

SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF NEW ACYL-OXIMINES DERIVATIVES

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Abstract

The present study is a follow up of previous research referring to the synthesis and characterization of new acyl-oximines derivatives with tricyclic structure and their pharmacological activity. The structures of these new compounds were confirmed by NMR spectra, IR spectra and elemental analysis. We continued our research by testing the biological activity of these new compounds. The pharmacological studies of these new O-acyl-oximine derivatives with dibenzo[a,d]cycloheptadienic structure revealed their amitriptyline-like antidepressant activity.

Rezumat

Acest studiu reprezintă o continuare a cercetărilor anterioare referitoare la sinteza și caracterizarea unor noi acil-oximine cu structură triciclică și la acțiunea lor farmacologică. Structurile noilor derivați au fost confirmate prin analiza spectrală în IR și RMN și analiza elementală. Am continuat cercetările prin determinarea activității biologice a noilor compuși. Studiile farmacologice efectuate pe noii compuși O-acil-oximinici cu structură dibenzo[a,d]cicloheptadienică au evidențiat efectul antidepressiv asemănător amitriptilinei.

Keywords: oximines, dibenzocycloheptadienes, antidepressant, tranquilizer, analgesic

Introduction

Depression is a psychiatric quantitative disorder of the central nervous system (CNS), which affects more and more people around the world [15]. The mood disorders are a category of illnesses that describe a serious change in mood and include: major depressive disorder, bipolar disorder, persistent depressive disorder and seasonal affective disorder. Moderate levels of physical activity can treat depression by increasing the levels of endorphins and the neurotransmitters serotonin, dopamine and norepinephrine [9, 17, 22].

Dibenzocycloheptadienic and dibenzocycloheptatrienic derivatives have certain pharmacological activities: antidepressant effect [23], analgesic properties [2, 14], they are used for attention deficit hyperactivity disorders (ADHD) [25]; some researchers studied new methods for treating functional dyspepsia and they discovered that dibenzocycloheptenic derivatives had an important role in the second-step therapy [19]. Researchers have also shown that some tricyclic antidepressants can reduce pain in peripheral neuropathy caused by cancer chemotherapy [26, 27]. For many years the tricyclic antidepressants (TCAs) were the first choice for the pharmacological treatment of clinical depression.

Although they are still considered to be highly effective, they have been predominantly replaced by antidepressants with an improved safety and side effect profile, such as the selective serotonin reuptake inhibitors (SSRIs) and other newer antidepressants such as the novel reversible monoamine-oxidase inhibitors (MAOI). However, tricyclic antidepressants are still more successful in treating melancholic depression than other antidepressant drug classes [4]. Newer antidepressants are thought to have fewer and less severe side effects and are also thought to be less likely to result in injury or death if used in a suicide attempt, as the doses required for clinical treatment and potentially lethal overdose are far wider in comparison [3].

Nonetheless, the TCAs are still used for treatment-resistant depression that has failed to respond to therapy with newer antidepressants [4]. They are not considered addictive and are somehow preferable to the monoamine-oxidase inhibitors. The side effects of the TCAs usually come to prominence before the therapeutic benefits against depression and/or anxiety, and for this reason, they may be potentially dangerous, possibly giving the patient a greater desire to attempt or commit suicide [24].

Beside this major side effect, tricyclic antidepressants have also less dangerous side effects, mostly caused by their anticholinergic activity, including: changes in appetite, muscle stiffness, nausea, constipation, tremor, dizziness, urinary retention, blurred vision, changes in sexual function; some rare side effects are: hypotension, tinnitus, heart block, arrhythmias, lips and mouth ulcers, extrapyramidal symptoms [13].

In this paper we present the continuation of our previous research [7-10] in order to investigate the therapeutic effects for the new tricyclic O-acyl-oximines synthesized in our laboratory.

We tested the therapeutic effects on central nervous system for three of the new O-acyl-oximines with

dibenzo[a,d]cycloheptadienic structure, which were synthesized in our previous research [7-10].

The synthesis of these three new derivatives started with a condensation reaction between dibenzo-suberone (10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one) (I) and hydroxylamine hydrochloride, in order to obtain 5-oximino-10,11-dihydro-5H-dibenzo[a,d]cycloheptene (II), which was treated afterwards with substituted aromatic acid chlorides in anhydrous benzene and in the presence of anhydrous pyridine as a proton fixator [7-10].

The new O-acyl-oximines were obtained in accordance with the following general procedure described in Figure 1:

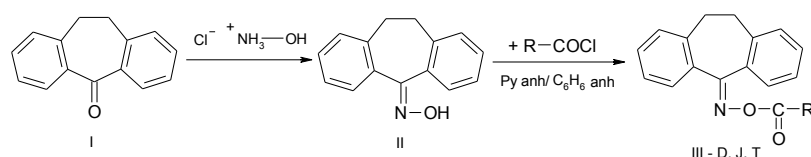


Figure 1.

Synthesis of new O-acyl-oximines (III-D, J, T)

The method used for the synthesis of these new O-acyl-oximines were described in our previous articles; also, their chemical structures were confirmed by NMR and IR spectra and elemental

analysis and the results were presented in previous papers [6-8].

The meaning of radical R, chemical names and molecular formulas are presented in Table I:

Table I

New O-acyl-oximines tested for therapeutic activity on central nervous system

Compound	R	Molecular formula	Chemical name
III - D		C ₂₃ H ₁₆ F ₃ NO ₂	O-(2-Trifluoromethyl-benzoyl)-5-oximino-10,11-dihydro-5H-dibenzo[a,d]cycloheptene
III - J		C ₂₃ H ₁₅ F ₄ NO ₂	O-(2-Fluoro-5-trifluoromethyl-benzoyl)-5-oximino-10,11-dihydro-5H-dibenzo[a,d]cycloheptene
III - T		C ₂₆ H ₂₅ NO ₄	O-(2,5-Diethoxy-benzoyl)-5-oximino-10,11-dihydro-5H-dibenzo[a,d]cycloheptene

Materials and Methods

We investigated the antidepressant activity, sedative effects and analgesic properties for new acyl-oximines derivatives, conventionally named III-D, III-J and III-T, after *per os* (p.o.) acute and subacute administration in mice.

Male NMRI mice, weighing 33 ± 6 g, were supplied by the rodent farm of the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. Groups of 12 animals were housed in plexiglass cages with sawdust litter, having free access to food and water. Experiments were carried out between 2nd and 12th April 2013 and every day

the animals were brought in the testing room one hour before the behavioural experiment. The temperature and relative humidity were continuously monitored using an electronic hygro-thermometer. The temperature was $21 \pm 2^\circ\text{C}$ and the relative humidity was generally maintained at $55 \pm 5\%$.

All experimental procedures were carried out in accordance with the Directive 2010/63/UE of 22nd September 2010, regarding the protection of animals used for experimental and other scientific purposes. All experimental procedures were approved by the Ethical Committee of the Faculty of Pharmacy Bucharest.

We used 5 groups of mice, each group having 12 animals. The test substances were administered in one dose. The route of administration was *per os* (p.o.) and doses were as it follows:

Group 1: blank control group - distilled water and tween 0.1 mL/10 g b.w. p.o.

Group 2: reference treated group - amitriptyline 10 mg/kg b.w. suspension 0.1% p.o.

Group 3: tested treated group - compound III-D 100 mg/kg b.w. suspension 1% p.o.

Group 4: tested treated group - compound III-J 100 mg/kg b.w. suspension 1% p.o.

Group 5: tested treated group - compound III-T 100 mg/kg b.w. suspension 1% p.o.

After one hour the animals were tested for the motor activity (horizontal and vertical motor activity), using the Activity Cage *Ugo Basile* and for the antidepressant effect using the forced swimming test (FST).

After three days without any treatment, the mice were treated with the same doses of reference, tested and blank substances to evaluate their anxiolytic effect using elevated plus-maze test (EPM) and the analgesic effect using the hot plate test.

Determination of immobility time of mice by the forced swimming test (FST) was originally described by Porsolt [6, 18, 20, 21]. The mice were individually placed into a glass cylinder (25 cm height, 30 cm diameter) containing water at a temperature of $22 \pm 2^\circ\text{C}$. The duration of the test was 6 minutes, but the immobility time was recorded only for 4 minutes; the first 2 minutes were for accommodation. Each mouse was considered immobile when it ceased struggling and remained floating motionless in the water, making only those necessary moves for keeping its head above water.

Determination of curiosity was performed on an elevated plus-maze system (EPM), in order to evaluate the anxiolytic effect of the new acyl-oximines derivatives. The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40-70 cm from the floor. The model is based on

rodents' aversion of open spaces. Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms) and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms). Total number of arm entries and number of closed-arm entries are usually employed as measures of general activity [5, 16].

Determination of motor activity aimed to evaluate the effect on central nervous system by recording the horizontal and vertical movements of mice in the *Ugo Basile* activity cage. The duration of this test was 5 minutes for each mouse [18, 20].

Evaluation of pain sensitivity was performed using the modified hot-plate test [1, 12, 18]. The threshold of pain perception was determined by measuring the time to first sign of pain (hind paw lifting) when the animal was placed on a hot plate heated at 53°C .

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 5 software. Statistical comparison between groups used the Student *t* test (for normal distribution) for 2 groups. Statistical evaluation for multiple groups was performed using the ANOVA test.

Normality of response distribution in collectivity was tested with D'Agostino & Pearson test.

Results and Discussion

Antidepressant effect

Determination of immobility time of mice was performed after acute administration, because it is well known that the forced swimming test (FST) gives positive results after only one dose of antidepressant.

Table II shows the mean values of immobility time for mice subjected to forced swimming test and the comparison of this parameter to blank control group. The statistical significance of this variation is also showed.

Table II
Immobility time (sec.) in FST

	Control	Amitriptyline (reference)	III - D	III - J	III - T
Mean $\pm$ SD	124.2 $\pm$ 41.07	63.00 $\pm$ 20.42	72.83 $\pm$ 17.65	79.08 $\pm$ 20.91	74.42 $\pm$ 21.37
SEM	11.860	5.896	5.094	6.036	6.170
Normal distribution	YES	YES	YES	YES	YES
Antidepressant effect (%) compared to control group		49.27%	41.52%	36.32%	40.08%
ANOVA	p < 0.0001 (***)				
Bonferroni		Amitriptyline/Control: p < 0.001	III - D/Control: p < 0.001	III - J/Control: p < 0.001	III - T/Control: p < 0.001

All three tested compounds and amitriptyline used as reference significantly decreased the immobility time in the FST ($p < 0.001$).

The new acyl-oximine compounds proved to have an antidepressant effect similar to amitriptyline used as reference.

Anxiolytic effect

Table III shows the mean values for all measured parameters for the 5 groups of animals: time spent in open arms, time spent in closed arms, entries into open arms, entries into closed arms, time spent in centre, exits outside open arms.

Table II

Mean values for 6 measured parameters in elevated plus-maze test

Group	Time spent in open arms (sec.)	Time spent in centre (sec.)	Time spent in closed arms (sec.)	Number of entries into open arms	Number of entries into closed arms	Number of exits outside open arms
Blank	26.50	83.92	189.80	7.67	11.25	6.67
Reference (Amitriptyline)	45.08	77.33	177.60	8.67	9.75	6.83
	Effect (%) compared to control					
	70.11%	-7.85%	-6.42%	13.04%	-13.33%	2.48%
	Student t Test					
Compound III – D	p = 0.04	NS	NS	NS	NS	NS
	34.42	82.25	183.30	8.83	10.92	5.27
	Effect (%) compared to control					
	29.88%	-1.98%	-3.42%	15.20%	-2.93%	-20.99%
Compound III – J	Student t Test					
	NS	NS	NS	NS	NS	NS
	41.92	78.67	179.40	7.33	10.67	8.67
	Effect (%) compared to control					
Compound III – T	58.18%	-6.25%	-5.47%	-4.35%	-5.15%	29.99%
	Student t Test					
	NS	NS	NS	NS	NS	NS
	26.22	76.25	189.60	8.58	10.92	7.58
Compound III – T	Effect (%) compared to control					
	-1.06%	-9.13%	-0.10%	11.94%	-2.93%	13.73%
	Student t Test					
	NS	NS	NS	NS	NS	NS

NS = non-significant

The results show that the new acyl-oximines have no significant influence on the animals' behaviour in the elevated plus-maze test compared to control group. The tested compounds III-D and III-J increased the time spent in open arms compared to control. Compound III-D determined the highest number of entries into open arms (with 15.2% higher than control group). In the same time, compound III-J determined the highest number of exits outside

open arms (with 29.99% higher than control group and higher than amitriptyline used as reference).

The values obtained for the measured parameters sustain that the new compounds III-D and III-J determine some positive changes in animals' behaviour which explain a weak anxiolytic effect.

Effect on motor activity

Table IV indicates the mean values for each group for the horizontal movements of mice.

Table IV

Experimental results of motor activity – number of horizontal movements

	Control	Reference (amitriptyline)	III - D	III - J	III - T
Mean $\pm$ SD	480.70 $\pm$ 118.20	495.80 $\pm$ 66.89	561.30 $\pm$ 54.84	569.90 $\pm$ 129.10	557.90 $\pm$ 123.80
SEM	34.11	19.31	15.83	37.28	35.74
Normal distribution	YES	YES	YES	YES	YES
Effect (%) compared to control		3.14%	16.76%	18.55%	16.05%
ANOVA	NS				

For the three tested compounds the horizontal motor activity was similar and was statistically non-significant (between 16 and 19%), but higher than amitriptyline (3.14%).

Table V indicates the mean values for each group for the vertical movements of mice.

The activity cage recorded an improved vertical motor activity for the new tested acyl-oximines compared to control (between 35 and 51%). Mice treated with compound III-T had the highest vertical activity compared to control (50.95%; $p < 0.05$).

Table V

Experimental results of motor activity – vertical movements

	Control	Reference (amitriptyline)	III - D	III - J	III - T
Mean $\pm$ SD	78.83 $\pm$ 33.29	91.50 $\pm$ 25.05	109.80 $\pm$ 18.84	106.30 $\pm$ 39.94	119.00 $\pm$ 31.81
SEM	9.61	7.23	5.44	11.53	9.84
Normal distribution	YES	YES	YES	YES	YES
Effect (%) compared to control		16.07 %	39.28 %	34.84 %	50.95 %
ANOVA	p < 0.0188 (*)				
Bonferroni		NS	NS	NS	p < 0.05

The results obtained by recording the horizontal and especially the vertical motor activity for mice treated with control, reference and tested substances confirm the absence of the sedative side effect for the tested acyl-oximines and a potential increase of animal curiosity.

Analgesic effect

Table VI presents the mean values of time to first sign of pain for the tested compounds, reference and control groups. The analgesic effect was determined compared to control group.

Table VI

Time to first sign of pain (sec.)

	Control	Reference (amitriptyline)	III - D	III - J	III - T
Mean $\pm$ SD	4.38 $\pm$ 1.49	4.63 $\pm$ 1.96	5.12 $\pm$ 1.41	4.47 $\pm$ 1.67	5.46 $\pm$ 1.37
SEM	0.43	0.57	0.41	0.48	0.39
Normal distribution	YES	YES	YES	YES	YES
Analgesic effect (%) compared to control		5.70%	16.92%	2.09%	24.52%
ANOVA	NS				

Time to the first sign of pain for the tested groups did not modify statistically significant compared to control group. Compound III-T produced the highest increase of time to first sign of pain (24.52% compared to control), possibly explained by an inhibition of norepinephrine and serotonin reuptake in descendent pain fibres.

Conclusions

For three new acyl-oximine derivatives there have been evaluated antidepressant effect, anxiolytic effect, analgesic effect and the presence or absence of sedative side effect. The three new compounds produced a significant decrease of the immobility time in the forced swimming test, similar to the effect produced by amitriptyline used as reference substance. It is possible that the tricyclic structure, as well as the oximine group, could generate a high affinity for norepinephrine and serotonin reuptake transporters.

The results obtained for the measured parameters in elevated plus-maze test showed that the new compounds III-D and III-J determined a weak anxiolytic effect, but it is not a significant action.

Compound III-T determined a significant increase of vertical movements recorded by the activity cage. This result can lead us to a further research concerning a possible stereotypical behaviour determined by this compound.

The hot-plate test confirmed positive modifications of time to first sign of pain for the new compound

III-T, possibly explained by an inhibition of norepinephrine and serotonin reuptake in descendent pain fibres. Although, the new acyl-oximines did not modify statistically significant the animal reaction to thermal stimulus compared to the control group.

In conclusion, these results suggest that the three new tested acyl-oximine compounds modify some pharmacological parameters at the central level, with significant antidepressant action, but without significant analgesic and anxiolytic actions. These CNS actions need further evaluation.

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References

1. Arsene A.L., Cristea A., Mitrea N., Negreș S., Analgesic effect of melatonin in association with ondansetron, petidine and tramadol in a murine model of behavior. *Acta Endocrinol.*, 2010; 2: 143-150.
2. Bădiceanu C.D., Dimcevic Poesina N., Missir A.V., Stecoza C.E., Dinu M., The phytobiological testing of some new thiourea derivatives. *Farmacia*, 2014; 62(1): 23-33.
3. Benbouzid M., Gaveriaux-Ruff C., Yalcin I., Waltisperger E., Tessier L.H., Muller A., Kieffer B.L., Freund-Mercier M.J., Barrot M., Delta-opioid receptors are critical for tricyclic antidepressants

- treatment of neuropathic allodynia. *Biol. Psychiat.*, 2008; 63(6): 633-636.
4. Brouquet K., Status of treatment of depression. *South Med. J.*, 1999; 92(9): 846-856.
 5. Casobrez A.P., Bertoglio L.J., Ethological and temporal analysis of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci. Behav. Rev.*, 2005; 29: 1193-1205.
 6. Chiriță C., Cristea A.N., Militaru M., Negreș S., Zbârcea C.E., Nuță C.D., Pharmacological evaluation of acute and subacute toxicity and antidepressants effect after acute administration of novel N-substituted benzamides. *Farmacia*, 2010; 58(1): 21-28.
 7. Cioroianu D.M., Morușciag L., Căproiu M.T., Chiriță I.C., Synthesis and characterization of some new acyl-oximines derivatives. *Studia Universitatis „Vasile Goldiș”*, 2012; 22(4): 495-501.
 8. Cioroianu D.M., Morușciag L., Căproiu M.T., Limban C., Chiriță I.C., Nuță C.D., Synthesis and characterization of new acyl-oximines derivatives with potential pharmacological activity. *Farmacia*, 2013; 61(3): 469-480.
 9. Cioroianu D.M., Nuță C.D., Căproiu M.T., Missir A.V., Limban C., Chiriță I.C., Morușciag L., New synthesis in the acyl-oximine derivatives seria. *Farmacia*, 2014; 62(2): 366-375.
 10. Cioroianu D.M., Nuță C.D., Morușciag L., Căproiu M.T., Chiriță I.C., Limban C., The synthesis of some new acyl-oximines compounds with potential pharmacological activity. *Farmacia*, 2013; 61(2): 298-312.
 11. Craft L.L., Perna F.M., The benefits of exercise for the clinically depressed. *Prim Care Companion J. Clin. Psychiatry*, 2004; 6: 104-111.
 12. Eddy N.B., Leimbach D., Synthetic analgesics. II Diethienylbutenyl- and diethienylbutylamines. *J. Pharmacol. Exp. Ther.*, 1953; 107(3): 385-393.
 13. Healy D., The Antidepressants Drama: The treatment of depression: bridging the 21st century. *Am. Psychiatric Pub.*, 2011; 10-11.
 14. Jones C.K., Eastwood B.J., Need A.B., Shanon H.E., Analgesic effects of serotonergic, noradrenergic or dual uptake inhibitors in the carrageenan test in rats: Evidence for synergism between serotonergic and noradrenergic reuptake inhibition. *Neuropharmacology*, 2006; 51(7-8): 1172-1180.
 15. Kessler R.C., Berglund P., Demler O., The epidemiology of major depressive disorder, Results from the National Comorbidity Survey Replication (NCS-R). *JAMA*, 2003; 289(203): 3095-3105.
 16. Lalonde R., Strazielle C., Relations between open-field elevated plus-maze and emergence test as displayed by C57/BL6J and BALB/C mice. *J. Neurosci. Meth.*, 2008; 171(1): 48-52.
 17. Murray E.D., Buttner N., Price B.H., Depression and psychosis in neurological practice. In: Bradley's Neurology in Clinical Practice, 6th Ed., 2012, 292.
 18. Negreș S., Zbârcea C.E., Arsene A., Chiriță C., Buzescu A., Velescu B.Ș., Ștefănescu E., Șeremet O., Nicolescu F., Experimental pharmacological model for inducing and quantifying depression in mouse. *Farmacia*, 2013; 61(6): 1102-1116.
 19. Otaka M., Jin M., Odashima M., New strategy of therapy for functional dyspepsia using famotidine, mosapide and amitriptyline. *Aliment Pharm. Ther.*, 2005; 21(2): 42-46.
 20. Papp M., Wieronska J., Antidepressants-like activity of amisulpride in two animal models of depression. *J. Psychopharmacol.*, 2000; 14(1): 45-62.
 21. Porsolt R.D., Bertin A., Jalfre M., Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.*, 1977; 229: 327-336.
 22. Saravane D., Feve B., Frances Y., Corruble E., Lancou C., Chanson P., Maison P., Terra J.L., Azorin J.M., Drawing up guidelines for the attendance of physical health of patients with severe mental illness. *L'Encephale*, 2012; 35(4): 330-339.
 23. Tatsumi G.K., Blakely R.D., Richelson E., Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur. J. Pharmacol.*, 1997; 340(2-3): 249-258.
 24. Teicher M., Glod C., Cole J., Antidepressants drugs and the emergence of suicidal tendencies. *Drug Saf.*, 1993; 8(3): 186-212.
 25. Wilens T., Biederman J., Geist D., Steingard R., Spencer T., Nortriptyline in the treatment of ADHD. A Chart Review of 58 cases. *J. Am. Acad. Child Psy.*, 1993; 32(2): 343-349.
 26. Zbârcea C.E., Negreș S., Chiriță C., Gabapentine, alone and associated with tramadol peripheral paclitaxel-induced neuropathy in rats. *Farmacia*, 2011; 59(3): 414-423.
 27. Zbârcea C.E., Negreș S., Cristea A.N., Chiriță C., The effect of dextromethorphan, gabapentine, amitriptyline and tramadol on mouse model of vincristine-induced peripheral neuropathy. *Farmacia*, 2011; 59(6): 809-818.