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**Design, Synthesis and In-Vivo Anticonvulsant Evaluation Of 5-[(E)-
 (3,4,5-Trimethoxybenzylidene) Amino]-1,3,4-Thiadiazole-2-Thiol**

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ABSTRACT

5-[(E)-(3,4,5-trimethoxybenzylidene)amino]-1,3,4-thiadiazole-2-thiol was designed based on ADME prediction and a proposed pharmacophore model, and synthesized by condensation reaction. The structure of the compound was established by means of FTIR, UV-Visible, ¹H and ¹³C nuclear magnetic resonance spectroscopy, mass spectroscopy and elemental analyses. The compound was evaluated for its anticonvulsant activity using maximal electroshock (MES) and subcutaneous pentylene tetrazole (PTZ) test models and its acute toxicity and neurotoxicity studies were determined. The compound showed no sign of neurotoxicity, and it exhibits significant protection, showing potent activity in both test models. The present findings suggest that 5-[(E)-(3,4,5-trimethoxybenzylidene)amino]-1,3,4-thiadiazole-2-thiol possesses anticonvulsant activity and is a candidate for further optimization and evaluation. Furthermore, the results evidenced the significance of Schiff bases in disubstituted 1,3,4-thiadiazole compounds by improving lipophylicity and thus, showing better anticonvulsant activity.

Keywords: Anticonvulsant, 1,3,4-thiadiazole-2-thiol, lipophylicity.

1.0 INTRODUCTION

1.1 Epilepsy

Epilepsy is a collective term that includes over 40 different types of human seizure disorders, and 50 million people worldwide are affected with this neurological disorder (Raj, 2014). Untreated epilepsy can lead to impaired intellectual function or death and is typically accompanied with psychosocial prejudices and other psycho pathological consequences such as loss of self-esteem and poor quality of life (Shindikar *et al.*, 2006). The typical behavioural manifestation common to all forms of epilepsy is the aberrant synchronized discharge of a neuronal population termed the 'seizures' (Errington *et al.*, 2005).

1.2 Anticonvulsant Drugs

Anticonvulsant drugs are employed in the management of epilepsy. They exert their actions by different mechanisms which include enhancement of the GABAergic neurotransmission, and effects on neuronal voltage gated sodium and/or calcium channels (Pasquale & Salvatore, 2009). However over 30% of people with epilepsy do not have seizure control even with the best available medications,

while others do so only at the expense of significant dose related toxicity and peculiar adverse effects (Tripathi *et al.*, 2012). Thus, new concepts and original ideas for developing antiepileptic drugs are urgently needed which was the reason to focus on the search for new targets. Overall, about a third of cases of epilepsy are considered symptomatic, the rest being idiopathic or cryptogenic. Starting from phenobarbital and phenytoin almost 100 years ago, much research has been devoted to the development of new, more active drugs to treat such a broad category of symptom complexes (Krasowski, 2010).

1.3 Literature Review

During the recent years, there has been intense investigation of different classes of thiadiazole compounds, many of which were found to be pharmacologically active (Chapleo *et al.*, 1986). Several disubstituted 1,3,4-thiadiazole compounds were investigated for anticonvulsant activity and did not exhibit good results, as well as having poor lipophylicity (Bahar and Yusuf, 2010). Schiff bases have gained importance in medicinal and pharmaceutical fields due to a

broad spectrum of biological activities including anticonvulsant (Chaubey & Pandeya, 2012). Hybrid design is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates (Claudio *et al.*, 2007).

1.4 Hypothesis

In this research, Schiff base of aromatic aldehyde imine derivative of 5-Amino-1,3,4-thiadiazole-2-thiol was designed via hybrid approach, synthesized and evaluated for anticonvulsant activity. The formation of Schiff base is expected to increase the lipophylicity and exhibit better results.

2.0 MATERIALS AND METHODS

2.1 Material

Gallenkamp melting point apparatus, reflux machine, mass spectrophotometer, UV absorption spectrophotometer, 500MHz NMR spectrometer, Shimadzu FITR spectrometer, Ugo Basile electroconvulsive unit (ECT800) fitted with ear clip electrodes, Analytical balance (Mettler AE-240), reflux condenser, thin layer chromatographic (aluminium) plates pre-coated with 0.2mm silica gel (Merck) and glass wares.

2.2 Chemicals

5-Amino-1,3,4-thiadiazol-2-thiol, 3,4,5-trimethoxy benzaldehyde, ethanol and glacial acetic acid were purchased from Sigma Aldrich

and were of analytical grades. Therefore, they were used without further purification.

2.3 Animals

72 swiss albino (male) mice in the weight range 16-24g and day-old chicks (80) were used. The animals were inbred in the animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

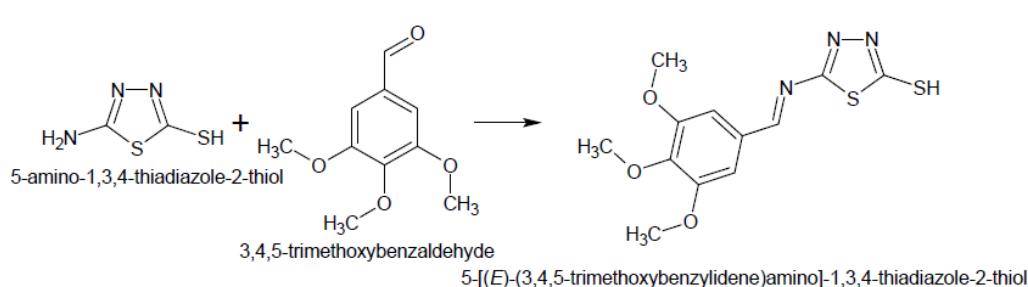
The animals were kept under standard laboratory conditions (temperature $25\pm30^{\circ}\text{C}$; humidity 45 – 60% - and 12-hours light/dark cycle) and were allowed free access to both food (Vital feeds) and water except when they were removed from their cages for the experiments.

2.4 Computational Methods

ACD/ChemSketch version 12.1 software downloaded from www.acdlabs.com was used for drawing three-dimensional optimized chemical structure of the proposed compound. Then, the bond lengths between the structural features of anticonvulsant pharmacophore model were measured. The anticonvulsant structural features include; distal ring, donor group and hydrogen bonding domain.

2.5 Synthesis

The derivative of 5-Amino-1,3,4-thiadiazol-2-thiol was synthesized via condensation reaction using ethanol and glacial acetic acid as the catalyst. The chemical equation for the synthesis is as shown in scheme 1. Thin layer chromatography (TLC) with silica gel was used to monitor the progress of the reaction.



Scheme 1. Synthesis of 5-[(E)-(3,4,5-trimethoxybenzylidene)amino]-1,3,4-thiadiazole-2-thiol.

2.6 Preparation of 5-[(E)-(3,4,5-trimethoxybenzylidene)amino]-1,3,4-thiadiazole-2-thiol

5-Amino-1,3,4-thiadiazol-2-thiol (0.015mol) and 3,4,5-trimethoxy benzaldehyde (0.015mol) were

heated under reflux for 4hrs. The reaction was concentrated and kept overnight below 20°C for crystallisation, the solid that separated was filtered under vacuum and recrystallised using ethanol.

2.7 Characterization

Detailed structural analysis of the synthesized compound was performed using Fourier Transformed Infrared Spectroscopy (FTIR), UV-Visible spectroscopy, melting point determination apparatus, ¹H and ¹³C Nuclear Magnetic Resonance (NMR) and mass spectroscopy experiments.

2.8 Determination of Acute Toxicity in Mice

Mean Lethal doses (LD₅₀) of the compound was estimated using the method of Lorke (1983). Briefly in the first phase of the experiment, 9 mice were divided into 3 groups and treated with 10, 100, and 1000 mg/kg body weight (i.p) of the synthesized compound. The mice were observed for 24 hours for signs and symptoms of toxicity and death. In the second phase, three groups consisting of one animal were treated with 1600, 2900 and 5000 mg/kg body weight (i.p) of the synthesized compound based on the result of the first phase. The LD₅₀ was calculated as the square root of the product of the lowest lethal dose (for which the animal died) and the highest non-lethal dose for which the animal survived by all the animals in the group. The median lethal doses obtained were used as guide for the selection of dose for the pharmacological tests.

2.9 Neurotoxicity Studies in Mice

Beam walking neurotoxicity test was carried out as described by Stanley (2005) by training 30 Swiss albino mice severally (about 6 times) to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports to a metal box at the other end until a memory of the path has been established in the mice.

The mice were grouped into 5 sub-groups each containing 6 mice, group I was administered i.p diazepam 1.5 mg/kg as positive control and group II was administered distilled water 10 ml/kg i.p as negative control. Groups III-V were administered different doses of 400, 200 and 100 mg/kg i.p of the synthesized compound and placed to walk on a narrow beam 30 minutes after the drug was administered. The mice were carefully observed for three basic parameters namely; time taken to complete the task, number of foot slips and number of falls for a period of 30 minutes.

The time taken to complete the task and foot slips predict motor coordination deficit with the number of foot slips having the potential to predict clinical sedation and is being used to evaluate for motor coordination deficit. The fall from the beam is an index of neurotoxicity (Stanley, 2005).

2.10 In-vivo Anticonvulsant Evaluation

2.10.1 Maximal Electroshock Induced Seizure Test in Chicks

The method of Swinyard (1985) was employed. Fifty cockerels were divided into five groups of ten cockerels each. Group I (negative control) was given 10 ml kg⁻¹ body weight of distilled water (i.p). Group II (positive control) was given 20 mg/kg body weight of phenytoin. Groups III-V received the synthesized compound (100, 200 and 400 mg/kg body weight).

Thirty minutes post treatment, maximum electroshock was delivered to induce seizure in all the groups using Ugo basile electroconvulsive machine. Corneal electrodes were placed in the upper eyelids of the cockerels. The current, shock duration, frequency, and pulse width were set and maintained at 80 mA, 0.6 sec, 100 pulses per second (100 Hz), and 0.6 ms, respectively. Hind limb tonic extension (HLTE) was selected as the end point of the experiment and the abolition of the HLTE was considered as protection from electroshock induced convulsion. The recovery time was determined in unprotected animals. (Swinyard *et al.*, 1985).

2.10.2 Pentylenetetrazole (scPTZ) Induced Seizure Test in Mice

The method of Swinyard *et al.* (1989) was employed. Thirty mice were divided into five groups of six mice each. Group I (negative control) received 10 mg/kg body weight of distilled water intraperitoneally (i.p). Group II (positive control) received sodium valproate 200 mgkg⁻¹ body weight (i.p). Group III, IV, and V received 100, 200, and 400 mgkg⁻¹ of the synthesized compound respectively. Thirty minutes post treatment; all the groups (I-V) were administered 85 mgkg⁻¹ (CD97) freshly prepared PTZ subcutaneously. All the mice were observed for a period of 30 minutes post PTZ administration. An episode of clonic spasm of at least 5 seconds duration with loss of righting reflex was considered an index of seizure (Swinyard *et al.* 1989). The percentages of

protection, onset of convulsion and time of death if any, were observed and recorded.

2.11 Statistical Analysis

The mean onset of seizure, mean time of death, and mean recovery time were presented as mean \pm standard error of mean (SEM). The mean values of the control groups were compared with

the mean values of the groups treated with the test compound using analysis of variance (ANOVA) followed by post hoc dunnet t-test for multiple comparison. The differences were considered significant at $p < 0.05$.

3.0 RESULTS AND DISCUSSION

3.1. Computational Method

3.1.1 Bond Length

The distance between the distal ring and the hydrogen bonding domain for the compound is 4.559 Å. The following figure illustrates the 3D optimized structure of the compound using chemsketch:

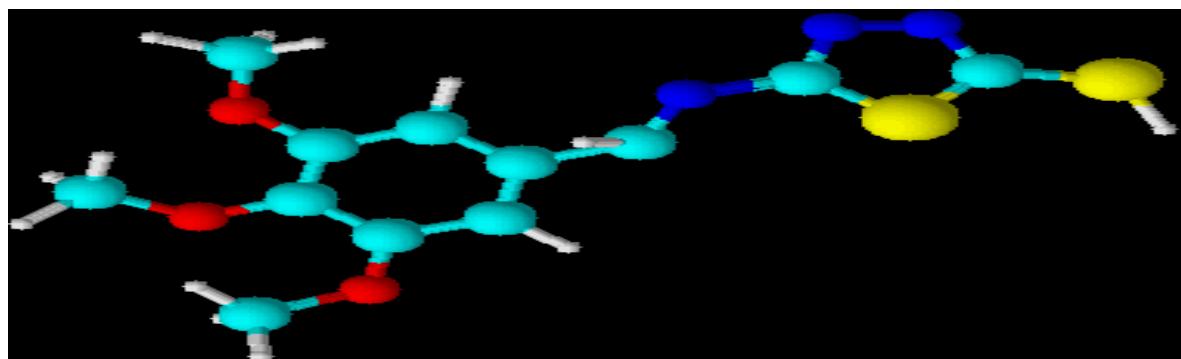


Fig 3.1 3D optimized structure of the synthesized compound

3.1.2 ADME Prediction

Table 3.1 ADME Prediction for the Compound

S/No.	Parameter	Synthesized Compound
1	Lipohylicity (Consensus Log P)	2.59
2	Water solubility	3.04 e-02 mg/ml; 9.77 e-05 mol/l
3	GI absorption	High
4	Lipinski rule of 5	Yes (0 violation)
5	Bioavailability score	0.55
6	Pains	0 alert
7	Leadlikeness	Yes
8	Synthetic accessibility	3.07

3.1.3 Microanalysis of the Compound

Table 3.2 Result from the Microanalysis of the Compound

E C	Microanalysis (%)					M W From MS	Formular
	C	H	N	O	S		
C, H, N, O, S	46.28	4.21	13.49	15.41	20.59	312.05 g/mol	C ₁₂ H ₁₃ N ₃ O ₃ S ₂

E C= Elemental Composition, M W=Molecular weight, M S=Mass Spec

3.1.4 Physicochemical Properties of the Compound

Table 3.1.4 Physicochemical Properties of the Compound

Physical appearance	Colour	Melting point	Yield (%)
Spongy	Lime	236 - 238 °C	22.83

3.1.5 FTIR

Table 3.4 Analysis of the functional group regions on the FTIR Spectrum of the Compound

S/NO	Vibration Frequencies (cm ⁻¹)	Functional Group
1	995.30	Ar OCH ₃ (methoxy) stretch
2	1519.96	Ar C-C stretch
3	1589.40	Ar C=C stretch
4	1681.98	C=N (imine) stretch
5	2592.41	S-H (thiol) stretch
6	3078.49	Ar C-H stretch

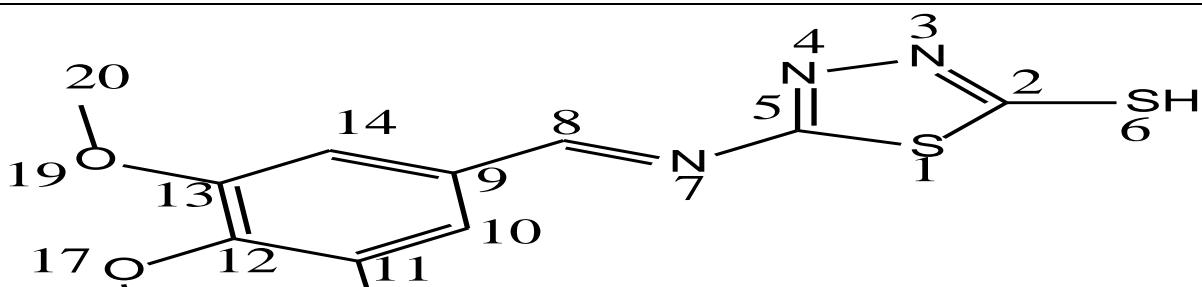
3.1.6 UV-Visible

The UV-Visible spectrum of the compound exhibited maximum absorbance at 407.20nm.

3.1.7 ¹H and ¹³C NMR

Table 3.5 ¹H and ¹³C NMR results of the compound

Position	¹ H NMR	¹³ C NMR
2		181.0
5		164.0
6	14.52	
8	8.64	162.0
9		129.5
10	7.45	107.0
11		153.0
12		142.5
13		153.0
14	7.40	107.0
16	3.90	56.0
18	3.85	60.5
20	3.90	56.0



3.1.8 Acute Toxicity Study (LD₅₀)
The LD₅₀ was calculated as 2807.89 (mg/kg).

3.1.9 Neurotoxicity Study in Mice

Table 3.6 Neurotoxicity Study in Mice

Treatment (mg/kg)	Mean Time Taken To Complete The Task (secs)	Mean Number of Footslips	Mean Number of Falls
Distilled Water	38.58 ± 5.67	6.96 ± 0.79	0.5 ± 0.34
Synthesized Compound 100	14.94 ± 3.52	2.50 ± 0.85	0.00

200	21.10 ± 6.63	4.82 ± 0.83	0.17 ± 0.17
400	11.01 ± 2.55	3.32 ± 1.14	0.00
Diazepam			
1.5	46.36 ± 6.50	9.44 ± 0.56	1.5 ± 0.56

Shows significant difference at p<0.05, n=6, Data presented as Mean ± SEM

3.1.10 In-vivo Anticonvulsant Evaluation

3.1.10.1 Maximal Electroshock Seizure Test in Chicks

Table 3.7 Maximal Electroshock Seizure Test in Chicks

Treatment (mg/kg)	Mean Time of Recovery (Mins)	Quantal Protection	Percentage Protection
Distilled Water			
10	6.77 ± 1.37	0/10	0.00 %
Synthesized Compound			
100	6.50 ± 1.13	8/10	80 %
200	5.73 ± 0.88	6/10	60 %
400	6.33 ± 0.96	4/10	40 %
Phenytoin			
20	4.12 ± 0.90	10/10	100 %

Shows significant difference at p<0.05, n=6, Data presented as Mean ± SEM

3.1.10.2 Subcutaneous Pentylenetetrazol Induced Seizures in Mice

Table 3.8 Subcutaneous Pentylenetetrazol Induced Seizures Mice

Treatment (mg/kg)	Mean Onset of Seizure (mins)	Quantal Protection	Percentage Protection
Distilled Water			
10	5.79 ± 1.66	0/6	0.00 %
Synthesized Compound			
100	12.96 ± 6.5	4/6	66.67 %
200	9.11 ± 3.84	3/6	50 %
400	15.93 ± 1.97	2/6	33.33 %

Sodium Valproate
200

6/6

100 %

Shows significant difference at $p<0.05$, $n=6$, Data presented as Mean \pm SEM

4.0 DISCUSSION

Shindikar (2006) proposed a two-point pharmacophore, consisting of an aromatic centre separated from an H-bonding acceptor/donor group within a distance range of 4.18-6.88 Å, which agrees with Unverferth's proposal. Computational study of the optimised three-dimensional structure of the synthesized compound in Figure 3.1 showed the distance relationship of the features postulated as essential for the anticonvulsant activity, as also proposed by Unverferth *et al.* (1998) showed that the synthesized compound meets the requirement of the anti-MES pharmacophore.

The synthesis carried out was achieved via the formation of schiff base, and the compound has a poor yield of 22.83%. The low yield is due to the physical texture of the compound; it is spongy and very light. The FTIR spectra obtained revealed the vibration frequencies; 3078.49 (Ar C-H stretch), 2592.41 (S-H stretch), 1589.40 (Ar C=C stretch), 1681.98 (C=N stretch imine), 1519.96 (Ar C-C stretch). This result agrees with the work of Bahar and Yusuf (2010) and Thrilochana, (2014). The UV-Visible spectra showed absorption maxima at 407.20nm. The absorption maximum for the compound falls under the visible region. Also, the high and broad absorption peak was due to the extended conjugation of the compound. The ¹H NMR spectrum obtained revealed signals for compound; 7.40-7.45 (2H, aromatic benzene), 8.64 (1H, imine), 3.85-3.90 (9H, Aromatic OCH₃) and 14.52 (1H, thiol). The ¹³C NMR spectrum obtained revealed the peaks for twelve carbon atoms; 181.0, 164.0, 162.0, 129.5, 107.0, 153.0, 142.5, 153.0, 107.0, 56.0, 60.5 and 56.0. The molecular weight of the compound was calculated as 311.41g/mol, and the mass spec showed 312.05 g/mol. Therefore, the calculated value agrees with the mass spec result of the compound.

The LD₅₀ was calculated for the compound as 3807.87mg/kg. This falls within the range of drugs classified as slightly toxic as all the LD₅₀ values fall between 500-5000 mg/kg (Hollinger, 2003). The neurotoxicity test showed the

synthesized compound to be neurologically non-toxic by having a shorter time to complete the task and fewer foot slips as similar with distilled water, thus making it safe for use which agrees with the results obtained in the work of Bahar & Yusuf (2010). In the in-vivo anticonvulsant screening using MES method, the synthesized compound showed 66.67% quantal protection against occurrence of seizures at 100mg/kg. While in the PTZ method, the synthesized compound showed 80% quantal protection against occurrence of seizures at 100mg/kg, and activity increases as the dose decreases.

4.0 CONCLUSION

The compound was synthesized in a yield of about 22.83% via condensation reaction, and tested for anticonvulsant activity. The compound fits to the Shindikar's postulated pharmacophore model, and exhibits protection at both the test models. It is thus, concluded that the synthesized compound was proven to be active for both MES and PTZ test methods and has a mixed molecular target; Voltage-gated ion channels and GABA inhibition. To the best of our knowledge, the synthesized compound is new, we found no literature on it. Therefore, we recommend further biological activities to be carried out on the compound.

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