaluated for anti-depressant activity on mice, and it has been observed that 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide showed low immobility rate in tail suspension method, forced swim test & social interaction, which proved that this compound is having excellent activity of anti-depressant. The 30g/kg dose of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzylacetamide showed more effective action than 15mg/kg.

CONCLUSION

The study and all the observations concluded that 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide has potential activity as an anxiolytic and anti-depressant drug. Also, it has been reported that a 30mg/k dose of 2-[4(4-nitro phenyl) piperazine-1-yl]-N-benzyl acetamide is more efficient than 15mg/kg.

REFERENCES

1. Stewart, R.E., & D.L. Chambless. (2009). Journal of Consulting and Clinical Psychology. 77(4), 595–606. https://doi.org/10.1037/a0016032
2. Xu, M., D. Liu, Z. Dong, X. Wang, X.Wang, Y. Liu, P.W. Baas, M. Liu. (2014). Cytoskeleton, 71(10), 555–563. https://doi.org/10.1002/cm.21193
3. Kumar, S., A.K.Wahi, R.Singh. (2016). Arabian Journal of Chemistry. 9, S1450–S1456. https://doi.org /10.1016/ j.arabjc.2012.03.016
4. Mathew, B., J.M.Oh, R.S.Baty,G.E.S. Batiha, D.G.T. Parambi, N. Gambacorta, O. Nicolotti, H. Kim. (2021). Environmental Science and Pollution Research. 28(29), 38855–38866. https://doi.org/10.1007/s11356-021-13320-y

5. Ajala, A., A. Uzairu, G.A. Shallangwa, S.E.Abechi. (2022). Molecular Docking and Drug Kinetics Evaluation. Brain Disorders, 7, 100041. https://doi.org/10.1016/j.dscb.2022.100041
6. Nandi, N.K., R. Bhatia, S. Saini, R. Rawat, S. Sharma, K. Raj, N. Rangra, B.Kumar. (2023). Journal of Molecular Structure. 1276, 134671. https://doi.org/10.1016/j.molstruc.2022.134671
7. Erhirhie, E.O., C.P. Ihekwereme, E.E.Ilodigwe.(2018). Interdisciplinary Toxicology.11(1), 5–12. https:// doi.org/10.2478/intox-2018-0001
8. Archer, J. Animal Behaviour, 1973. 21(2), 205–235. https://doi.org/10.1016/s0003-3472(73)80065-x
9. De Brito, A.F., J.L.R. Martins, J.O. Fajemiroye, P. M.Galdino, T.C.M. De Lima, R.Menegatti, E.A.Costa. (2012). Life Sciences. 90(23–24), 910–916. https://doi.org/10.1016/j.lfs.2012.04.037
10. Pellow, S., P. Chopin, S.E. File, M. Briley. (1985). Journal of Neuroscience Methods. 14(3), 149–167. https://doi.org /10.1016/0165-0270(85)90031-7
11. Crawley, J., F.K. Goodwin, (1980). Pharmacology Biochemistry and Behavior. 13(2), 167–170. https://doi.org /10.1016/0091-3057(80)90067-2
12. Pytka, K., A. Partyka, M. Jastrzębska-Więsek, A. Siwek, M. Głuch-Lutwin, B. Mordyl, G. Kazek, A. Rapacz, A. Olczyk, A. Gałuszka, M. Błachuta, A. Waszkielewicz, H. Marona, J. Sapa, B. Filipek, A. Wesołowska. (2015). PLOS ONE. 10(11), e0142499. https://doi.org/10.1371/journal.pone.0142499
13. Porsolt, R.D., A. Bertin, M. Jalfre. (1977). Archives internationales de pharmacodynamie et de therapie, 229(2), 327–336.
14. Steru, L., R. Chermat, B. Thierry, P. Simon. (1985). Psychopharmacology. 85(3), 367–370. https://doi.org/10.1007/BF00428203
15. Can, A., D.T. Dao, C.E.Terrillion, S. Piantadosi, C.S. Bhat &T. D. Gould.(2011). Journal of Visualized Experiments, 58. https://doi.org/10.3791/3769