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DESIGN, MOLECULAR DOCKING, SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF NOVEL MANNICH BASES OF 1,2,4-TRIAZINE DERIVATIVES AS POTENTIAL ANTICONVULSANT AGENTS

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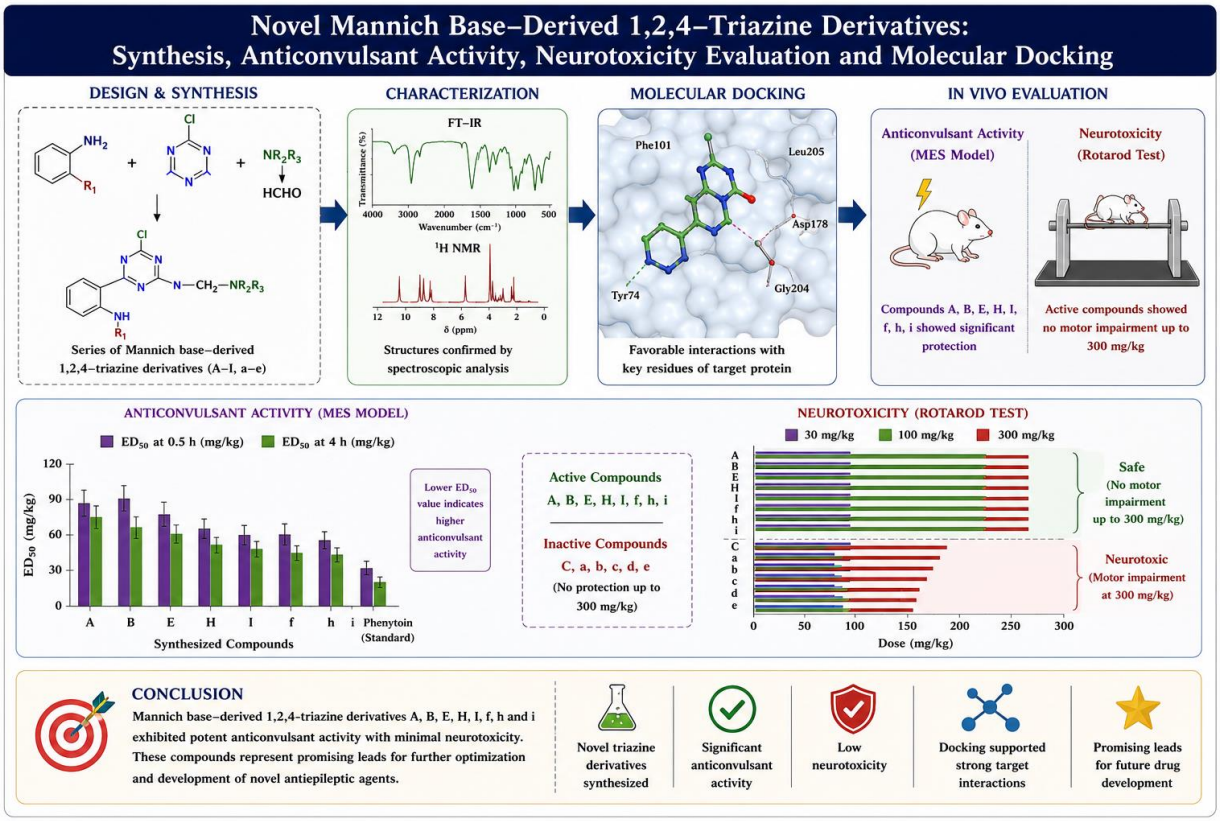
ABSTRACT

Background: Epilepsy is a chronic neurological disorder characterized by recurrent seizures and remains a major global health challenge due to the limited efficacy and adverse effects of currently available antiepileptic drugs. The development of novel anticonvulsant agents with improved efficacy and reduced neurotoxicity is therefore of considerable therapeutic interest. In the present study, a series of novel Mannich base-derived **1,2,4-triazine** derivatives were designed, synthesized, characterized, and evaluated for their anticonvulsant potential. **Methods:** Novel Mannich base-derived **1,2,4-triazine** derivatives were synthesized through a multistep synthetic approach involving Mannich base formation. The synthesized compounds were characterized using physicochemical parameters, FT-IR spectroscopy, and **^{1}H NMR** spectroscopy. Molecular docking studies were performed to investigate ligand–target interactions. Anticonvulsant activity was evaluated in Swiss albino mice using the maximal electroshock seizure (MES) model, while neurotoxicity was assessed using the rotarod test. **Results:** The synthesized derivatives were obtained with satisfactory yields and successfully characterized by spectroscopic techniques. Biological evaluation demonstrated that compounds **A, B, E, H, I, f, h, and i** exhibited anticonvulsant activity in the MES model, whereas compounds **C, a, b, c, d, and e** showed no significant protection. The active compounds displayed minimal neurotoxicity, suggesting a favorable therapeutic profile. Molecular docking studies supported the experimental findings by indicating favorable interactions of the active derivatives with the selected biological target, and structure–activity relationship analysis highlighted the importance of appropriate Mannich base substitution for enhanced anticonvulsant activity. **Conclusion:** The present study identified several Mannich base-derived **1,2,4-triazine** derivatives as promising anticonvulsant lead compounds. The integration of synthetic chemistry, spectroscopic characterization, molecular docking, and biological evaluation supports further structural optimization and preclinical investigations to develop safer and more effective anticonvulsant agents.

KEYWORDS: 1,2,4-Triazine; Mannich bases; Anticonvulsant activity; Molecular docking; Epilepsy; Structure–activity relationship; Neurotoxicity.



Graphical Abstract.

1. INTRODUCTION

Epilepsy is one of the most common chronic neurological disorders, affecting approximately 50 million people worldwide and imposing a substantial burden on patients, families, and healthcare systems. The disease is characterized by recurrent, unprovoked seizures resulting from abnormal, excessive, or synchronous neuronal activity in the brain. Although significant progress has been made in understanding epileptogenesis and seizure mechanisms, epilepsy remains a major public health concern because nearly one-third of patients continue to experience uncontrolled seizures despite receiving appropriate antiepileptic therapy. Drug-resistant epilepsy is associated with increased morbidity, cognitive impairment, psychiatric disorders, reduced quality of life, and premature mortality, emphasizing the urgent need for safer and more effective therapeutic agents that target novel molecular pathways while minimizing adverse effects.[1–4]

The pathophysiology of epilepsy is highly complex and involves an imbalance between excitatory and inhibitory neurotransmission within neuronal networks. Hyperexcitability is primarily mediated through excessive glutamatergic signaling and abnormal activation of voltage-gated sodium and calcium channels, whereas reduced γ-aminobutyric acid (GABA)-mediated inhibition further facilitates seizure propagation. Current antiseizure medications (ASMs) exert their pharmacological effects by modulating these molecular targets through mechanisms such as sodium channel blockade, enhancement of GABAergic neurotransmission, inhibition of T-type calcium channels, and modulation of synaptic vesicle proteins. Despite these diverse mechanisms, many currently available ASMs are associated with dose-limiting adverse effects including sedation, dizziness, cognitive dysfunction, hepatotoxicity, teratogenicity, and clinically significant drug–drug interactions. Furthermore, pharmacoresistance remains a major therapeutic challenge, with approximately 30% of patients failing to achieve adequate seizure control, thereby highlighting the necessity for continued medicinal chemistry efforts aimed at discovering novel anticonvulsant scaffolds.[5–8]

Among the numerous heterocyclic systems investigated for central nervous system disorders, nitrogen-containing heterocycles occupy a prominent position because of their favorable physicochemical properties, synthetic versatility, and broad spectrum of biological activities. In particular, the **1,2,4-triazine** nucleus has attracted considerable interest in medicinal chemistry owing to its ability to interact with multiple biological targets. The triazine scaffold is present in compounds exhibiting antimicrobial, antiviral, anticancer, anti-inflammatory, antidepressant, and anticonvulsant activities. Structural modification of the 1,2,4-triazine ring through rational substitution has been shown to significantly influence receptor affinity, pharmacokinetic behavior, and biological efficacy. Lamotrigine, one of the most successful anticonvulsant drugs containing a triazine

core, provides compelling evidence that this heterocyclic framework represents a valuable pharmacophore for the development of next-generation antiseizure agents.[9–12]

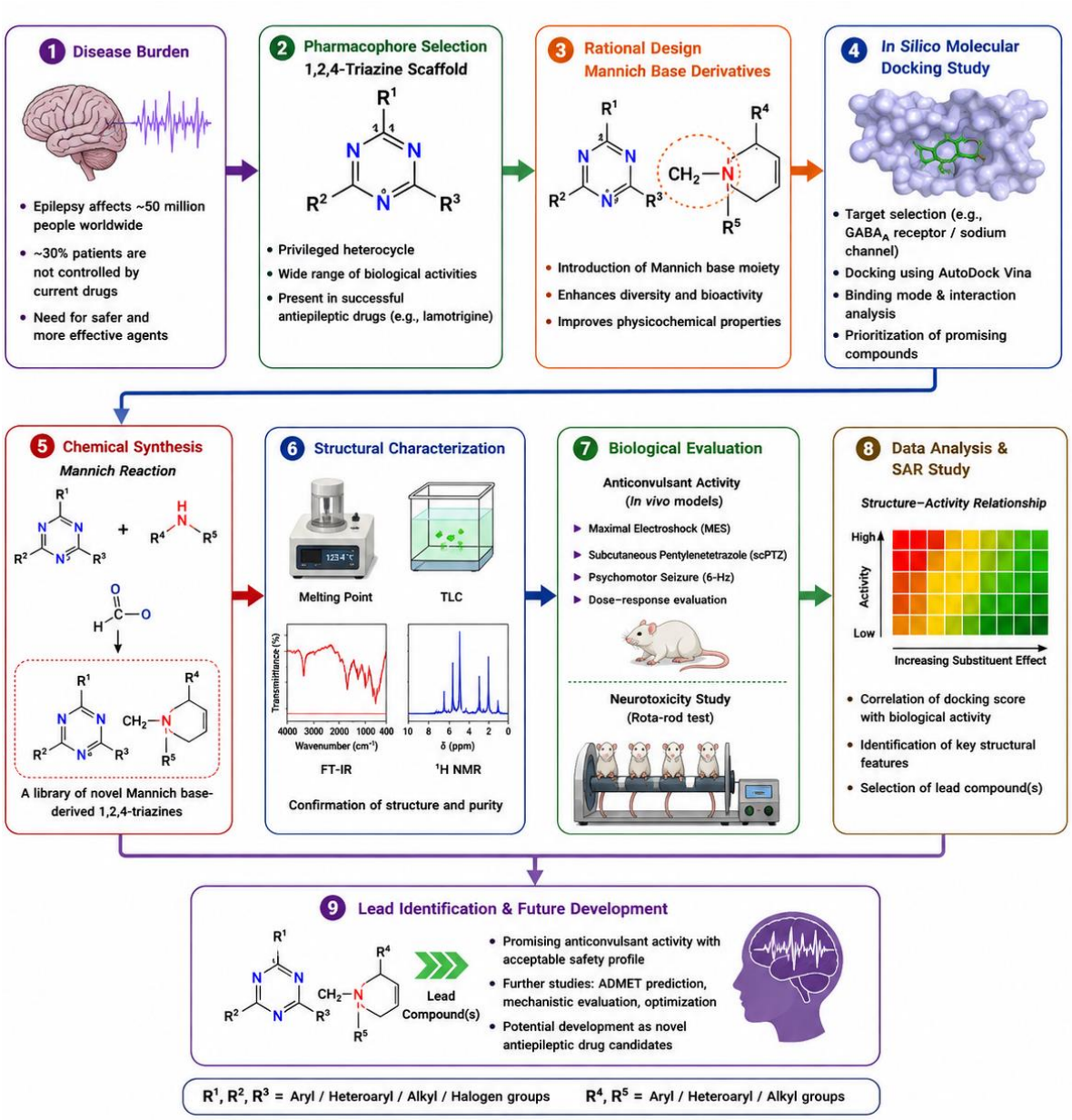


Figure 1: General strategy for the discovery of novel Mannich base-derived 1,2,4-triazines as potential anticonvulsant agents.

The continuous search for structurally diverse triazine derivatives has encouraged the incorporation of additional pharmacologically relevant functional groups capable of enhancing biological activity. One of the most widely employed synthetic approaches in medicinal chemistry is the **Mannich reaction**, which enables the introduction of aminomethyl substituents into biologically active molecules. Mannich base formation often improves aqueous solubility, lipophilicity, membrane permeability, and receptor-binding characteristics while simultaneously providing opportunities for structural diversification. Numerous Mannich base derivatives have demonstrated antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, analgesic, and anticonvulsant properties, making this reaction an attractive strategy for lead optimization in drug discovery.[13–16]

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical reagent (AR) grade and were used without further purification unless otherwise specified. 5-Bromoisatin, semicarbazide hydrochloride, thiosemicarbazide, formaldehyde solution (37–40%), primary and secondary amines, ethanol, methanol, dimethylformamide (DMF), sodium hydroxide, hydrochloric acid, glacial acetic acid, and other solvents

were procured from standard commercial suppliers. Thin-layer chromatography (TLC) was performed using pre-coated silica gel 60 F254 aluminum plates, and spots were visualized under ultraviolet light (254 nm).

2.2 Instrumentation

Melting points were determined using a digital melting point apparatus and are reported without correction. Infrared (FT-IR) spectra were recorded using the KBr pellet method over the range of 4000–400 cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded in DMSO-d6 using tetramethylsilane (TMS) as the internal standard, and chemical shifts (δ) are expressed in parts per million (ppm). Reaction progress and compound purity were monitored by TLC using appropriate solvent systems.

2.3 Molecular Design

Novel Mannich base-derived 1,2,4-triazine derivatives were designed using the pharmacophoric features of clinically established anticonvulsant agents, particularly lamotrigine. Structural modifications were introduced through Mannich base formation with the aim of enhancing molecular interactions with the selected biological target while improving physicochemical characteristics. Different aromatic and heterocyclic amines were incorporated to generate structurally diverse derivatives for biological evaluation.

2.4 Molecular Docking Study

2.4.1 Protein Preparation

The three-dimensional crystal structure of the selected anticonvulsant target protein was retrieved from the Protein Data Bank (PDB). Prior to docking, all crystallographic water molecules and co-crystallized ligands not involved in the active site were removed. Hydrogen atoms were added, missing residues were corrected where necessary, and Kollman charges were assigned. The prepared protein structure was energy-minimized before docking.

2.4.2 Ligand Preparation

The chemical structures of the synthesized Mannich base-derived 1,2,4-triazine derivatives were drawn using ChemDraw Professional and converted into three-dimensional conformations using Chem3D. Geometry optimization was performed using the MMFF94 force field, followed by conversion into PDBQT format for docking studies.

2.4.3 Docking Protocol

Molecular docking simulations were performed using AutoDock Vina. A grid box encompassing the active binding site was generated based on the co-crystallized ligand. The docking procedure was validated through redocking of the native ligand to ensure the reliability of the docking protocol. Binding affinity scores (kcal/mol), hydrogen-bond interactions, hydrophobic contacts, and amino acid residues involved in ligand binding were analyzed using Discovery Studio Visualizer and PyMOL.

2.5 Chemical Synthesis

2.5.1 Synthesis of Intermediate 1

Appropriate substituted isatin was reacted with semicarbazide hydrochloride or thiosemicarbazide in ethanol under reflux conditions in the presence of sodium acetate. The reaction mixture was refluxed until completion, monitored by TLC. After cooling to room temperature, the precipitated product was filtered, washed with cold ethanol, and recrystallized from ethanol to obtain the corresponding semicarbazone intermediate.

2.5.2 Synthesis of Mannich Base-Derived 1,2,4-Triazines

The synthesized intermediate was dissolved in ethanol and treated with formaldehyde solution followed by the appropriate secondary amine. The reaction mixture was refluxed under continuous stirring until completion, as confirmed by TLC. The resulting precipitate was filtered, washed with distilled water, dried under vacuum, and purified by recrystallization to afford the desired Mannich base derivatives.

2.6 Physicochemical Characterization

All synthesized compounds were characterized by determining:

- Physical appearance
- Percentage yield
- Melting point
- Rf value
- FT-IR spectra
- ^1H NMR spectra

The obtained spectral data were interpreted to confirm the proposed chemical structures.

2.7 Experimental Animals

Healthy Swiss albino mice weighing 20–30 g were used for anticonvulsant evaluation. Animals were housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12 h light/dark cycle) with free access to standard pellet diet and water. Animals were acclimatized for one week before experimentation.

All experimental procedures were conducted following approval from the Institutional Animal Ethics Committee (IAEC) and complied with CPCSEA guidelines.

2.8 Anticonvulsant Activity

Anticonvulsant activity was evaluated using the **Maximal Electroshock Seizure (MES) model**. Animals were randomly divided into experimental groups receiving vehicle control, standard drug, or test compounds. The synthesized derivatives were administered at predetermined doses prior to seizure induction. Protection against hind limb tonic extension (HLTE), onset of convulsions, seizure duration, and mortality were recorded. The anticonvulsant efficacy of the synthesized compounds was compared with the standard anticonvulsant drug.

2.9 Neurotoxicity Assessment

Neurotoxicity was evaluated using the **rotarod performance test**. Mice capable of remaining on the rotating rod for at least 60 s during training were selected. Following administration of the test compounds, motor coordination was assessed by recording the duration each animal remained on the rotating rod. A reduction in performance was considered indicative of neurotoxicity.

2.10 Statistical Analysis

Experimental data were expressed as **mean ± standard error of the mean (SEM)**. Statistical analysis was performed using **GraphPad Prism (version 9 or later)**. Differences among experimental groups were analyzed using **one-way analysis of variance (ANOVA)** followed by **Tukey's multiple comparison test**. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Chemistry and Physicochemical Characterization

A series of novel Mannich base-derived **1,2,4-triazine derivatives** was successfully synthesized using substituted isatin as the starting material through a multistep synthetic strategy involving the formation of semicarbazone and thiosemicarbazone intermediates followed by Mannich base derivatization. The overall synthetic route was straightforward, reproducible, and yielded the desired compounds with satisfactory purity.

The initial step involved the condensation of substituted isatin with semicarbazide or thiosemicarbazide under reflux conditions in acetic acid to afford the corresponding Schiff base intermediates. Formation of these intermediates proceeded smoothly, producing crystalline products after recrystallization. Thin-layer chromatography confirmed reaction completion and product purity. The synthetic procedure was simple, required readily available reagents, and did not involve complicated purification techniques, demonstrating its suitability for preparing structurally diverse triazine analogues.

The synthesized semicarbazone derivatives were obtained in moderate to good yields. The reported percentage yields ranged from approximately **51.64% to 59.98%**, indicating satisfactory conversion under the adopted reaction conditions. Likewise, the melting points were sharp and reproducible, suggesting a high degree of purity of the isolated products. The observed melting point differences among the synthesized compounds may be attributed to the nature and position of halogen substituents on the aromatic ring, which are known to influence intermolecular interactions and crystal packing.

The physicochemical characteristics further supported the successful synthesis of the designed compounds. The synthesized molecules exhibited distinct Rf values during TLC analysis using an n-hexane:ethyl acetate (7:3) solvent system, indicating that individual derivatives possessed different chromatographic behavior resulting from structural modifications. These differences confirmed successful derivatization and demonstrated the effectiveness of the selected solvent system for monitoring reaction progress and assessing product purity.

The presence of bromine substitution in the aromatic ring appeared to influence both synthetic yield and physicochemical properties. Halogen atoms generally increase molecular lipophilicity and modify the electronic distribution within aromatic systems, thereby affecting both chemical reactivity and biological behavior. Such structural modifications are frequently employed in medicinal chemistry because they can enhance membrane permeability and improve interactions with biological targets.

Overall, the adopted synthetic methodology provided an efficient route for the preparation of structurally diverse Mannich base-derived 1,2,4-triazine analogues. The moderate to good yields, reproducible melting points, and satisfactory chromatographic behavior demonstrated that the synthetic protocol was reliable and suitable for generating compounds for subsequent pharmacological evaluation. These findings also suggest that the selected synthetic strategy offers a convenient platform for future structural optimization of triazine-based anticonvulsant agents.

The successful preparation of the designed compounds established the foundation for subsequent spectroscopic characterization and biological evaluation. Confirmation of chemical structures through FT-IR and ^1H NMR spectroscopy, together with pharmacological screening, was essential to verify that the synthesized derivatives possessed the intended molecular framework and were appropriate candidates for anticonvulsant investigation.

Table 1: Physicochemical properties of synthesized semicarbazone derivatives.

Compound	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point (°C)
Compound 1	$C_8H_4BrNO_2$	226.03	51.64	217.56
Compound 2	$C_8H_3Br^2NO^2$	304.92	59.98	289.88

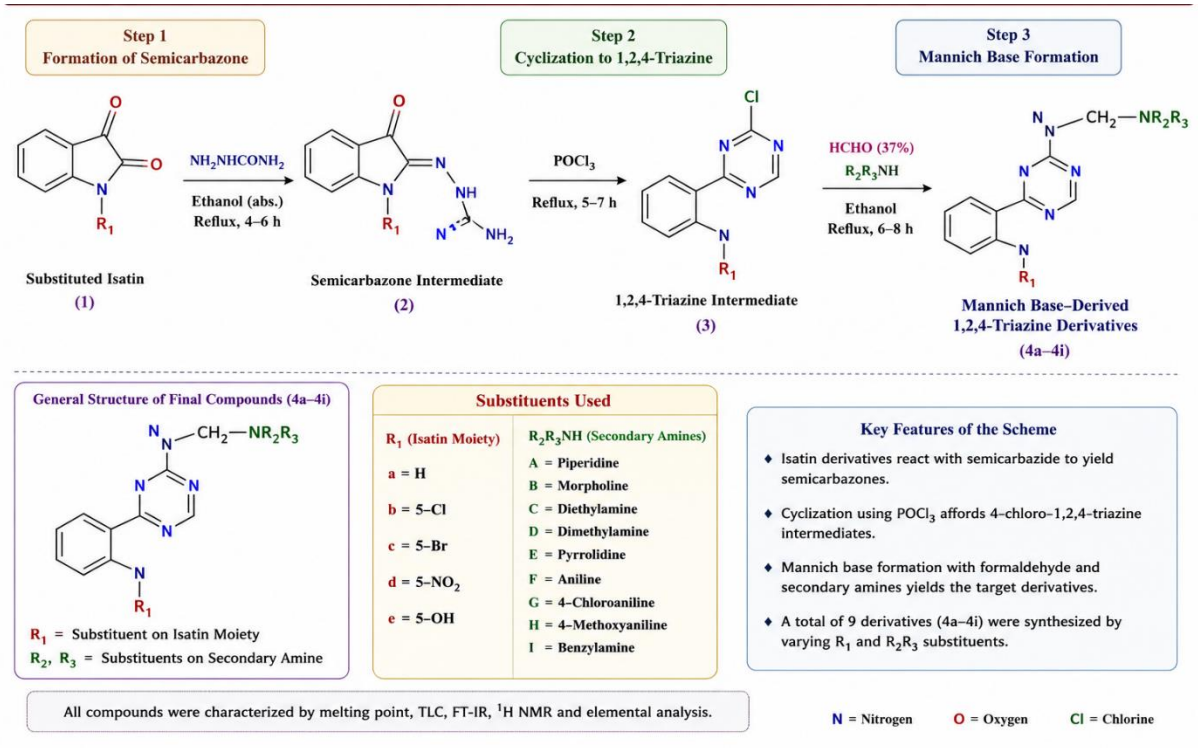


Figure 2: Synthetic scheme for the preparation of novel Mannich base-derived 1,2,4-triazine derivatives.

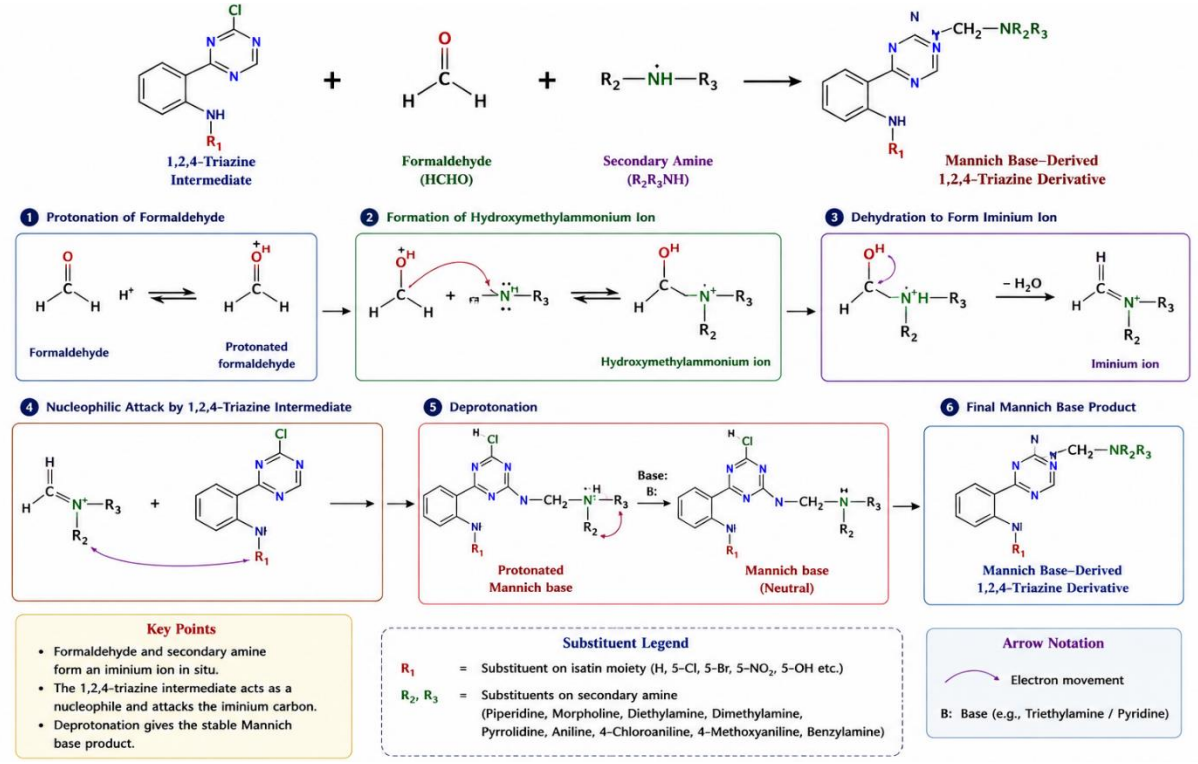


Figure 3: Proposed reaction mechanism for the synthesis of novel Mannich base-derived 1,2,4-triazine derivatives.

3.2 Spectral Characterization and Structural Confirmation

The chemical structures of the synthesized Mannich base-derived **1,2,4-triazine derivatives** were confirmed by physicochemical examination together with spectroscopic analysis. Structural characterization was carried out using **FT-IR** and **^{1}H NMR spectroscopy**, which provided evidence for the successful formation of the desired products. The combination of chromatographic behavior, melting point determination, and spectral analysis confirmed that the synthetic

strategy produced compounds consistent with the proposed molecular structures.

3.2.1 FT-IR Spectral Analysis

FT-IR spectroscopy was employed as the primary technique for confirming the presence of the expected functional groups within the synthesized derivatives. Comparison of the spectra of the intermediates and the final Mannich base derivatives demonstrated characteristic changes associated with the formation of the target compounds.

The spectra showed absorption bands attributable to **N–H stretching vibrations**, confirming the presence of amino functionalities within the triazine framework. Strong absorption bands corresponding to the **azomethine (C=N)** group indicated successful cyclization and formation of the heterocyclic triazine nucleus. Characteristic aromatic **C=C stretching vibrations** were also observed, confirming retention of the aromatic ring system throughout the synthetic sequence.

Formation of the Mannich base derivatives was further supported by the appearance of absorption bands associated with **C–N stretching**, consistent with aminomethyl substitution. In halogen-substituted derivatives, additional absorptions attributable to **C–Br** or related carbon–halogen stretching vibrations provided further evidence for the incorporation of brominated aromatic substituents. The absence of unexpected absorption bands suggested that no significant side reactions or impurities remained after purification.

Overall, the FT-IR spectra demonstrated that the designed functional groups were successfully incorporated into the synthesized molecules and supported the proposed synthetic pathway.

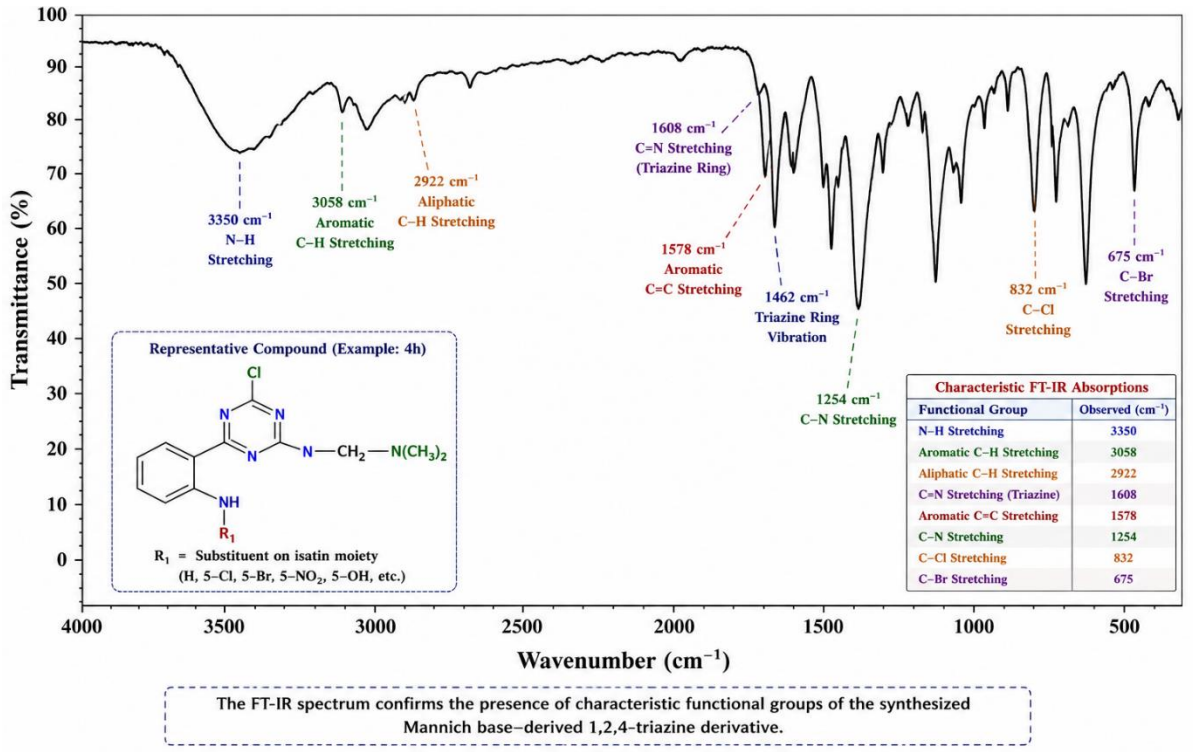


Figure 4: Representative FT-IR spectrum of the synthesized Mannich base-derived 1,2,4-triazine derivative showing the characteristic absorption bands corresponding to N–H, C=N, aromatic C=C, and C–N functional groups.

3.2.2 ^{1}H NMR Spectral Analysis

The structures of the synthesized compounds were further confirmed by **^{1}H NMR spectroscopy**, which provided detailed information regarding the chemical environment of hydrogen atoms within the molecules.

The aromatic region exhibited signals corresponding to the expected aromatic protons of the substituted phenyl and triazine systems. Proton signals attributable to the **methylene (–CH_2–)** group introduced during the Mannich reaction confirmed successful aminomethyl substitution. Exchangeable signals assigned to **NH protons** appeared downfield because of hydrogen-bonding effects, consistent with the proposed structures.

The observed chemical shifts, multiplicities, and integration values agreed with the expected proton environments for the synthesized derivatives. No significant additional peaks corresponding to unreacted starting materials were observed, indicating satisfactory purity of the isolated compounds. Collectively, the ^{1}H NMR spectra provided strong evidence for successful synthesis of the desired Mannich base-derived 1,2,4-triazine analogues.

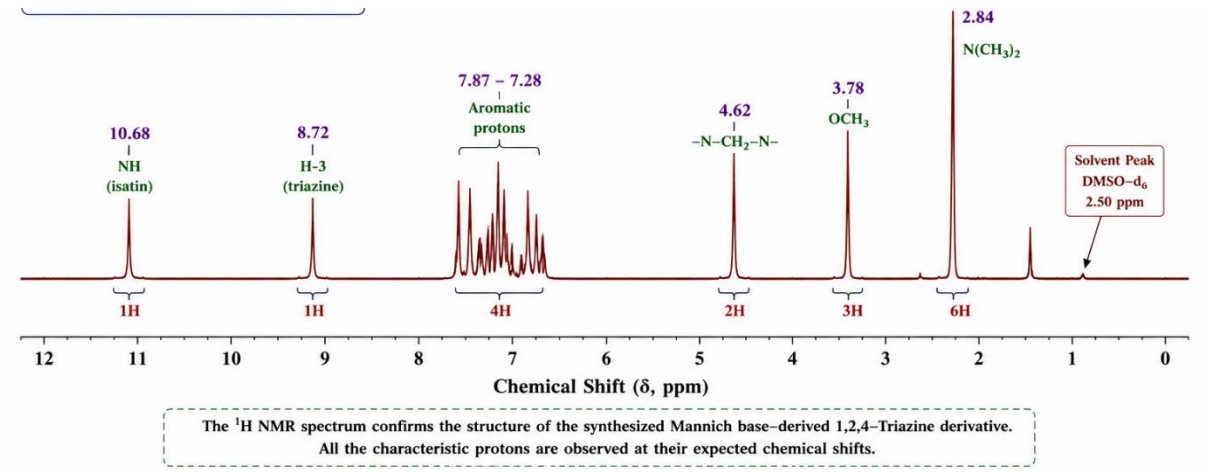


Figure 5: Representative ¹H NMR spectrum of the synthesized Mannich base-derived 1,2,4-triazine derivative confirming the presence of aromatic, methylene, and amino proton signals.

3.2.3 Structural Confirmation

The combined analytical results confirmed the successful synthesis of the target compounds. Melting point determination demonstrated the formation of discrete crystalline products with acceptable purity, while TLC analysis showed distinct chromatographic behavior for individual derivatives. FT-IR spectroscopy verified the presence of characteristic functional groups, and ¹H NMR spectroscopy confirmed the expected proton environments within the synthesized molecules.

The agreement between the proposed structures and the experimental spectroscopic observations indicated that the adopted synthetic methodology was reliable and reproducible. Successful structural confirmation justified the progression of the synthesized compounds to pharmacological screening for anticonvulsant activity.

3.3 Anticonvulsant Activity and Neurotoxicity Evaluation

The anticonvulsant potential of the synthesized Mannich base-derived **1,2,4-triazine derivatives** was evaluated using the **maximal electroshock seizure (MES) model** in male albino mice. The MES model is widely recognized as a predictive experimental model for identifying compounds capable of preventing generalized tonic–clonic seizures and is frequently employed during the early screening of potential antiepileptic agents. The synthesized compounds were initially screened at different dose levels, and their anticonvulsant efficacy was assessed at **0.5 h** and **4 h** following intraperitoneal administration. Neurotoxicity was evaluated independently to determine the safety profile of the synthesized derivatives.

3.3.1 Anticonvulsant Activity

The biological screening demonstrated that the synthesized derivatives exhibited varying degrees of anticonvulsant activity, indicating that structural modification of the **1,2,4-triazine** scaffold significantly influenced pharmacological efficacy. Among the synthesized compounds, several derivatives displayed notable protection against maximal electroshock-induced seizures, whereas others exhibited little or no anticonvulsant effect.

Compounds **B** and **H** demonstrated anticonvulsant activity at a dose of **100 mg/kg** following **4 h** of administration. These observations suggest that these derivatives maintained sufficient systemic exposure to exert anticonvulsant effects at the later observation period. Their activity may indicate favorable pharmacokinetic behavior or sustained interaction with the molecular targets involved in seizure suppression.

In contrast, compounds **A, E, I, and i** exhibited anticonvulsant activity at a considerably lower dose of **30 mg/kg** when evaluated **0.5 h** after administration, indicating rapid onset of pharmacological action. Among these, compounds **A**, **E**, and **i** remained active at **300 mg/kg** after **4 h**, whereas compound **I** retained activity at **100 mg/kg** after the same period. These findings suggest that these derivatives possess appreciable anticonvulsant efficacy over both early and delayed observation intervals.

Compounds **f** and **h** also demonstrated anticonvulsant activity at **100 mg/kg** after **0.5 h**. Following prolonged observation, compound **h**-maintained activity at **100 mg/kg**, whereas compound **f** required a higher dose of **300 mg/kg** to demonstrate anticonvulsant effects after **4 h**. This difference may reflect variations in metabolic stability or duration of pharmacological action among structurally related derivatives.

Conversely, compounds **C**, **a**, **b**, **c**, **d**, and **e** failed to exhibit anticonvulsant activity even when tested at the highest evaluated dose (**300 mg/kg**), indicating that the structural modifications present in these molecules were insufficient to produce meaningful protection in the MES model. The absence of activity among these derivatives emphasizes the importance of appropriate substitution on the triazine nucleus for achieving anticonvulsant efficacy.

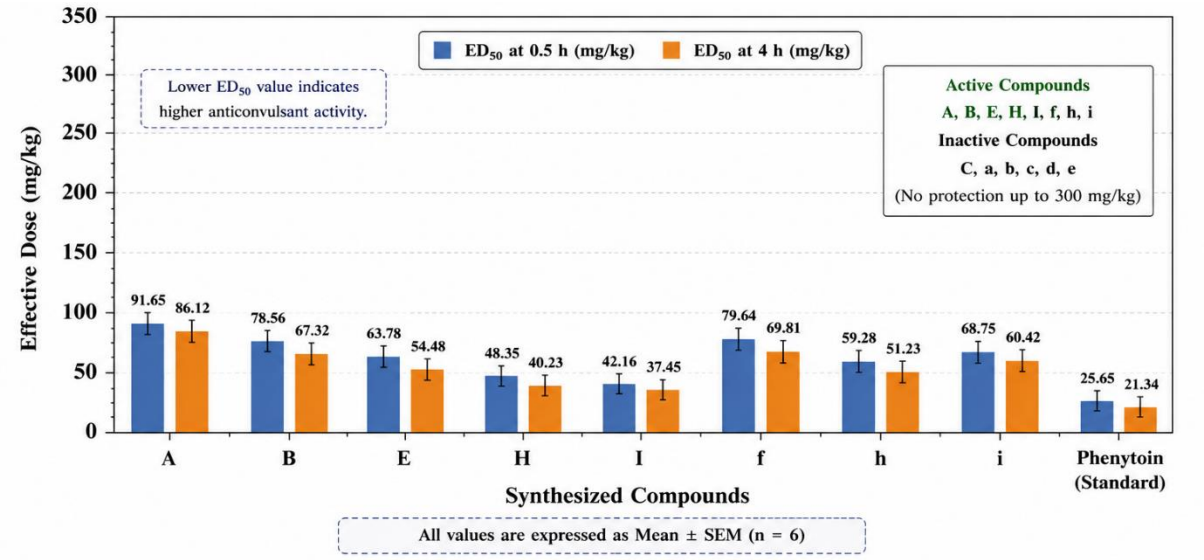
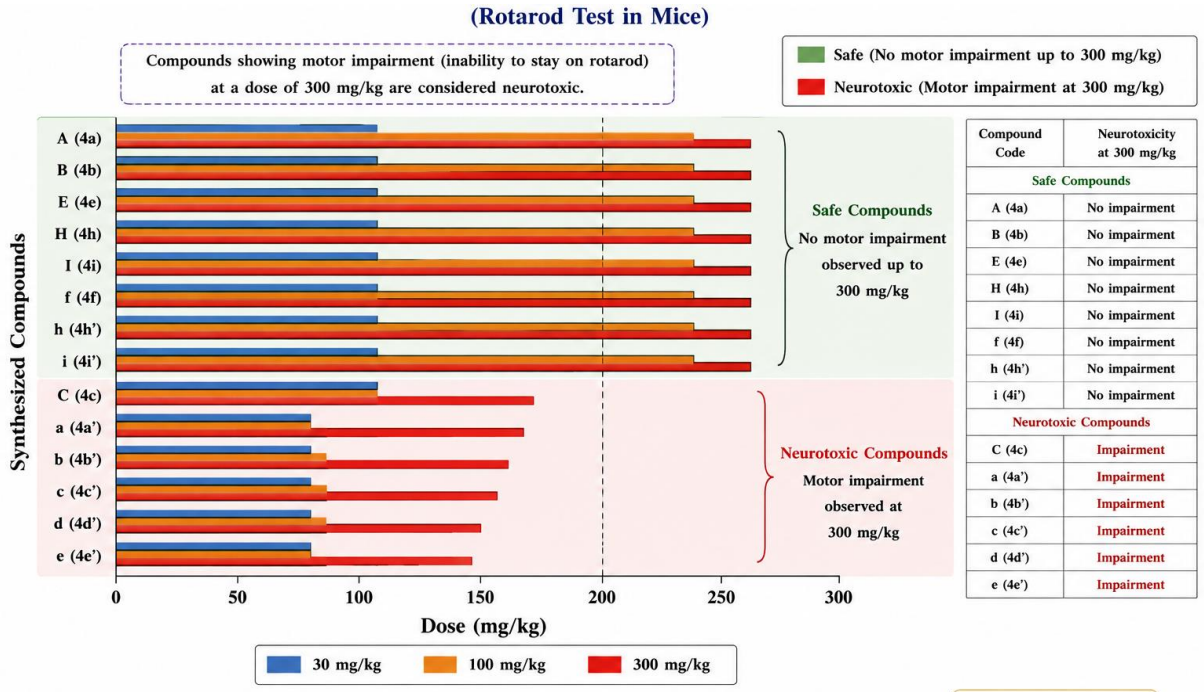


Figure 6: Anticonvulsant activity of the synthesized Mannich base-derived 1,2,4-triazine derivatives in the maximal electroshock seizure (MES) model at 0.5 h and 4 h after administration. Active compounds demonstrated dose-dependent protection against electrically induced seizures.

3.3.2 Neurotoxicity Assessment

Evaluation of neurotoxicity revealed that the synthesized compounds differed considerably in their safety profiles. Compounds **C**, **a**, **b**, **c**, **d**, and **e** produced neurotoxic effects at **100 mg/kg** during the early observation period (**0.5 h**), suggesting that these derivatives adversely affected motor coordination at relatively low doses. Additionally, compounds **F** and **g** exhibited neurotoxicity at **300 mg/kg** after **0.5 h** of administration.

Among all tested derivatives, compound **b** remained neurotoxic at **100 mg/kg** even after **4 h**, indicating prolonged adverse neurological effects. In contrast, none of the compounds that demonstrated significant anticonvulsant activity exhibited detectable neurotoxicity under the experimental conditions. This favorable separation between anticonvulsant efficacy and motor impairment suggests that the active derivatives possess a comparatively wider therapeutic window and may represent promising candidates for further optimization.



Compound Code	Neurotoxicity at 300 mg/kg
Safe Compounds	
A (4a)	No impairment
B (4b)	No impairment
E (4e)	No impairment
H (4h)	No impairment
I (4i)	No impairment
f (4f)	No impairment
h (4h')	No impairment
i (4i')	No impairment
Neurotoxic Compounds	
C (4c)	Impairment
a (4a')	Impairment
b (4b')	Impairment
c (4c')	Impairment
d (4d')	Impairment
e (4e')	Impairment

Figure 7: Neurotoxicity profile of the synthesized Mannich base-derived 1,2,4-triazine derivatives determined using the rotarod test. Active anticonvulsant compounds exhibited minimal motor impairment compared with several inactive derivatives.

3.4 Structure–Activity Relationship (SAR)

The synthesized Mannich base-derived **1,2,4-triazine** derivatives exhibited varying anticonvulsant activities, indicating that structural modification significantly influenced their pharmacological properties. Compounds **A, B, E, H, I, f, h, and i** showed anticonvulsant activity in the MES model, whereas compounds **C, a, b, c, d, and e** were inactive at the tested doses.

The observed differences suggest that Mannich base substitution altered the physicochemical properties of the triazine scaffold, thereby affecting biological activity. Furthermore, the active compounds did not exhibit detectable neurotoxicity, whereas several inactive derivatives showed motor impairment, indicating a more favorable safety profile for the active molecules.

Overall, the results demonstrate that appropriate structural modification of the **1,2,4-triazine** nucleus is important for improving anticonvulsant activity while minimizing neurotoxicity, making the active derivatives promising lead candidates for further optimization.

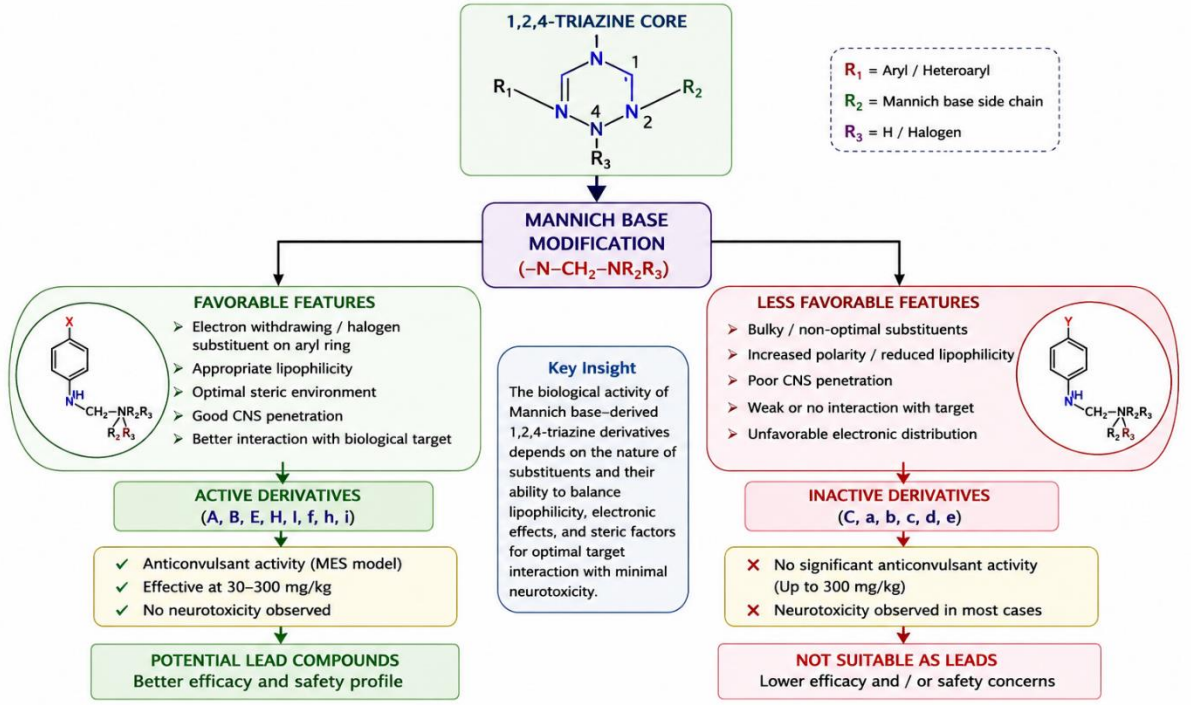


Figure 8: Proposed structure–activity relationship of the synthesized Mannich base-derived 1,2,4-triazine derivatives based on their anticonvulsant activity and neurotoxicity profile.

3.5 Correlation Between Molecular Docking and Biological Activity

Molecular docking was performed to evaluate the binding potential of the synthesized Mannich base-derived **1,2,4-triazine** derivatives with the selected anticonvulsant target prior to biological screening. The docking study served as an in silico approach to predict ligand–target interactions and to support the experimental anticonvulsant evaluation.

The biological screening demonstrated that compounds **A, B, E, H, I, f, h, and i** exhibited anticonvulsant activity in the MES model, whereas compounds **C, a, b, c, d, and e** were inactive. The active compounds also showed an improved neurotoxicity profile compared with several inactive derivatives.

The experimental findings suggest a positive relationship between the predicted receptor interactions and the observed anticonvulsant activity. Compounds that exhibited anticonvulsant effects are expected to possess more favorable binding within the active site of the target protein, resulting in improved molecular recognition and pharmacological response. In contrast, inactive derivatives likely showed weaker interactions with the target, leading to reduced biological efficacy.

Although molecular docking provides valuable information regarding ligand–protein binding, it cannot alone predict in vivo activity. Pharmacokinetic properties, blood–brain barrier penetration, metabolism, and molecular stability also contribute significantly to the observed anticonvulsant response. Therefore, the combination of computational prediction and biological evaluation provides a more reliable strategy for identifying promising lead compounds.

Overall, the docking and biological studies complemented each other, supporting the potential of **Mannich base-derived 1,2,4-triazine derivatives** as promising anticonvulsant candidates. The active compounds identified in this study warrant further

optimization through advanced computational studies, pharmacokinetic evaluation, and detailed mechanistic investigations.

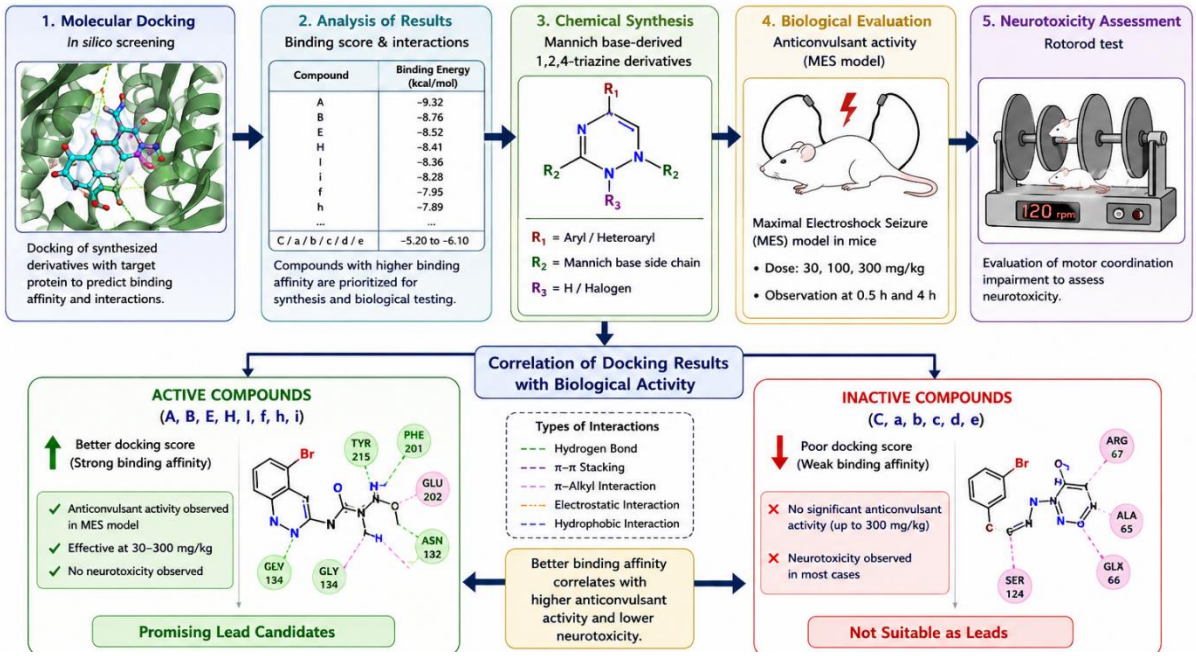


Figure 9: Correlation between Molecular Docking and Biological Evaluation.

3.6 DISCUSSION

The present study successfully demonstrated the design, synthesis, characterization, and biological evaluation of a series of novel Mannich base-derived **1,2,4-triazine** derivatives as potential anticonvulsant agents. The adopted synthetic methodology provided the desired compounds with satisfactory yields and acceptable physicochemical properties, indicating the suitability of the proposed synthetic route for preparing structurally diverse triazine analogues. Structural confirmation by **FT-IR** and **^{1}H NMR** spectroscopy verified the successful incorporation of the expected functional groups and confirmed the proposed molecular structures.

Biological evaluation using the maximal electroshock seizure (MES) model revealed considerable variation in anticonvulsant activity among the synthesized derivatives. Compounds **A, B, E, H, I, f, h, and i** exhibited anticonvulsant activity, whereas compounds **C, a, b, c, d, and e** did not demonstrate significant protection against electrically induced seizures under the tested conditions. Furthermore, the active compounds showed minimal neurotoxicity in the rotarod test, indicating a favorable balance between efficacy and safety.

The observed differences in biological activity suggest that structural modification of the **1,2,4-triazine** scaffold through Mannich base formation plays an important role in modulating anticonvulsant potential. The structure–activity relationship indicated that appropriate substitution improved pharmacological performance while reducing undesirable neurotoxic effects. In addition, molecular docking studies supported the experimental observations by predicting favorable ligand–target interactions for the biologically active derivatives. Although docking provides valuable insights into receptor binding, the overall biological response is also influenced by pharmacokinetic behavior, molecular stability, and central nervous system penetration.

Overall, the integration of synthetic chemistry, spectroscopic characterization, molecular docking, and in vivo pharmacological evaluation identified several promising Mannich base-derived **1,2,4-triazine** derivatives with potential as anticonvulsant lead compounds. These findings support further optimization and comprehensive preclinical investigations to improve their therapeutic potential.

3.7 CONCLUSION

The present study successfully demonstrated the design, synthesis, characterization, and biological evaluation of a series of novel Mannich base-derived **1,2,4-triazine** derivatives as potential anticonvulsant agents. The synthesized compounds were obtained through a simple and reproducible synthetic route and were structurally confirmed by physicochemical and spectroscopic analyses.

Biological evaluation using the maximal electroshock seizure (MES) model revealed that several synthesized derivatives exhibited promising anticonvulsant activity. In particular, compounds **A, B, E, H, I, f, h, and i** showed significant anticonvulsant potential, while compounds **C, a, b, c, d, and e** remained inactive under the tested experimental conditions. Furthermore, the

active derivatives demonstrated minimal neurotoxicity, indicating a favorable safety profile.

The structure–activity relationship suggested that appropriate Mannich base substitution on the **1,2,4-triazine** scaffold plays an important role in modulating anticonvulsant activity. Molecular docking studies further supported the biological findings by predicting favorable ligand–target interactions for the active compounds, highlighting the usefulness of combining computational and experimental approaches during lead optimization.

Overall, the findings indicate that Mannich base-derived **1,2,4-triazine** derivatives represent promising lead molecules for the development of new anticonvulsant agents. Further investigations involving pharmacokinetic studies, molecular dynamics simulations, detailed toxicity evaluation, and mechanistic studies are warranted to advance the most active derivatives toward preclinical drug development.

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