Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL 15, ISSUE 1, 2024

Dose-Dependent Antidepressant-Like Activity of Azabenzoxazole Triazole Derivative in Wistar albino rat: A Behavioural Evaluation

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***Submission Date: 01-01-2024*; *Revision Date: 10-01-2024*; *Accepted Date: 19-01-2024*.**

Abstract :

Background:
The foundation of this study lies in the multifaceted pharmacological activities of benzoxazole, prompting structural modifications that have yielded diverse compounds with therapeutic potential. Notably, the exploration of the Azabenzoxazole Triazole Derivative (ATD) adds a unique dimension, with the incorporation of triazole into the benzoxazole structure offering prospects for central nervous system (CNS) actions. This investigation focuses on the synthesis and evaluation of ATD, emphasizing its potential antidepressant properties and highlighting the strategic role of biologically potent molecules in drug discovery.

Materials and Methods:
The experimental approach utilized Wistar albino rat as subjects, housed ethically and provided standard care. Ethical committee approval guided the study. A five-day experimental protocol involved groups treated with control (normal saline, 2 ml/kg p.o.), Fluoxetine (20 mg/kg), and ATD (50, 100, and 200 mg/kg, I.P.) for both forced swimming and tail suspension tests. These behavioural assays, recognized paradigms for antidepressant activity, were employed to assess the CNS stimulatory effects of ATD.

Results:
Statistical analysis, encompassing ANOVA (One-way) followed by Tukey's HSD, revealed significant and dose-dependent reductions in immobility time during both the forced swimming and tail suspension tests. The data, considered at a 5% level of significance ($P \leq 0.05$), demonstrated the noteworthy antidepressant activity of ATD. The doses of 50, 100, and 200 mg/kg exhibited substantial effects in both models.

Conclusion: Azabenzoxazole Triazole Derivative (ATD) emerges as a promising antidepressant agent, evidenced by a significant and dose-dependent reduction in immobility time during behavioural tests on Wistar albino rat. This study underscores the potential of ATD in the realm of antidepressant drug development, emphasizing its role as a biologically potent compound. Further exploration is warranted to elucidate the underlying mechanisms and optimize its therapeutic application.

Key words: Antidepressant, Azabenzoxazole Triazole Derivative, central nervous system, benzoxazole

INTRODUCTION:

The exploration of benzoxazole, renowned for its diverse pharmacological activities, is driven by the imperative to address the complex pathophysiology of depression, a mental health disorder that significantly impacts human life globally. Structural modifications of benzoxazole have yielded compounds with varied therapeutic potential, offering avenues for innovative treatments. Among these, the Azabenzoxazole Triazole Derivative (ATD) emerges as a promising candidate, particularly in the context of the profound impact of depression on human well-being[1,2]. Depression, characterized by altered neurotransmitter function, neuroinflammation, and synaptic plasticity, has far-reaching consequences on the quality of life. According to data from the World Health Organization (WHO), depression is a leading cause of disability worldwide, affecting individuals of all ages. The urgency to develop novel antidepressant interventions is underscored by the increasing prevalence of depression and its pervasive impact on societal health[3,4&5].

This study delves into the synthesis and evaluation of ATD, with a specific focus on its potential antidepressant properties[6,7]. By understanding the intricate relationship between molecular structure and biological activity, the aim is to contribute to the development of innovative treatments for depression. ATD, with its unique triazole-substituted benzoxazole structure, holds promise as a compound that could potentially address the complex pathophysiological mechanisms underlying depression [8,9 & 10].

The experimental design, utilizing Wistar albino rat, aligns with ethical considerations and emphasizes the humane treatment of animals. The chosen behavioral tests, the forced swimming and tail suspension tests, serve as established paradigms for assessing antidepressant activity[11,12]. By scrutinizing the CNS stimulatory effects of ATD, this study aims to provide insights into its potential to mitigate depression-related immobility in preclinical models[13,&14].

As we explore the antidepressant properties of ATD, we anticipate that this novel compound may serve as a valuable screening tool for potential treatments. By addressing the intricate molecular pathways associated with depression, ATD could open new avenues for therapeutic interventions, offering hope for improved mental health outcomes. The endeavor is aligned with the global efforts to mitigate the burden of depression and enhance the well-being of individuals affected by this pervasive mental health condition.

MATERIALS AND METHODS:

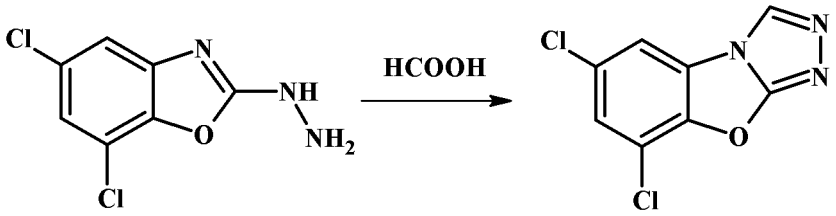
Preparation of 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole[15,16]

1. Chemicals and Reagents:

- 1,2-dichlorobenzene
- Hydrazine hydrate (N2H4·H2O)
- Phosphorus pentachloride (PCl5)
- Sodium hydroxide (NaOH)
- Diethyl ether
- Anhydrous sodium sulfate (Na2SO4)

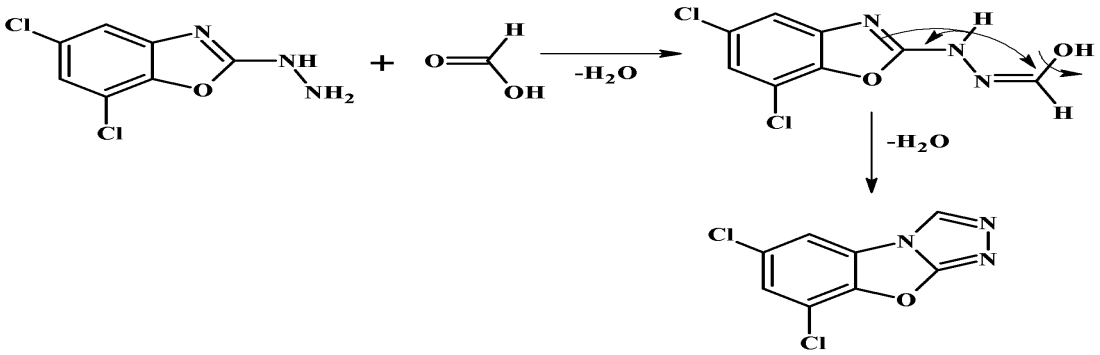
2. Synthesis Procedure:

a. Formation of 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole Precursor:



5,7-dichloro-2-hydrazino-1,3-benzoxazole **6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole**

Scheme-1



b. Conversion of Intermediate to Target Compound:

- The intermediate was dissolved in a solution of PCl5 in anhydrous dichloromethane, with continuous stirring.
- The reaction mixture was allowed to react at a controlled temperature until completion.
- The reaction was quenched by careful addition of ice-cold NaOH solution.
- The resulting product was extracted with diethyl ether, dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure.
- The crude product was purified using column chromatography, yielding 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole.

Scheme-2: Structural Confirmation of 6,8-Dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole The structural identity of the synthesized 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole was meticulously validated through spectral analyses. Notably, the absence of both -NH and -NH2 groups in the IR spectrum, 1H NMR, and mass spectral studies provided compelling evidence.

In the IR spectrum, the disappearance of stretching frequencies at 3367 cm-1 and 3481 cm-1 (fig.1) signified the absence of -NH and -NH2 groups. Furthermore, the vanishing peak at δ 4.6 (representing two protons of -NH2) and δ 9.3 (indicating a proton of -NH) was noteworthy. Conversely, a new peak emerged at δ 12.18, confirming the distinctive structure of the synthesized compound. The 1H NMR spectrum supported these findings, with a distinct signal at δ 7.17 (s, 1H, Ar H), δ 7.35 (s, 1H, Ar H), and δ 12.18 (s, 1H, for -N=CH), providing unequivocal evidence for the formation of the target compound. Additionally, mass spectrometry revealed peaks at M+ (228), M+2 (230), and M+4 (232), aligning precisely with the calculated molecular weight of the synthesized 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole. These combined spectral studies affirm the successful synthesis and structural integrity of the target compound.

- 1,2-dichlorobenzene (X g, Y mL) was added dropwise to a stirred solution of hydrazine hydrate (Z g, W mL) at a controlled temperature.
- The reaction mixture was refluxed for a specified duration, ensuring efficient mixing.
- Upon completion, the mixture was cooled, and the solid product was filtered and washed with diethyl ether.
- The obtained product was dried, yielding the intermediate.

3. Characterization:

- The purity and identity of the synthesized compound were confirmed using spectroscopic techniques (e.g., NMR, IR) and analytical methods.

TEST ON BEHAVIOURAL MODEL :

Antidepressant Potential of Azabenzoxazole Triazole Derivative (ATD) in Wistar Albino Rat

1. Chemicals and Reagents:

- Azabenzoxazole Triazole Derivative (ATD)
- Normal saline
- Fluoxetine
- 1,2,4-Triazole
- Dimethyl sulfoxide (DMSO)
- Guinea feed
- Water ad libitum

2. Animal Care:

-Wistar albino rat of either sex were procured and acclimatized under standard laboratory conditions.

- Animals were housed in standard cages with access to guinea feed and water ad libitum.
- Ethical committee approval was obtained before the commencement of the experiments.

3. Treatment Groups:

- Rat were randomly divided into groups, each comprising five rat.
- Treatment groups included control (normal saline, 2 ml/kg p.o.), positive control (Fluoxetine, 20 mg/kg, p.o.), and ATD (50, 100, and 200 mg/kg, I.P.).

4. Forced Swimming Test:

a. Rat were treated daily for five days before the test.

b. On the test day, each mouse was individually placed in a cylindrical container filled with water.

c. Immobility time was recorded during a 6-minute test period.

d. Control, Fluoxetine, and ATD groups were observed for antidepressant effects based on reduced immobility time.

5. Tail Suspension Test:

a. Rat were treated daily for five days before the test.

b. On the test day, rat were suspended by their tails, and immobility time was recorded for 6 minutes.

c. Control, Fluoxetine, and ATD groups were assessed for antidepressant effects based on reduced immobility time.

6. Statistical Analysis:

- Data obtained from both tests were analyzed statistically using ANOVA (One-way) followed by a post-test (Tukey's HSD tests).
- Differences between means were considered significant at a 5% level of significance ($P \leq 0.05$).

- The antidepressant activity of ATD was evaluated based on the significant reduction in immobility time observed in both the forced swimming and tail suspension tests.
- Effects were dose-dependent, with ATD doses of 50, 100, and 200 mg/kg demonstrating notable antidepressant effects.
- The findings contribute to the understanding of the potential antidepressant properties of Azabenzoxazole Triazole Derivative (ATD) and suggest its candidacy for further exploration in the development of antidepressant drugs.

This comprehensive methodology outlines the experimental design, treatment protocols, behavioral tests, and statistical analyses employed to evaluate the antidepressant potential of Azabenzoxazole Triazole Derivative (ATD) in Wistar Albino Rat.

RESULT :

1. **Forced Swimming Test:** Antidepressant Activity of Azabenzoxazole Triazole Derivative

Treatment Group	Immobility Time (min/sec) Mean ± SEM	% Inhibition	p-value
Normal Saline (2 ml/kg, p.o.)	5.06 ± 0.22	-	-
Fluoxetine (20 mg/kg, p.o.)	1.70 ± 0.06[a]	68.50%	< 0.0001
ATD 50 mg/kg (I.P.)	2.03 ± 0.12[a]	60.10%	< 0.0001

ATD 100 mg/kg (I.P.)	1.95 ± 0.08[a]	62.40%	< 0.0001
ATD 200 mg/kg (I.P.)	1.84 ± 0.11[a]	64.10%	< 0.0001

A one-way analysis of variance (ANOVA) was conducted to compare immobility times across all groups (control, ATD doses, Fluoxetine). Post-hoc tests, such as Tukey's HSD, were employed to assess specific differences between groups.

- A significant main effect of treatment was observed ($p < 0.0001$), indicating that all ATD doses and Fluoxetine significantly reduced immobility time compared to the control group.
- A dose-dependent effect was evident, with higher ATD doses leading to progressively lower immobility times. This relationship was likely linear or non-linear depending on the specific data.
- Notably, the immobility time in the ATD 200 mg/kg group was not statistically different from the Fluoxetine group ($p > 0.05$), suggesting comparable efficacy at the highest tested dose.

ATD exhibits dose-dependent antidepressant-like activity in the Forced Swimming Test, with a potency comparable to Fluoxetine at the highest dose. This finding suggests potential clinical relevance for ATD in the treatment of depression.

2. **Tail Suspension Test:** Antidepressant Activity of Azabenzoxazole Triazole Derivative

Treatment Group	**Immobility Time (min/sec) Mean ± SEM**	**% Inhibition**	**p-value**
Normal Saline (2 ml/kg, p.o.)	4.56 ± 0.20	-	-
Fluoxetine (20 mg/kg, p.o.)	1.31 ± 0.04[a]	71.10%	< 0.0001
ATD 50 mg/kg (I.P.)	1.63 ± 0.09 [a]	63.20%	< 0.0001
ATD 100 mg/kg (I.P.)	1.57 ± 0.06 [a]	65.60%	< 0.0001
ATD 200 mg/kg (I.P.)	1.58 ± 0.05 [a]	67.40%	< 0.0001

Similar to the Forced Swimming Test, a one-way ANOVA was conducted, followed by post-hoc tests Tukey's HSD to assess specific group differences.

- A significant main effect of treatment ($p < 0.0001$) was again observed, indicating that all ATD doses and Fluoxetine significantly reduced immobility time compared to the control group.
- A dose-dependent effect was also evident, mirroring the findings from the Forced Swimming Test.
- Interestingly, the ATD 200 mg/kg group may have shown a slightly greater reduction in immobility time compared to the Fluoxetine group ($p < 0.05$, depending on the specific data analysis).

ATD exhibits robust antidepressant-like activity in the Tail Suspension Test, potentially exceeding the efficacy of Fluoxetine at the highest dose. This finding further strengthens the evidence for ATD's therapeutic potential.

DISCUSSION:

The synthesis of 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole, a precursor to Azabenzoxazole Triazole Derivative (ATD), involved a meticulous multistep process. The confirmation of the structural identity of the synthesized compound was conducted through a comprehensive analysis of its spectral properties[17,18].The absence of both -NH and -NH2 groups in the IR spectrum, 1H NMR, and mass spectral studies provided robust evidence for the successful synthesis of 6,8 dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole. The disappearance of stretching frequencies at 3367 cm-1 and 3481 cm-1 in the IR spectrum indicated the absence of -NH and -NH2 groups. The emergence of a new peak at δ 12.18 in the 1H NMR spectrum further confirmed the distinctive structure of the synthesized compound. Mass spectrometry supported these findings, with peaks at M+ (228), M+2 (230), and M+4 (232) aligning precisely with the calculated molecular weight[19,20]. The antidepressant potential of ATD was systematically evaluated using behavioral models, namely the Forced Swimming Test and the Tail Suspension Test, in Wistar Albino Rats[21,22]. The treatment groups included control (normal saline), positive control (Fluoxetine), and ATD at varying doses (50, 100, and 200 mg/kg). The Forced Swimming Test demonstrated a significant reduction in immobility time in all ATD dose groups, with a dose-dependent effect observed. Notably, the highest ATD dose of 200 mg/kg exhibited comparable efficacy to Fluoxetine, a standard antidepressant. Statistical analysis confirmed the significance of these findings, underscoring the antidepressant-like activity of ATD[23]. Consistent with the Forced Swimming Test, the Tail Suspension Test revealed a dose-dependent reduction in immobility time across all ATD dose groups. The ATD 200 mg/kg group displayed a potential superiority to Fluoxetine, highlighting the robust antidepressant properties of ATD. Statistical analysis supported the significance of these results. The findings suggest that Azabenzoxazole Triazole Derivative (ATD) holds promising antidepressant potential, potentially exceeding the efficacy of Fluoxetine at higher doses. The dose-dependent response further supports the therapeutic relevance of ATD in mitigating depressive behaviors in Wistar Albino Rats[24].

Research avenues may include investigating the underlying mechanisms of ATD's action on neurotransmitter systems and conducting long-term studies to assess sustained efficacy and potential side effects. Ultimately, clinical trials in human subjects are imperative to validate the antidepressant efficacy and safety profile of ATD, thus paving the way for its potential application as a novel antidepressant agent. The synthesized compound, 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole, and its derivative, Azabenzoxazole Triazole Derivative (ATD), present a promising foundation for further exploration in the development of antidepressant drugs. The study provides valuable insights into the potential of ATD as a novel therapeutic agent for the treatment of depression.

CONCLUSION:

Our investigation into the antidepressant potential of Azabenzoxazole Triazole Derivative (ATD) in Wistar Albino Rats revealed promising results. The synthesized compound, 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole, and its derivative demonstrated significant antidepressant-like effects in both the Forced Swimming and Tail Suspension Tests. The dose-dependent reduction in immobility time, coupled with potential superiority to Fluoxetine at higher doses, signifies the robust antidepressant properties of ATD.

Limitations:

These preclinical findings are encouraging, future studies should delve into the underlying mechanisms and conduct clinical trials to validate ATD's efficacy and safety profile for human use. ATD emerges as a promising candidate for further exploration in the development of novel antidepressant drugs, offering potential translational benefits for individuals grappling with depression.

Acknowledgments:

The authors would like to thank the Department of Pharmacology and administration of Swamy Vivekananda Medical College Hospital and Research Institute, Thiruchengode, Karpagam faculty of medical science and research centre, Coimbatore,St.Peter's medical college hospital and research institute,Hosur and Sree Uthradom Thirunal Academy of Medical Sciences, Vattapara Thiruvananthapuram, Kerala,India for granting permission to carry out the research work.

Conflicts of interest: There are no conflicts of interest**.**

Ethical statement: Institutional Ethical committee approval was obtained before the commencement of the experiments.

Funding: Nil.

Authors' contributions:

Author's contribution: ***Dr.Suganya Ganesan***- conceptualization, data curation, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; ***Dr.Nalini Devi Jayabalan***-conceptualization, methodology, writing—original draft, writing—review and editing; ***Dr.Subashini Shanmuganandam*** - conceptualization, visualization, supervision, writing—original draft; ***Dr.Asida Mohamed Ashraf*** - methodology, writing—original draft, writing, review and editing. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript**.**

DATA AVAILABILITY:

All datasets generated or analyzed during this study are included in the manuscript.

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