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SYNTHESIS OF NEW THIOPHENE INCORPORATED 1, 3-OXAZEPINE DERIVATIVES WITH ANTIDEPRESSANT POTENTIAL

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ABSTRACT

Depression is a medical condition that can have serious consequences if it persists for an extended period. The serotonin receptor family plays a role in transporting serotonin across the synaptic cleft, with SERT (or 5-HTT) and 5-HT2A being two key members. Antagonizing these receptors would result in an antidepressant effect on individuals suffering from depression. A series of thiophene-incorporated 1,3-oxazepine derivatives were synthesized by reacting 2-amino thiophene derivatives by Gewald reaction with various substituted aromatic benzaldehydes to form Thienyl Schiff bases. The desired thiophene incorporated 1,3-oxazepine derivatives were obtained by cyclizing Thienyl Schiff bases using maleic anhydride, which was then characterized using spectroscopic techniques like IR, Mass, and ^{1}H NMR. Among the synthesized compounds, Forced swimming and tail suspension experiments were used to assess TOM3's in vivo antidepressant efficacy. It significantly decreased immobility time in low, medium, and high doses compared to the control group**.** This research contributes to Sustainable Development Goal 3 (Good Health and Well-Being) by addressing mental health through the development of novel therapeutic agents. Overall, the findings of this study could assist with developing strong 5-HTT and 5-HT2AR inhibitors for the treatment of depression.

Keywords: Depression, Thiophene, Schiff Base, Oxazepine, Antidepressant

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INTRODUCTION

More than 280 million people worldwide suffer from depression, yet the effectiveness of existing antidepressants remains limited for nearly one-third of patients. Elevation or decrease in mood is a frequent mental health condition called depression. It includes interaction between a person's social and psychological behavior. Features of depression include trouble in thinking, grief, loss of attentiveness, feelings of incompetence, dejection, suicidal notions, variations in appetite, etc., which may be caused by mental and sexual abuse, negligence, partial parental treatment of siblings, unemployment, jealousy, separation, stress, medical diagnosis, and catastrophic injury. Depression can be graded as mild, moderate, severe, or psychotic. Two categories of depression, as per DSM-IV-TR, are bipolar depression and unipolar depression.[1] Mild depression as such may not require medical intervention, but a depressed mood for an extended period, particularly when paired with other symptoms, might lead to a diagnosis of a psychiatric or medical illness that requires treatment. Antidepressants and psychotherapy are frequently used in conjunction to treat depression. Among the several drugs that are frequently prescribed are tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin and noradrenaline reuptake inhibitors, and reversible inhibitors of MAO-A.[2] Besides the effectiveness of the typical antidepressants, there exist potential side effects of the drug therapy, like serotonin toxicity, diabetes, drug interactions, drug-induced QT prolongation, etc. Due to the frequent dosing regimen, medication adherence is also found to be a major problem. This motivates the discovery of new therapeutic agents with lesser toxicity and better activity.[3] In support of the United Nations Sustainable Development Goal 3 (Good Health and Well-Being), which emphasizes the importance of mental health, this study aims to contribute to the development of safer and more effective treatments for depression. In the recent era of emerging diseases, naturally occurring heterocyclic molecules are intensively under investigation for their utility in the discovery of novel therapeutic agents. Thiophene was a family of molecules that caught the attention of

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medicinal chemists among a wide range of such heterocyclic compounds, which led to the discovery of its vast therapeutic values as an anticancer, anti-inflammatory, analgesic, antihypertensive, and antibacterial agent. Thiophenes, also called thiofuran, consist of a five-membered ring structure with sulfur as the heteroatom. It is aromatic and takes part in several substitution reactions. Certain plant pigments were found to be the derivatives of thiophene. There are many synthetic derivatives like Methapyrilene, Tipepidine, Dorzolamide, Citizolam, Benocyclidine, etc., with therapeutic importance.[4] Oxazepines are a group of cyclic compounds with seven atoms, one carbon replaced by nitrogen and another by oxygen. Due to their influencing biological activity, 1,3-oxazepines and their benzo-fused derivatives have been an object of investigation in the field of medicine. Activities like anticonvulsant, antifungal, anti-epileptic, anti-inflammatory, anti-thrombotic, analgesic, antipsychotic, etc., were investigated, resulting in promising observations that motivate the development of novel therapeutic molecules containing the Oxazepine nucleus.[5] For the purpose of identifying promising lead compounds that offer better efficacy and safety profiles than current antidepressant therapies, the present study aims to design, synthesize, and characterize a novel series of thiophene-incorporated 1,3-oxazepine derivatives and examine their potential antidepressant activity using in vivo models.

EXPERIMENTAL

Chemistry

Open capillary measurement yielded the melting points, which were not adjusted. Using the FT-IR approach in Alpha Bruker, all of the IR spectra were captured. A Bruker spectrometer (400 MHz) was used to record ^{1}H NMR spectra (DMSO-d 6) with TMS serving as the internal standard. Values for the chemical shift are expressed in δ scales. A JOEL JNM-ECZ400/L1 NMR Spectrometer operating at 400 MHz was used to record ^{1}H NMR spectra. The mass spectrum was captured using a TOF mass analyzer and an LC-MS (Waters, USA - Xevo G2-XS QT of) spectrometer that was produced using the ESI method. Thin layer chromatography (TLC) on silica gel-G coated plates was used to verify that the reaction was efficient. The reaction was examined under a UV lamp while using n-hexane: ethyl acetate (7:3) as the solvent. We used commercial-grade solvents and reagents with purity levels of at least 98% from Sigma-Aldrich, Loba Chemie, Himedia, and Sisco Research Laboratories.

Synthesis of Thiophene Incorporated 1,3-Oxazepine Derivatives

Synthesis of Amino Thiophene Derivatives[6]

A mixture of 10–30 mL of ethanol, 10 mL of diethylamine, 0.1M keto methylene, 0.1M malononitrile, 0.11M sulfur, and 45–50 °C was added and agitated for three hours. It is recommended to stay below the 60 °C reaction temperature. After that, the solid was rinsed and filtered. After drying, the resultant solid was filtered and washed with ethanol. It was then refined by recrystallization using the proper solvent, preferably ethanol.

Synthesis of Thienyl Schiff Base[7]

2-Aminothiophene derivatives (1 mmol) and substituted aromatic benzaldehyde (1 mmol) were dissolved in 20 mL of warm ethanol, containing a small amount of glacial acetic acid as a catalyst. After that, the mixture was boiled and given a 5–7-hour reflux period. The solid formed upon cooling was separated by filtration and washed with hot ethanol. Finally, the solid was dried and purified by recrystallization using ethanol.

Synthesis of Thiophene Incorporated 1,3-Oxazepine[8]

Maleic anhydride (0.002 mol) was added after thienyl Schiff base (0.001 mol) was first dissolved in 20 millilitres of ethanol. Until the reaction was completed, the resultant mixture was refluxed in a water bath for three to four hours. Thin-layer chromatography was used to track the reaction's development. After cooling, the precipitate was filtered and purified through recrystallization using dimethylformamide.

In vivo Antidepressant Activity

Selection of Animals

Swiss albino mice, aged between 8-12 weeks, of either gender and weighing approximately 25-30g, were procured from the central animal house at Nitte Centre for Animal Research and Experimentation

(NUCARE), Paneer, Mangalore. The mice were assigned to individual cages, split into five groups, and had their doses and routes of administration determined per the OECD 425 criteria. The experimental methodology was accepted by the IAEC, and rules for researching animals have been created by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) in New Delhi, India. It is registered under the number NGSMIPS/IAEC/JUNE-2021/241.

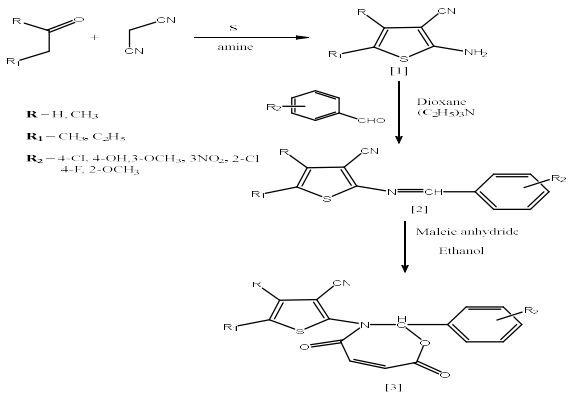

Fig.-1: Scheme for the Synthesis of Thiophene Incorporated 1,3-Oxazepine Derivative

Acute Oral Toxicity Studies

The acute oral toxicity investigation was carried out using the up-and-down method on adult Swiss albino mice weighing approximately 25–30g in accordance with OECD 425 criteria. Before dosage, the animals were fasted (food was withheld overnight, but not water). Each animal's fasting body weight was established, and the dose was computed based on that weight. Since there was no information on the substance's lethality available, dosing of the first animal was commenced at 175 mg/kg. The up-and-down method was used to fix the dose. Throughout the tests, the dose progressed at a constant rate, as determined by dividing the antilog of 1 by the predicted slope of the dose-response curve. Given that the default progression factor is employed, the following doses were chosen: 1.75, 5.5, 17.5, 55, 175, 550, and 2000. Doses were administered to the animals at predetermined times (every 48 hours, for example), and dosage was continued based on each animal's performance up to that point. When three successive animals survived the highest dose, the testing procedure was terminated. When the stopping criteria were met, the LD_{50} was calculated based on the outcomes of the animals.

Experimental Design

Screening of *in vivo* antidepressant activity on experimental animals was done by the following methods:

(a) Forced Swimming Test (FST)

The antidepressant potential of the synthesized compound was assessed using the forced swim test. Three groups of six animals each, receiving low, medium, and high dosages, were created from the fasted animals. The mice were given the standard medication, imipramine (10 mg/kg) in a Tween 80 suspension, 60 minutes before the test, and a suspension of the synthesized chemical was to be examined.[9,10] A control mouse was also included and trained to swim for six minutes in a cylindrical container measuring 25 cm in height and 10 cm in diameter. The container was filled with water up to an 18 cm height. The animal will struggle a great deal in the first two minutes. The last four minutes were when the immobility time was recorded.

The percentage change in immobility relative to the control group was calculated using the formula: % Change = [(Test / Control) × 100] – 100.

(b) Tail Suspension Test (TST)

Imipramine (10 mg/kg) is frequently used as a standard reference antidepressant in the tail suspension test (TST). Mice were given a Tween 80 suspension containing the standard medicine and the in-silico possible synthesized molecule to be evaluated (at low, medium, and high doses) half an hour before the test.[11] A control was retained throughout the study. On the experimental day, mice subjected to a pre-established fasting period (n = 6 per group) were systematically allocated into distinct treatment groups.

Paper sticky tape was used to hang each mouse 65 cm from the work surface. The apparatus was affixed about 1 cm from the tail tip, and each mouse was suspended for six minutes, with immobility measured during the final five minutes.[12]

The percentage change from control was determined using the following formula:

$$\% \text{ Change of immobility} = [(\text{Test/Control}) \times 100)-100]$$

Statistical Analysis

GraphPad Prism 8.0.2 was used to complete the calculations based on statistics for every value. A one-way analysis of variance (ANOVA) was performed on the six animal data points from each group. The mean ± standard deviation (SD) indicates the experimental outcomes. Significance was determined based on p values below 0.05 (***p < 0.001, **p < 0.01, and *p < 0.0).

RESULTS AND DISCUSSION

Compound TOP11: 2-(2-(4-hydroxyphenyl)-4,7-dioxo-4,7-dihydro-1,3-oxazepin-3(2*H*)-yl)-5-methylthiophine-3-carbonitrile

IR (cm^{-1}): 3135(Ar C-H str), 1478(Ar C=C str),2999(aliphatic C-H str),3358(O-H str), 1220(C-N str),2215(C≡N str), 1645(C=O lactam str) ; ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.409-8.227 (m, 8H, Ar-H), 6.409 (d, 2H, oxazepine), 8.227 (s, 1H, oxazepine), 7.985 (s, 1H, thiophene), 5.433 (s, 1H, OH) ; MS (M^+):340

Compound TOM3: 2-(2-(4-chlorophenyl)-4,7-dioxo-4,7-dihydro-1,3-oxazepin-3(2*H*)-yl)-5-ethyl-4-methylthiophene-3-carbonitrile

IR (cm^{-1}): 3003(Ar C-H str), 1538(Ar C=C str), 2852(aliphatic C-H str), 636(C-Cl str), 1224(C-N str), 2218(C≡N str), 1647(C=O lactam str) ; ^{1}H NMR (400 MHz, DMSO-d_6): δ6.039-8.868 (m, 7H, Ar-H), 6.039 (d, 2H, oxazepine) 8.868 (s, 1H, oxazepine), 1.241-1.276 (s, 3H,CH_3); MS (M^+): 386

Compound TOM5: 5-ethyl-2-(2-(4-fluorophenyl)-4,7-dioxo-4,7-dihydro-1,3-oxazepin-3(2*H*)-yl)-4-methylthiophene-3-carbonitrile

IR (cm^{-1}): 1532(Ar C=C str),2995(aliphatic C-H str), 1219(C-F str), 1219(C-N str), 1734(C=O lactone str), 2216(C≡N str), 1660(C=O lactone str); ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.429-7.125 (m, 4H, Ar-H), 6.429 (d, 2H, oxazepine),7.125 (s, 1H, oxazepine); MS (M^+): 369

Table-1: Physical Data of Thiophene Incorporated 1,3-Oxazepine Derivatives

Comp.	R	R_1	R_2	Mol. Formula	Mol. Wt	m.p (°C)	% Yield
TOP3	H	CH_3	4-Cl	$C_{17}H_{11}ClN_2O_3S$	358.79	163	75%
TOP11	H	CH_3	4-OH	$C_{17}H_{12}N_2O_4S$	340.35	170	56%
TOP13	H	CH_3	4-OH, 3-OCH_3	$C_{18}H_{14}N_2O_5S$	370.37	153	43%
TOM1	CH_3	C_2H_5	3- NO_2	$C_{19}H_{15}N_3O_5S$	397.40	172	55%
TOM3	CH_3	C_2H_5	4-Cl	$C_{19}H_{15}ClN_2O_3S$	386.85	179	63%
TOM4	CH_3	C_2H_5	2-Cl	$C_{19}H_{15}ClN_2O_3S$	386.85	185	61%
TOM5	CH_3	C_2H_5	4-F	$C_{19}H_{15}FN_2O_3S$	369.39	177	72%
TOM8	CH_3	C_2H_5	2-OCH_3	$C_{20}H_{18}N_2O_4S$	382.43	192	53%
TOM11	CH_3	C_2H_5	4-OH	$C_{19}H_{16}N_2O_4S$	368.40	184	48%
TOM13	CH_3	C_2H_5	4-OH, 3-OCH_3	$C_{20}H_{18}N_2O_5S$	398.43	168	42%

Fig.-2: (A.) Forced Swimming Test; (B.) Antidepressant Evaluation of the Compound TOM3 using the Forced Swimming Test

Table-2: Antidepressant Evaluation of Compound TOM3 using Forced Swimming Test

Groups	Treatment	Dose(mg/kg)	Immobility time (sec)	% change in mobility
I	Control	0.6% w/v CMC10mL/kg	189±1.82	0
II	STD (Imipramine)	10	79±2.12**	58.2
III	Low	8.75	60.75±0.60***	67.85
IV	Medium	17.5	53.36±1.66**	71.76
IV	High	35	63.7125±1.22*	66.29

Values are expressed as mean ± SD for 6 animals; ***p < 0.001, **p < 0.01, and *p < 0.05 Vs control.

(b) Tail Suspension Test (TST)

The developed compounds' in-silico analysis has been published. Based on the *in-silico* studies of the designed molecules best ten compounds were selected for synthesis. The reaction of amino thiophene derivatives and substituted benzaldehyde in the presence of dioxane and glacial acetic acid prepared different thienyl Schiff base derivatives TLC, melting point, and IR spectrum of these compounds exhibited significant absorption bands at 1615 (C=N str) for the Schiff base. The title compounds thiophene thiophene-incorporated 1,3-oxazepine derivatives, were synthesized through the cyclization of a thienyl Schiff base with maleic anhydride. The lactam of 1,3-oxazepine, which was absent from the intermediate thienyl Schiff base, was represented by characteristic absorption bands in the IR spectra of 1,3-oxazepine derivatives at 1647-1660 cm^{-1}.

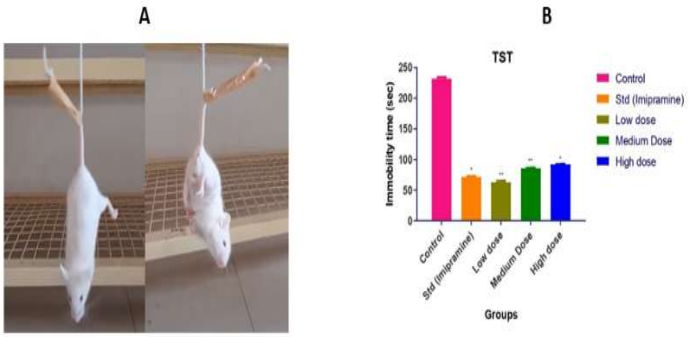

Fig.-3: (A.) Tail Suspension Test; (B.) Antidepressant Evaluation of the Compound TOM3 using the Tail Suspension Test

Table-3: Antidepressant Evaluation of Compound TOM3 using the Tail Suspension Test

Groups	Treatment	Dose(mg/kg)	Immobility time (sec)	% change in mobility
I	Control	0.6% w/v CMC 10mL/kg	232±2.35	0
II	STD (Imipramine)	10	71.6±1.28*	61.13
III	Low	8.75	62.73±2.10**	72.96
IV	Medium	17.5	85.6±0.73**	63.1
IV	High	35	92.27±0.51*	60.22

Values are expressed as mean ± SD for 6 animals; ***p < 0.001, **p < 0.01, and *p < 0.05 Vs control.

Similarly, the ^{1}H NMR of the synthesized 1,3-oxazepine derivatives showed one characteristic signal at δ 6.039 (d, 2H, oxazepine), 8.868 (s, 1H, oxazepine), which was absent in the ^{1}H NMR spectra of thienyl Schiff base. Therefore, mass spectra that comply with the molecular formula verified and further validated the generation of the compounds with the title compounds, benzodiazepines.

Based on the docking score, and physicochemical and ADME properties, compound TOM3 was found to be a better molecule for *in vivo* analysis of an antidepressant-like effect compared to the other designed molecules. In this study, compound TOM3 was investigated on the immobility time of mice in three different dosages (low, medium, and high) using the FST. The findings revealed that the TOM3 compound reduced mice's immobility duration at low ($p < 0.001$), medium ($p < 0.01$), and high ($p < 0.05$)

doses significantly compared to the control group, showing comparable effectiveness to the standard drug imipramine ($p < 0.01$). These findings imply that TOM3, in the FST, exhibited significant antidepressant-like effects at all three levels. The compound TOM3 was subjected to TST to determine its effect on the immobility frequencies of mice. It was observed that the TOM3 compound considerably reduced the immobility period in mice when given at low ($p < 0.01$), medium ($p < 0.01$), and high ($p < 0.05$) doses, which was comparable to the standard drug imipramine ($p < 0.05$) relative to the control group. The results supported the observations from the FST and pointed out the antidepressant-like activities of the test compound. A possible reason is that it exhibits strong antidepressant potential because of the presence of electron-donating methyl and ethyl groups in the thiophene ring and electron-withdrawing chloro group in the phenyl ring.

CONCLUSION

The synthesis of new thiophene incorporated 1,3-oxazepine derivatives was done by a conventional method with moderate yields. The compound TOM3 was selected to be analyzed for its antidepressant-like effect in two different *in vivo* models using mice. The in silico identified compound TOM3 demonstrated a significant antidepressant-like effect in both the Forced Swim Test (FST) and Tail Suspension Test (TST). It can be concluded that the compound TOM3 could be a promising molecule as an antidepressant agent compared to all other synthesized compounds. This research aligns with Sustainable Development Goal 3 (Good Health and Well-Being) by contributing to the development of safer, more effective treatments for mental health disorders such as depression.

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CONFLICT OF INTERESTS

The authors declare that there was no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. K. Singh, R. Pal, S. A. Khan, B. Kumar and M. J. Akhtar, *Journal of Molecular Structure*, **1237**,130369(2021), https://doi.org/10.1016/j.molstruc.2021.130369
2. L. Rao, V. Verma, S. Jain, K. K. Pinapati, S. S. Bhagyawant, S. Sharma, and N. Srivastava, *Cell Biochemistry and Biophysics*, **82(2),** 1335(2024), https://doi.org/10.21203/rs.3.rs-3999287/v1
3. M. P. Rochester, A. M. Kane, S. A. Linnebur and D. R. Fixen, *Therapeutic Advances in Drug Safety*, **9(6)**, 297(2018), https://doi.org/10.1177/2042098618772979
4. R. Shah and P. K. Verma, *Chemistry Central Journal*, **12(1)**, 137(2019), https://doi.org/10.30492/ijcce.2021.523685.4550
5. M. H. Jebur, E. A. Albdere, M. K. Al-Hussainawy and S. H. Alwan, *International Journal of Health Sciences,* **6(S4)**, 4578(2022), https://doi.org/10.53730/ijhs.v6nS4.9123
6. R. W. Sabnis, *Coloration Technology*, **132**, 49(2016), https://doi.org/10.1111/cote.12182
7. S. G. Nayak, B. Poojary and V. Kamat, *Archives of Pharmacal Research*, **353**, e2000103(2020), https://doi.org/10.1002/ardp.202000103

8. M. M Aftan, M. Q Jabbar, A. H. Dalaf and H. K. Salih, *Materials Today Proceedings*, **43**, 2040(2021), https://doi.org/10.1016/j.matpr.2020.11.838
9. D. A. Slattery, J. F. Cryan, *Nature Protocols*, **7**, 1009(2012), https://doi.org/10.1038/nprot.2012.044
10. R. Yankelevitch-Yahav, M. Franco, A. Huly and R. Doron, *Journal of Visualized Experiments*, **97**, 52587(2015), https://doi.org/10.3791/52587
11. A. Can, D. T. Dao, C. E. Terrillion, S. C. Piantadosi , S. Bhat, and T.D. Gould, *Journal of Visualized Experiments*, **59**, e3769(2012), https://doi.org/10.3791/3769
12. D. G. Machado, L.E. B. Bettio, M. P. Cunha, A R S. Santos, M. G. Pizzolatti, I. M. C. Brighente and A. L. S. Rodrigues, *European Journal of Pharmacol*ogy, **587(1-3)**, 163(2008), https://doi.org/10.1016/j.ejphar.2008.03.021

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