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By:

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Synthesis and Biological Evaluation of New Asymmetric Coumarin–1,3,4-Thiadiazole Hybrids

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To my beloved parents,

In gratitude for their endless love, sacrifices, and support.

To my dear wife,

For her patience and encouragement, and her constant presence by my side.

To my brothers,

For their love, support, and confidence.

I dedicate this work.

List of abbreviations

A_{0.5}	Concentration required to reach an absorbance of 0.5
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ABTS^{•+}	ABTS radical cation
AChE	Acetylcholinesterase
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
ATCI	Acetylthiocholine iodide
AutoDock Vina	Software used for molecular docking simulations
AutoDockTools	Software to prepare ligands and receptors for docking
BHA	Butylated Hydroxyanisole – standard antioxidant control
BHT	Butylated Hydroxytoluene – standard antioxidant control
BIOVIA Discovery Studio	Software used for visualization of docking interactions
CDCl₃	Deuterated chloroform – solvent for NMR measurements
Chloroform-d	Deuterated chloroform – solvent for NMR measurements
CPCM	Conductor-like Polarizable Continuum Model
d	Doublet
dd	Double doublet
DCM	Dichloromethane
DFT	Density Functional Theory
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)
ΔE	Energy gap
ΔG	Binding free energy – used to rank docking poses
d₆-DMSO	Deuterated dimethyl sulfoxide
EtOH	ethanol / Solvent for recrystallization
FTIR	Fourier Transform Infrared Spectroscopy
FeCl₃	Ferric chloride
Gal	Galantamine hydrobromide

HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
m	Multiplet
Microplate	96-well plate used for performing assays in triplicate
MP	Melting point
NaCl	Sodium chloride
Na₂SO₄	Sodium sulfate
NMR	Nuclear Magnetic Resonance
OriginPro	Software used for graphical representation of data
PDB	Protein Data Bank
PDBQT	AutoDock format – input format for docking
ppm	Parts per million
q	Quartet
RMSD	Root Mean Square Deviation
Rf	Retention factor
SD	Standard deviation – measure of data variability
s	Singlet
t	Triplet
TPSA	Topological Polar Surface Area
XLSTAT	Software for regression and statistical analysis
BTC	Butyrylthiocholine chloride
Lipinski's Rule of Five	Drug-likeness rules for oral bioavailability
GI	Gastrointestinal – absorption prediction
BBB	Blood–Brain Barrier
SwissADME	Online tool for ADMET and drug-likeness predictions
ADMETlab	Web-based platform for ADMET predictions
ProTox-II	Online tool for toxicity prediction (LD ₅₀ , toxicity class)

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Abstract

General introduction

Introduction

Natural products have long represented one of the most important sources of bioactive molecules and continue to play a pivotal role in modern drug discovery [1]. Among these naturally occurring compounds, coumarins constitute an important class of oxygen-containing heterocyclic metabolites owing to their remarkable structural diversity and broad spectrum of pharmacological activities [2]. They are widely distributed throughout the plant kingdom and have been isolated from various plant organs, including leaves, stems, roots, seeds, fruits, and flowers, with the latter representing the richest natural sources [3] [4]. Biosynthetically, coumarins originate from phenylalanine through the shikimate pathway, which is responsible for the formation of numerous secondary metabolites involved in plant growth, defense, and adaptation to environmental stress [5].

Structurally, coumarins are classified into several subclasses, including simple coumarins, furocoumarins, dihydrofurocoumarins, linear and angular pyranocoumarins, phenylcoumarins, and biscoumarins [6]. This structural diversity largely accounts for their broad spectrum of biological activities [7]. Numerous studies have demonstrated that coumarin derivatives exhibit anticancer, antimicrobial, antiparasitic, antioxidant, and neuroprotective properties. Furthermore, they are capable of regulating oxidative stress and modulating the activity of several biologically important enzymes, making the coumarin scaffold an attractive template for the development of new therapeutic agents [8] [9].

Heterocyclic compounds constitute another fundamental class of molecules in medicinal chemistry because they are present in numerous biologically active compounds and marketed drugs [10]. Among sulfur-containing heterocycles, thiadiazoles have attracted considerable attention due to their synthetic accessibility, structural versatility, and broad pharmacological potential. Thiadiazoles are five-membered aromatic heterocycles containing two nitrogen atoms and one sulfur atom. Four constitutional isomers are commonly recognized, namely 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole. Among these isomers, the 1,3,4-thiadiazole scaffold has attracted particular attention because of its favorable pharmacological profile and its versatility as a privileged pharmacophore in medicinal chemistry [11]. Numerous investigations have demonstrated that 1,3,4-thiadiazole derivatives possess antifungal [12], antimicrobial

[13], anticancer [14], anti-inflammatory [15], and enzyme inhibitory effects [16]. The combination of two biologically active pharmacophores into a single molecular framework, commonly referred to as molecular hybridization, has become an effective strategy in medicinal chemistry for developing multifunctional compounds with enhanced therapeutic potential. In this regard, the hybridization of coumarin and 1,3,4-thiadiazole scaffolds is particularly attractive because both pharmacophores possess complementary biological activities. Consequently, coumarin–1,3,4-thiadiazole hybrids have emerged as promising multifunctional scaffolds for the development of novel therapeutic agents. Despite the considerable progress achieved in the development of coumarin and 1,3,4-thiadiazole derivatives, relatively little attention has been devoted to asymmetric coumarin–1,3,4-thiadiazole hybrids, particularly those evaluated through an integrated experimental and computational approach. Considering the multifactorial nature of neurodegenerative disorders, the development of multifunctional molecules capable of simultaneously modulating oxidative stress and cholinergic dysfunction remains an important research challenge.

Alzheimer's disease is one of the most prevalent neurodegenerative disorders and is characterized by progressive cognitive impairment associated with oxidative stress and cholinergic dysfunction. Because acetylcholinesterase (AChE) plays a central role in the hydrolysis of acetylcholine, its inhibition remains one of the principal therapeutic strategies for the symptomatic management of the disease. Consequently, compounds combining antioxidant activity with AChE inhibitory potential have attracted increasing interest as promising multifunctional therapeutic agents [17].

Alongside advances in synthetic chemistry, computational approaches have become indispensable tools in contemporary drug discovery. Computational chemistry facilitates molecular modeling, predicts physicochemical and biological properties, and supports the rational design of new therapeutic candidates. Computational methods play a crucial role in predicting molecular behavior, guiding experimental design, and accelerating the identification of potential drug candidates [18]. Among these techniques, molecular docking is widely employed to investigate ligand–protein interactions by predicting the preferred binding orientation of small molecules within the active site of target proteins and estimating their binding affinities [19]. In addition, in silico prediction of pharmacokinetic properties has become an essential step in early

drug development. Web-based platforms such as SwissADME and ProTox-II provide valuable information concerning absorption, distribution, metabolism, excretion, toxicity (ADMET), drug-likeness, oral bioavailability, and toxicity profiles, thereby facilitating the early identification of promising lead compounds with favorable pharmacokinetic characteristics according to the Lipinski, Veber, and Egan rules [20]. Based on these considerations, the present work aimed to design and synthesize a new series of asymmetric coumarin–1,3,4-thiadiazole hybrids and to evaluate their biological potential. Accordingly, twelve novel asymmetric coumarin–1,3,4-thiadiazole hybrids (T1a–T1g, T2a–T2c, and T3a–T3b) were synthesized and structurally characterized by ^1H and ^{13}C NMR spectroscopy. Their antioxidant activity was evaluated using ABTS radical scavenging and o-phenanthroline reducing power assays, while acetylcholinesterase inhibitory activity was investigated as a potential therapeutic strategy for Alzheimer's disease. Furthermore, molecular docking studies were performed against three therapeutically relevant protein targets (PDB IDs: 4EY7, 1P0I, and 1DNU), and complementary in silico ADMET and toxicity analyses were carried out using the SwissADME and ProTox-II platforms to evaluate the pharmacokinetic behavior, drug-likeness, and safety profiles of the synthesized compounds [21]. Overall, the present work integrates synthetic organic chemistry, biological evaluation, and computational approaches to provide a comprehensive assessment of the pharmacological potential of asymmetric coumarin–1,3,4-thiadiazole hybrids. The integration of these complementary approaches is expected to provide valuable insights into their structure–activity relationships and to facilitate the identification of promising lead compounds for the future development of therapeutic agents against Alzheimer's disease and other oxidative stress-related neurodegenerative disorders. [13].

CHAPTER I:

***Coumarin and Its Derivatives:
Structure, Synthesis, and Medicinal
Importance***

I.1. Introduction:

Coumarins, whether of natural origin or obtained through synthetic procedures, constitute a large and structurally diverse family of heterocyclic compounds characterised by a benzo- α -pyrone core [22]. These compounds are distributed extensively throughout the plant kingdom and have long been recognised for their remarkable chemical and biological significance. The parent coumarin molecule, which serves as the most representative member of this class [4], was first identified in tonka beans and was subsequently the subject of extensive biochemical and pharmacological investigations [23].

Among the naturally occurring coumarin derivatives, dicoumarol is of particular significance due to its potent anticoagulant activity. The substance was initially identified in damp, decomposing sweet clover straw, where it is understood to be produced through the microbial transformation of naturally occurring coumarin [24]. Another prominent derivative, ostol, has also received increasing attention in scientific research in recent years, due to its wide range of pharmacological activities, including anti-inflammatory, anti-cancer, neuroprotective, and antioxidant activity [25]. Osthole, a naturally occurring organic compound, was isolated from *Cnidium monnieri*, a medicinal plant that has been used extensively in traditional medicine for centuries. In addition, scoparone, which has demonstrated promising pharmacological properties – especially its immunosuppressive and vasodilatory effects – was identified in *Artemisia scoparia* [26]. Representative chemical structures of selected coumarin derivatives are illustrated in Figure 1.

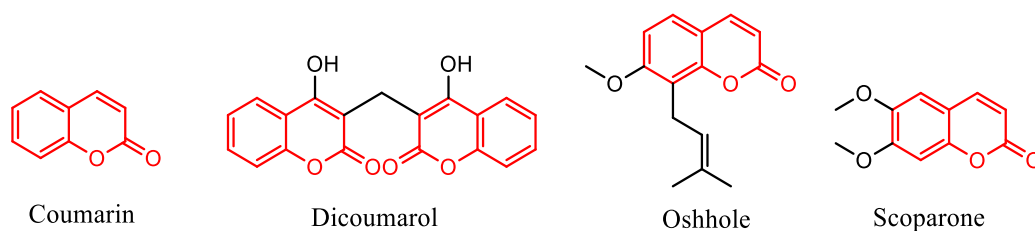


Figure 1. Examples of biologically active compounds containing coumarin as a basic motif

In recent decades, the structural chemistry of coumarin and its derivatives has been a subject of significant interest in the field of pharmaceutical research. This sustained interest is primarily attributed to the remarkably wide range of biological and pharmacological activities exhibited by these compounds. A substantial body of

research has demonstrated the significant antidepressant [27], antimicrobial [28] and antioxidant [29] activities of coumarin derivatives, in addition to other therapeutic effects such as anti-inflammatory, anticoagulant, anticancer and neuroprotective properties. The versatility of the coumarin scaffold, in conjunction with its ease of chemical modification, renders it a highly attractive platform for the design and development of new bioactive molecules [30].

I.2. Chemical Structure and Nomenclature

I.2.1 Molecular structure of coumarin:

Coumarin is a heterocyclic organic compound consisting of a benzene ring attached to a pyran molecule, forming a bicyclic system known as benzo- α -pyrone. This structure includes a carbonyl (ketone) group located at the α position relative to the oxygen atom in the heterocyclic ring, which is essential for determining the chemical reactions and biological properties of coumarin and its derivatives [31].

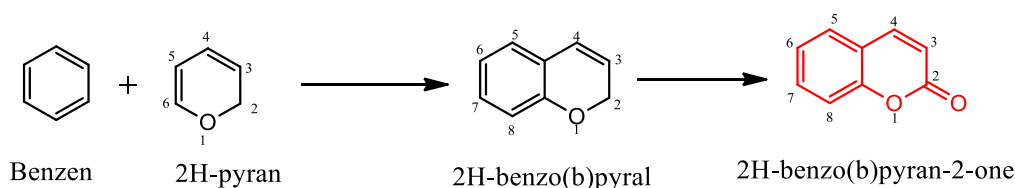


Figure 2. Combination of benzene with pyran to form coumarin

I.2.2 Nomenclature:

Molecular formula: $C_9H_6O_2$

2H-chromen-2-one or 2H-1-benzopyran-2-one (IUPAC name)

Others: 1,2-benzopyrone (α -benzopyrone) [31]

Based on the nomenclature adopted by the International Union of Pure and Applied Chemistry (IUPAC), coumarin is classified as a derivative of 2H-chromen-2-one. This structure belongs to the chromene family and represents one of the unsaturated forms of chromane [32].

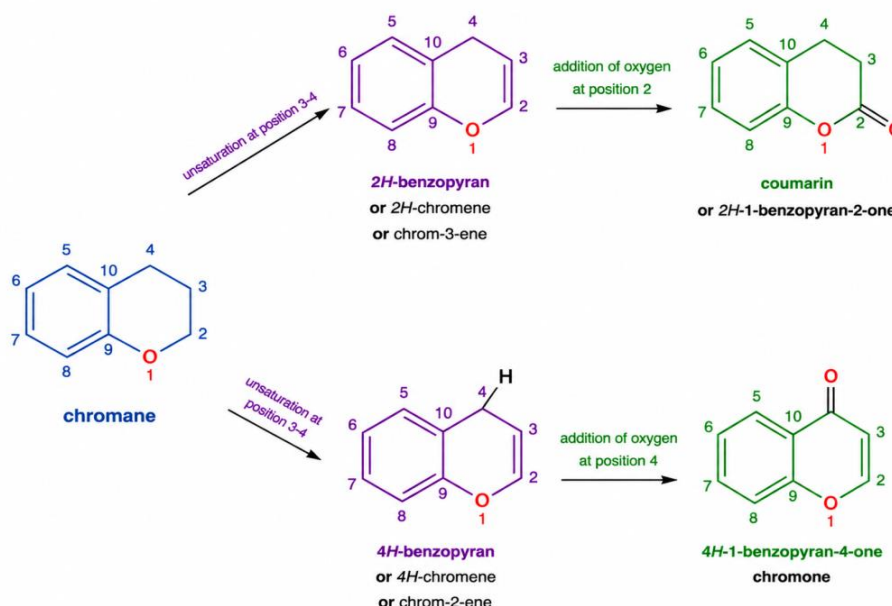


Figure 3. Chemical relationship between coumarin and its structural isomer

I.3. Classification of coumarins:

Coumarins contain one or more phenolic functional groups, whose hydroxyl moieties may exist in the free form, be etherified, or participate in heterosidic linkages [33]. The structural complexity of coumarins is typically used to differentiate between two primary classifications: simple coumarins and complex coumarins [34].

I.3.1. Simple coumarins:

These represent the most widespread class of coumarins in the plant kingdom. They are generally classified into two main types: genins (aglycones) and heterosides (glycosides) [34]. Representative structures of these compounds are summarized in Tables 1 and 2.

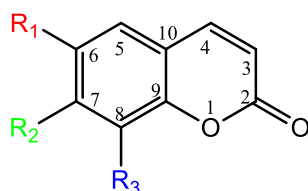


Figure 4 . General simple coumarine structure

Table 1. Some examples of genines

Coumarins	R1	R2	R3
Umbelliferone	H	OH	H
Esculetin	OH	OH	H
Scopoletin	OCH ₃	OH	H
Herniarin	H	OCH ₃	H
Fraxetin	OCH ₃	OH	OH

Table 2. Some examples of glycosides

Coumarins	R1	R2	R3
Esculoside (Aesculin)	O-Glu	OH	H
Cichorioside (Cichorin)	OH	O-Glu	H
Scopoloside (Scopoline)	OCH ₃	O-Glu	H
Fraxoside	OCH ₃	O-Glu	OH

I.3.2. Complex Coumarins:

Complex coumarins are characterized by the presence of an additional furan or pyran ring fused to or linked to a benzo- α -pyrone nucleus. Several structural subclasses have been identified, such as furanocoumarins, pyranocoumarins, dicoumarins, and tricoumarins [34]. Figure 5 shows typical molecular structures of these complex coumarins.

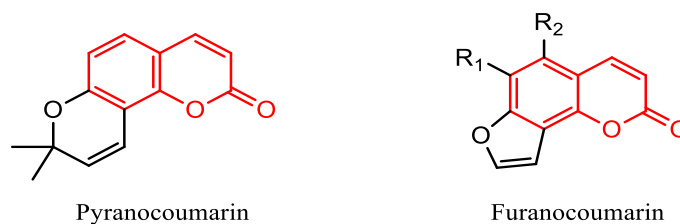


Figure 5. General structure of some complex coumarins

I.4. Physicochemical Properties:

Coumarins in their free form are readily soluble in alcohols and a variety of organic solvents, including ethyl dioxides and chlorinated solvents, which allow efficient

extraction. Conversely, their heterosidic derivatives exhibit partial solubility in water [35].

Coumarins possess a distinctive UV absorption profile that is highly dependent on both the nature and the position of substituents and undergoes significant modification in alkaline environments (KOH, NaOCH₃) [36]. Upon UV examination, thin-layer chromatography of coumarin-based substances produces colored spots whose intensity is enhanced by ammonia, displaying hues ranging from blue and yellow to purple [35].

I.5. Synthetic Methods of Coumarin Derivatives:

Several reactions provide access to a wide range of coumarin derivatives. The schematic below summarizes the primary synthetic pathways for coumarin compounds [37].

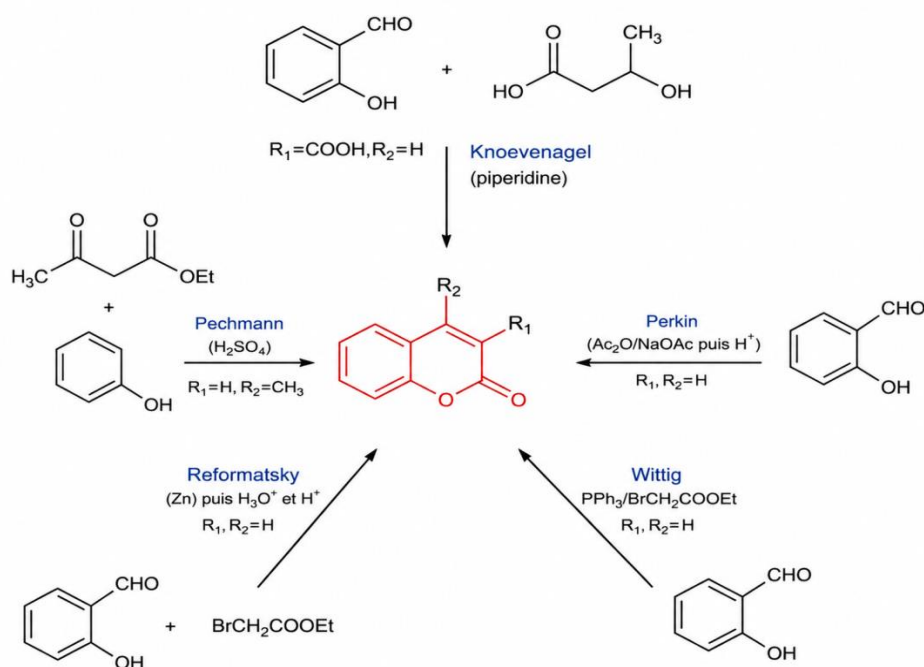


Figure 6. Different pathways for coumarin synthesis

I.5.1. Synthesis of coumarins by the Pechmann reaction:

The Pechmann reaction [38] is one of the simplest and most common methods for coumarin synthesis. This conventional technique involves the condensation of phenol with a beta-keto ester, utilising a range of reagents, typically yielding a 4-position substituted coumarin [38]. A plethora of acid catalysts have been employed, including H₂SO₄, HClO₄, and P₂O₅-CF₃COOH [39]. Alternative approaches, such as the

utilisation of ionic liquids or microwave irradiation, frequently yield highly acidic byproducts and/or necessitate the use of costly, non-reusable agents. Recent research has demonstrated the utilisation of a range of heterogeneous catalysts in the Pechmann reaction, including H-BETA zeolite, montmorillonite clay, amberlist 15, Navion-H, alumina, and silica-sulfuric acid, in conjunction with the application of ultrasound ³⁶.

In accordance with the paradigm shift towards greener chemistry, research has been directed towards the development of eco-friendly, reusable catalysts derived from natural biopolymers. Copolymers, particularly starch and its derivatives, exhibit unique properties that make them promising alternatives to conventional organic or inorganic supports for solid-supported catalysis [38].

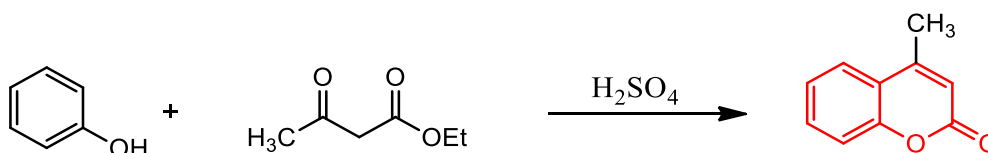


Figure 7. Pechmann condensation from phenol

I.5.2. Synthesis of coumarins by the Perkin reaction:

Perkin reported the synthesis of coumarins by heating the sodium salt of salicylic aldehyde with acetic acid, and subsequent studies contributed to the development of a widely used method for preparing cinnamic acid and its derivatives, now known as the Perkin reaction. This reaction, which has been extensively studied, typically begins with an aromatic aldehyde [37].

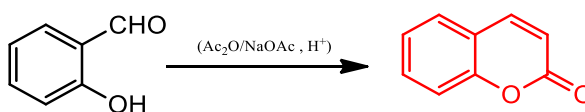


Figure 8. Schematic diagram of coumarin synthesis via the Perkin reaction

I.5.3. Synthesis of coumarins by the Wittig reaction:

The Wittig reaction is considered a nucleophilic addition. It involves the reaction of a phosphorus ylide (phosphorane) with a carbonyl compound to form an alkene. This reaction has been further developed by various researchers and can be conveniently applied to the synthesis of coumarins. For example, salicylaldehyde reacts

with ethoxycarbonylmethylene triphenylphosphorane to produce coumarin derivatives [39].

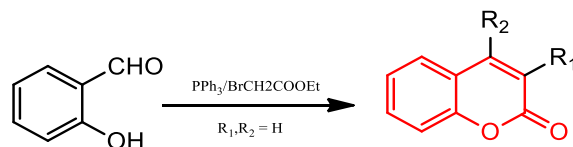


Figure 9 . Schematic diagram of coumarin synthesis via the Wittig reaction

I.5.4. Synthesis of coumarins by the Knoevenagel reaction:

The Knoevenagel reaction is a prevalent method for the formation of double bonds (C=C). This reaction involves the combination of a compound containing an active methylene group with an aldehyde, such as malonic acid or its esters, in the presence of a catalyst that typically acts as a weak base. The reaction also involves the removal of a water molecule, thereby facilitating the formation of alpha and beta unsaturated compounds [37].

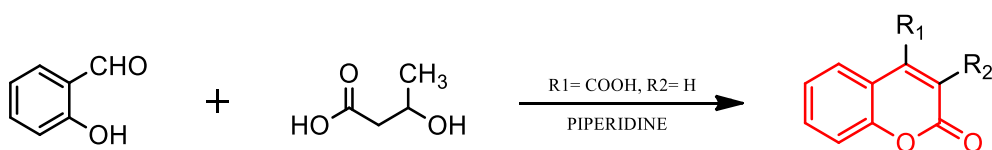


Figure 10. Schematic diagram of coumarin synthesis via the Knoevenagel reaction

I.5.5. Synthesis of coumarins by the Reformatsky reaction:

This reaction occurs between a carbonyl compound-such as an aldehyde, ketone, or ester-and an alpha-halo compound in the presence of zinc. It represents an extension of the reactions of carbonyl compounds with dialkyl zinc or allylic zinc halides. The process forms a new carbon-carbon bond and typically involves:

- From the halide
- Carbonyl group addition in aldehyde and ketone
- Transesterification followed by dehydration using dilute acids [35].

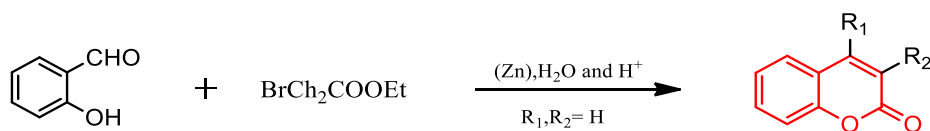


Figure 11. Schematic diagram of coumarin synthesis via the Reformatsky reaction

I.5.6. Synthesis of coumarin by ring-closing metathesis (RCM):

In another method, Debenedetti and colleagues [40] synthesized substituted coumarin from phenol via a series of five steps [41] [42], achieving overall yields ranging from 20% to 67%.

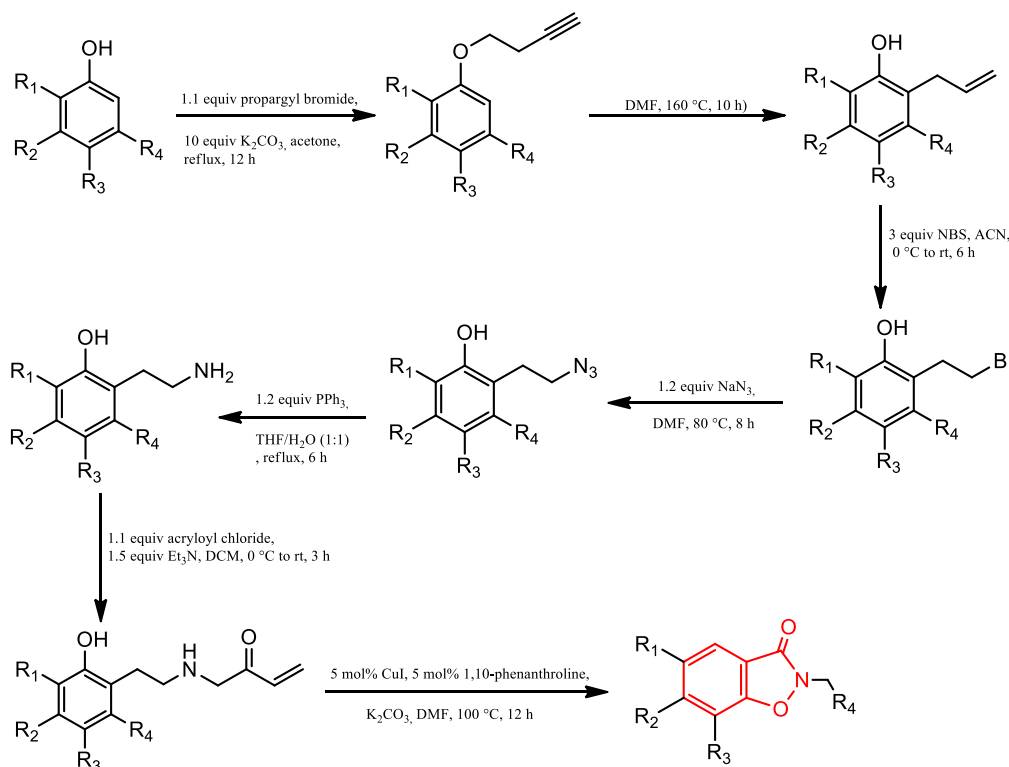


Figure 12 . Synthesis of coumarin by ring-closing metathesis (RCM)

I.5.7. Synthesis of coumarin by cathodic reduction of o-hydroxycarbonyl trichloroacetylester:

Direct synthesis of 3-chloro-4-alkylcoumarin derivatives: Dolly and colleagues reported on this approach, achieving moderate to average yields [43].

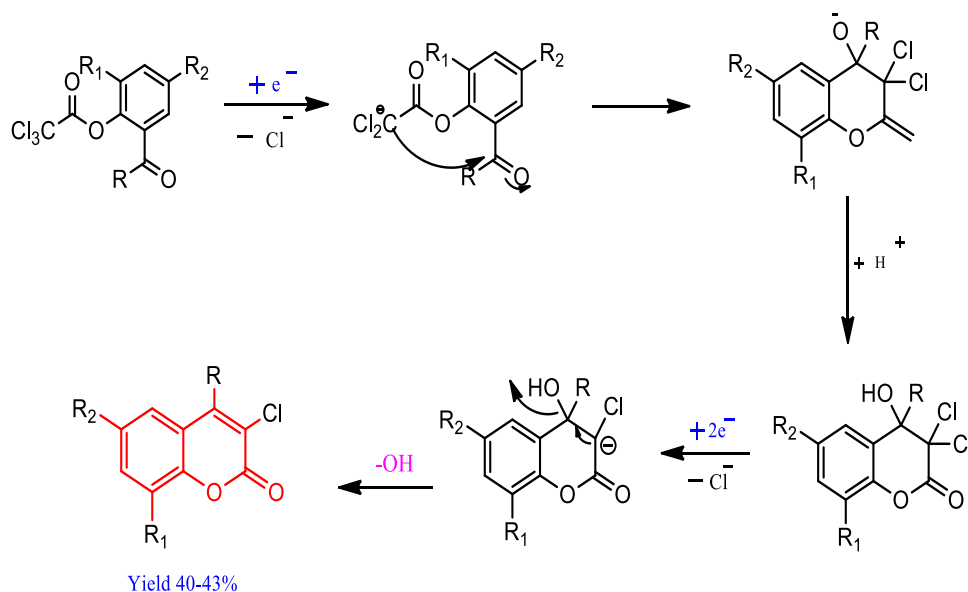


Figure 13 . Synthesis of coumarin by cathodic reduction of o-hydroxycarbonyl trichloroacetyl ester

I.5.8. Other reactions:

a) By introducing carbon monoxide:

In 2003, Kadnikov and Richard reported a method based on the annulation of an internal alkyne, involving the reaction of alkyne with an o-iodophenol in the presence of carbon monoxide and a Pd(0) catalyst. This process affords coumarin derivatives exclusively and in good yields [44] [45].

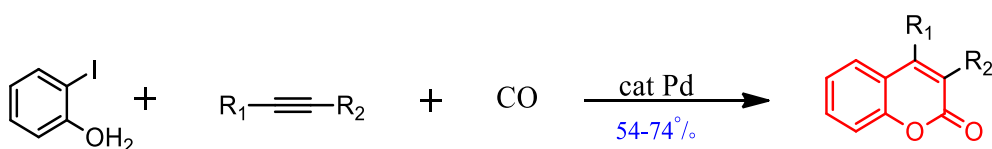


Figure 14 . Synthesis of coumarin by introducing carbon monoxide

b) Synthesis of coumarin via the Suzuki cross-coupling reaction:

This reaction requires an electrophilic palladium catalyst, Pd(II). Fujiwara [46]. reported that electrophilic palladium promotes the cycloisomerization of aryl brominated acetylenic esters, leading to the preparation of coumarins in good yields.

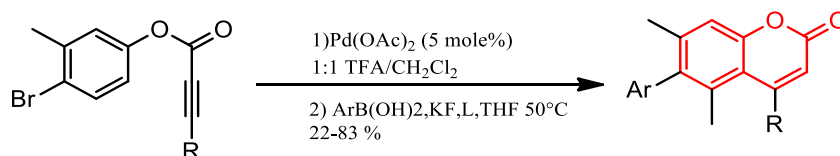


Figure 15. Synthesis of coumarin by via the Suzuki cross-coupling reaction

I.6. Synthesis of coumarin derivatives:

The synthesis of coumarin and its derivatives has historically relied on a variety of reactions, including Perkin, Pechmann, Knoevenagel, Reformatsky, and Wittig. Whilst these approaches are well-established, they frequently necessitate the use of costly reagents, specialised catalysts, or aggressive reaction conditions, thus limiting their practical application. This emphasises the necessity for a more efficient synthetic strategy. Such a strategy should be straightforward, utilise readily accessible materials, be economically viable, and align with green chemistry principles.

I.6. 1. Synthesis Strategy:

Coumarin derivatives are prepared via a stepwise reaction pathway, where the first stage involves the formation of an intermediate compound, followed in the second stage by a cyclization reaction that leads to the desired substituted coumarin product.

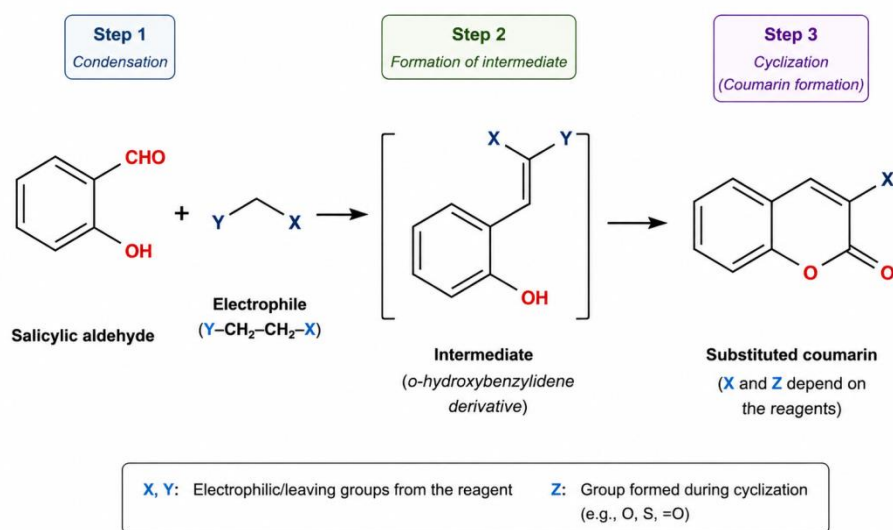


Figure 16. Synthesis of coumarin derivatives

I.6.2. Alkylation reactions:

The synthesised coumarins have the typical structural framework of an α,β -unsaturated lactone, with a substituent positioned at the α -carbon. This versatile molecular scaffold is amenable to a broad spectrum of chemical modifications, enabling the synthesis of more complex, highly substituted derivatives. One especially valuable transformation strategy is the 1,4-Michael addition [47], which allows for the selective introduction of functional groups that can subsequently participate in intramolecular cyclisation

reactions. These sequential transformations provide a simple way to construct tricyclic systems, increasing the structural diversity and potential biological applications of coumarin derivatives.

In parallel, conversion of the carbon–carbon double bond at the 3-position into a single bond would enable the generation of an enolate under basic conditions, which could subsequently react with various alkylating agents at this position [48].

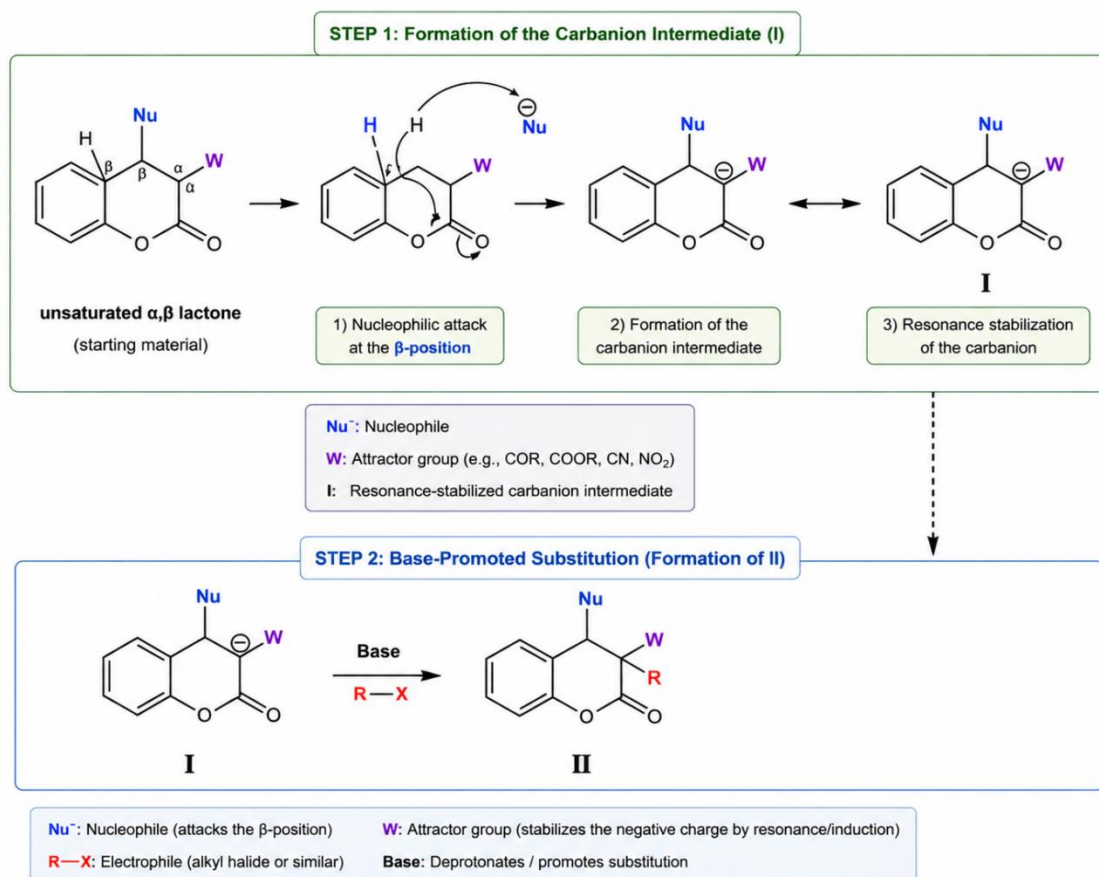


Figure 17. Synthesis of coumarin derivatives Alkylated

I.7. Medicinal Importance:

Coumarins are known for their wide range of biochemical and pharmacological activities, and their biological performance is mainly determined by the type and location of the substituents associated with the coumarin structure [49] [50]. Therefore, most coumarin derivatives, both natural and synthetic, have been subjected to extensive studies to assess their potential effects on human health, and many of these studies have revealed that they possess anti-HIV and anti-tumor properties [51], anticancer,

antimicrobial [52], anti-inflammatory, antifungal, antioxidant, and vasodilatory activities [53].

In addition to these effects, certain coumarins have been reported to display estrogenic [54] and antineoplastic properties. They are also known to inhibit platelet aggregation and suppress the activity of acetylcholinesterase [55], further highlighting their therapeutic potential.

The relationship between coumarin structure and antioxidant activity has been elucidated by the studies of Kaneko and Zhang, who demonstrated that the characteristic 1,2-pyrone moiety of the coumarin skeleton contributes only marginally to antioxidant potency. Their findings further revealed that the presence of a catechol functional group plays a decisive role in enhancing free radical scavenging activity. From a biological standpoint, coumarins are generally regarded as phytoalexins, synthesized by plants in response to pathogenic invasion or antibiotic stress [56].

Among the various subclasses of coumarins, furocoumarins have attracted particular attention in recent years due to their diverse therapeutic applications. These compounds have been employed in the symptomatic treatment of multiple sclerosis, in photochemotherapy of T-cell lymphomas [57], and in the chemotherapy of tumors resistant to conventional polychemotherapy regimens. Moreover, several investigations have confirmed the insecticidal and larvicidal activities of furocoumarins [58]. Their pronounced phototoxic properties contribute to plant defense mechanisms, offering protection against herbivores, insects, and pathogenic microorganisms.

We mention some of them.

I.7.1. Antispasmodic Activity:

Several plant-derived substances exhibiting hypotensive and antispasmodic properties have been shown to inhibit spasmodic contractions of gastrointestinal and urogenital smooth muscles, which are commonly associated with conditions such as cramps and colic. Notably, these bioactive compounds are derived from coumarin-containing plants, including *Viburnum prunifolium*, which is a natural source of scopoletin, and species of *Angelica*, from which antispasmodic constituents are obtained in the form of essential oils extracted from the roots [47].

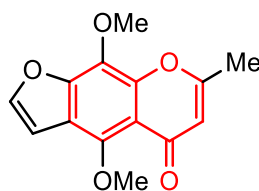


Figure 18. Chemical structure of khella (Ammi visnaga. L)

Khellin, a naturally occurring furanochromone is well recognized for its anti-anginal activity, exerting a pronounced spasmolytic effect specifically on the smooth muscle of the coronary vessels, thereby contributing to improved coronary blood flow.

I.7.2. Anti-tumor activity:

Coumarins have been widely recognized for their diverse biological activities, including chelating, anticoagulant, and antioxidant properties, which collectively contribute to their potential cytotoxic effects. These properties render coumarins particularly interesting as candidates for antitumor therapy, as they can interfere with cellular processes essential for tumor growth and survival. Notably, the cytotoxic effects coumarins are highly dependent on the type of cell, suggesting a degree of selectivity that could be advantageous in cancer treatment by minimizing harm to normal cells [59].

The Fells Cancer Research Institute investigated this possibility in greater detail by synthesizing a series of coumarin derivatives based on the 3-(N-aryl) carboxamide coumarin structure (see Figure 19). These derivatives were systematically evaluated for cytotoxicity in various cancer cell lines, and the results showed their ability to selectively inhibit cancer cell growth, demonstrating their potential as targeted antitumor agents [60]. The study highlights the role of structural modifications in enhancing the cytotoxic activity of coumarin, showing that the careful design of coumarin-based derivatives may enable the development of effective chemotherapeutic agents with improved selectivity and low systemic toxicity.

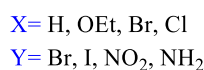
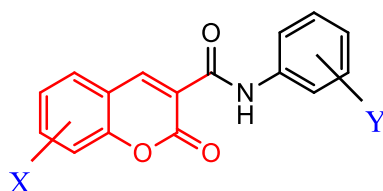


Figure 19 . Chemical structure of 3-(N-aryl) carboxamide coumarin derivatives

An initial investigation revealed that Auraptene, a geranylated coumarin, exhibited significantly greater efficacy in preventing skin tumorigenesis in murine models compared to umbelliferone, which lacks the geranyl moiety. This finding underscores the critical role of structural modifications, such as geranylation, in enhancing the antitumor potential of coumarin derivatives. Furthermore, the anticancer properties of coumarins have been corroborated by subsequent chemical studies, which demonstrated the potent antitumor activity of Geiparvarin, another coumarin-based compound [61]. These results collectively highlight the relevance of specific substituents in modulating the biological activity of coumarins and support their continued investigation as promising candidates for chemopreventive and chemotherapeutic applications.

I.7.3. Anti-inflammatory and analgesic activity:

Several studies have demonstrated that coumarin derivatives exhibit notable anti-inflammatory and analgesic properties. In particular, research conducted by Han and colleagues highlighted that specific coumarin analogs, such as cloricromene, are capable of significantly reducing inflammation and alleviating pain in experimental models [62]. The results indicate that the structural composition of coumarin can be effectively exploited to design novel compounds with dual anti-inflammatory and analgesic activity. Furthermore, the modulation of inflammatory pathways by coumarin derivatives highlights their potential as promising therapeutic agents targeting chronic inflammation and pain relief.

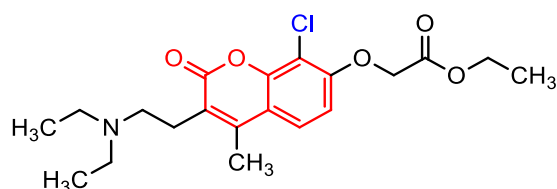


Figure 20 . Chemical structure of cloricromene

Another study demonstrated that the dimerization of coumarin into bicoumarin (Figure 21) [63] confers significant anti-inflammatory and analgesic properties to the resulting compound.

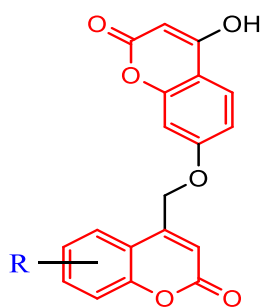


Figure 21 . Chemical structure of a bicoumarin (dimeric state)

I.7.4. Photosensitizing Activity:

Photosensitization refers to an abnormal skin reaction triggered by exposure to sunlight, which can often be induced by certain medications or chemical compounds. Among natural coumarins, two compounds extracted from bergamot-bergapten and psoralen (Figure 22) as well as 6,7-furanocoumarins such as xanthotoxin, exhibit pronounced photosensitizing effects. These compounds enhance the skin's responsiveness to ultraviolet (UV) radiation, particularly the UVA spectrum (320–400 nm), which is primarily responsible for pigmentation processes [64].

This property has been exploited in photodynamic therapy (PDT), a clinical intervention employed to stimulate skin pigmentation in conditions such as vitiligo, and, to a lesser extent, in psoriasis and alopecia areata. The therapeutic procedure involves the oral administration or topical application of photosensitizing coumarin preparations, followed by controlled UVA exposure. The resulting activation of these compounds leads to localized skin responses, promoting melanogenesis and offering a targeted approach for managing pigmentary disorders [35].

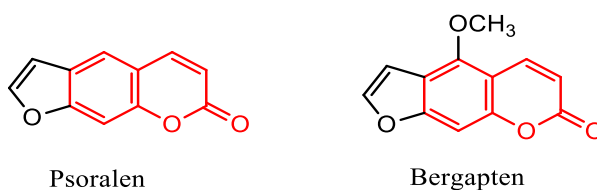


Figure 22 . Chemical structure of photosensitizing coumarins

I.7.5. Anticoagulant Activity:

Coumarin-based compounds are widely recognized for their anticoagulant properties, primarily employed to prevent the formation of blood clots or to inhibit their progression. A prominent example is Warfarin, also known as coumaphene (Figure 23),

which was first synthesized by S. Vilar and Ansell [65]. Warfarin remains the most extensively used oral anticoagulant, owing to its well-established efficacy in the prophylaxis and treatment of thromboembolic disorders. Its anticoagulant activity is mediated through the inhibition of vitamin K-dependent clotting factors, thereby reducing the risk of pathological clot formation while maintaining therapeutic safety [66]. The success of Warfarin has also inspired the development of numerous coumarin derivatives aimed at optimizing pharmacokinetic properties and minimizing adverse effects.

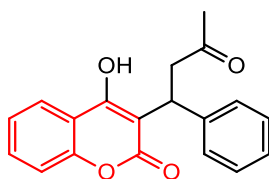


Figure 23 . Chemical structure of Warfarin

I.7.6. Vasodilatory Activity:

Several coumarin derivatives have been shown to exert significant vasodilatory effects, contributing to their cardiovascular therapeutic potential. Notably, Ansell and Chen investigated compounds such as carbochromene [66] and coumarin-7-yl α -methylene- γ -butyrolactone, demonstrating their pronounced ability to induce vasodilation. These effects are thought to result from interactions with vascular smooth muscle and endothelial pathways, leading to the relaxation of blood vessels and improved blood flow. The vasodilatory properties of such coumarin derivatives highlight their potential utility in managing cardiovascular disorders, including hypertension and peripheral vascular diseases.

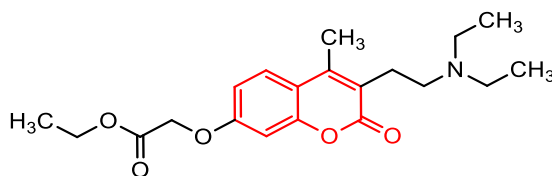


Figure 24. Chemical structure of a coumarin with vasodilatory activity (carbochromene)

I.7.7. Antibiotic Activity:

Several coumarin derivatives have demonstrated notable antibiotic properties, representing an important class of antimicrobial agents. Compounds such as novobiocin, chlorobiocin, and novenamine (Figure 20) [67] are coumarin-based antibiotics that exhibit a broad spectrum of activity, particularly against Gram-positive bacteria. Of special interest is their efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA), a pathogen notorious for its resistance to conventional staphylococcal antibiotics. The antibacterial activity of these coumarin derivatives is generally attributed to their ability to inhibit bacterial DNA gyrase or other critical enzymes, thereby interfering with bacterial replication and survival. These findings underscore the potential of coumarin scaffolds as templates for the development of novel antibiotics targeting resistant bacterial strains.

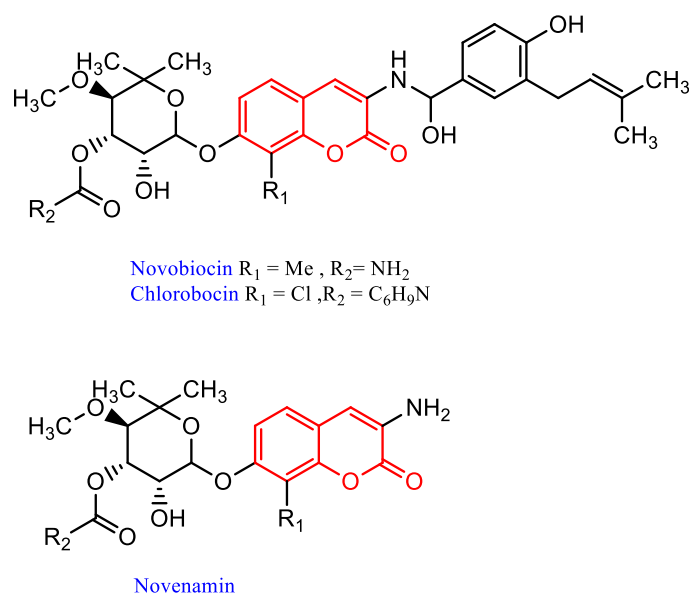


Figure 25. Chemical structure of some coumarins with antibiotic activity

I.7.8. Other Activities:

Coumarins have also been reported to exhibit anti-edematous activity [68], which is primarily attributed to their ability to enhance lymphatic drainage and stimulate the proteolytic activity of macrophages, thereby facilitating the resorption of excess interstitial fluids. Despite these beneficial effects, clinical use of coumarin at high doses has been associated with hepatotoxicity, as evidenced by an increased incidence of hepatitis in treated patients. This safety concern ultimately led to the withdrawal of coumarin-based pharmaceutical products from the market.

Nevertheless, coumarin continues to be employed in herbal medicine at significantly lower and safer doses, particularly in formulations derived from medicinal plants such as sweet clover (*Melilotus officinalis*), where it contributes to therapeutic effects without significant toxicity [69].

I.8. Conclusion:

Finally, coumarin and its derivatives are classified as a unique group of biologically active compounds. Their structural diversity and wide range of pharmacological activities make them important building blocks in medicinal chemistry. The versatility of the coumarin nucleus, combined with its well-established structure–activity relationships, provides a solid foundation for the rational development of novel molecules exhibiting enhanced therapeutic properties.

Contemporary drug design strategies increasingly rely on molecular hybridisation, involving the incorporation of two or more pharmacologically active moieties within a single molecular framework to improve biological performance and overcome the limitations of existing drugs. Among the heterocyclic systems frequently explored for this purpose, the 1,3,4-thiadiazole ring has attracted considerable attention due to its remarkable spectrum of biological activities.

Therefore, linking the coumarin nucleus to the 1,3,4-thiadiazole pharmacological group is a promising approach for developing novel hybrid compounds with synergistic biological effects and enhanced pharmacodynamic properties. The following chapter is devoted to the synthesis and biological evaluation of the coumarin-1,3,4-thiadiazole hybrid compound.

CHAPTER II:

1,3,4-Thiadiazoles: Structural Insights, Synthetic Strategies, and Biological Activities

II.1. Introduction:

Heterocyclic compounds, which include common structural motifs found in naturally occurring biologically active substances, have received sustained attention in medicinal chemistry and drug discovery. These compounds are systematically designed and assembled into chemical libraries that constitute valuable resources for the development of novel therapeutic agents. Among these heterocyclic systems, 1,3,4-thiadiazoles represent an important class of biologically active five-membered aromatic heterocyclic compounds, characterized by the presence of one sulfur atom and two nitrogen atoms within the ring [70] [71].

The presence of these heteroatoms significantly influences the physicochemical and pharmacokinetic properties of thiadiazole derivatives. In particular, the nitrogen and sulfur atoms enhance membrane permeability and enable these molecules to function as versatile hydrogen bond acceptors, thereby promoting efficient interactions with biological targets. Furthermore, the sulfur atom exerts a strong electron-withdrawing inductive effect, contributing to the weakly basic nature of the heterocyclic ring while preserving its aromatic stability [72]. These characteristics enhance both the chemical stability and biological compatibility of thiadiazole derivatives [73].

Additionally, thiadiazole derivatives are considered bioisosteres of pyrimidine and other nitrogen-containing heterocycles because of their comparable electronic properties and structural features. This bioisosteric relationship enhances their value as privileged scaffolds in medicinal chemistry, enabling them to mimic biologically relevant heterocyclic systems and participate in a wide range of molecular recognition processes [74]. Consequently, thiadiazole derivatives are increasingly exploited as key structural motifs in the design and development of biologically active molecules exhibiting a broad spectrum of pharmacological activities [75].

II.2. Structural overview of 1,3,4-Thiadiazole:

Thiadiazole rings can exist in four distinct regioisomeric configurations: 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole [76]. This structural variability, which arises from differences in the relative positions of the heteroatoms within the ring, results in diverse electronic distributions and local

polarization effects, thereby enhancing the chemical and biological versatility of thiadiazole derivatives (Figure 26) [77]. Among these isomeric forms, the 1,3,4-thiadiazole and 1,2,4-thiadiazole frameworks have been the most extensively studied and widely utilized in medicinal and pharmaceutical research.

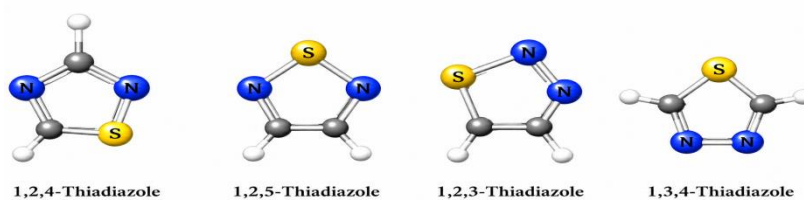


Figure 26 . Regioisomer Forms of Thiadiazoles

II.2.1. Structure and Nomenclature of 1,3,4-Thiadiazole:

1,3,4-Thiadiazole is one of the most important isomers of the thiadiazole family in pharmaceutical chemistry, as it is a heterocyclic aromatic compound with a wide range of biological applications. This compound consists of a five-membered ring with one sulfur atom and two nitrogen atoms at positions 3 and 4, with the ring atoms numbered according to standard nomenclature rules for heterocyclic compounds [78]. This unique structural arrangement of heteroatoms results in a rigid and constrained molecular framework, which gives 1,3,4-thiadiazole high efficacy as a potent pharmaceutical structure. Furthermore, the ring system acts as a hydrogen bonding site and an electron donor, contributing to its ability to react strongly and selectively with a broad spectrum of biological targets [71].

From a structural standpoint, the C–N bond length in the 1,3,4-thiadiazole structure closely resembles that observed in thiazole, whereas the C–S bond length is comparable to that in thiophene. Moreover, the N–N bond exhibits partial double-bond character. These bond length characteristics indicate significant π -electron delocalization across the ring, where formal single bonds acquire partial double-bond character and formal double bonds display partial single-bond behavior. This extensive electron delocalization contributes to the high aromaticity and exceptional stability of the 1,3,4-thiadiazole system [79].

Furthermore, 1,3,4-thiadiazole exists in two partially reduced (dihydrogen) forms known as 1,3,4-thiadiazole, depending on the position of the remaining double bond. In its fully reduced (tetrahydrogen) form, the compound is referred to as 1,3,4-thiazolidine, which is the saturated isotope of the original heterocyclic ring. These structural modifications contribute to the expanded chemical diversity and biological potential of 1,3,4-thiadiazole derivatives.

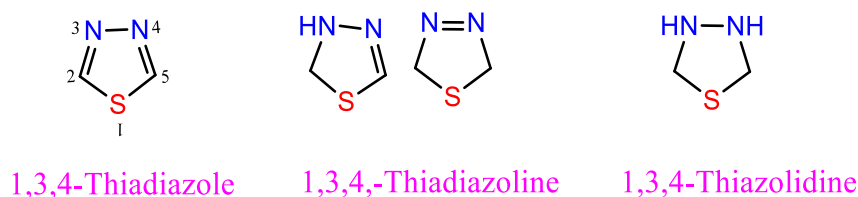


Figure 27. Structural forms of 1,3,4-thiadiazole and its reduced derivatives (dihydro and tetrahydro forms)

II.2.2. Properties of 1,3,4-Thiadiazoles:

The thiadiazole nucleus exhibits chemical properties closely resembling those of the pyrimidine ring, allowing it to be regarded as a bioisostere of this fundamental heterocycle. Since pyrimidine is an essential structural component of nucleobases—the building blocks of DNA and RNA—it is reasonable to assume that thiadiazole derivatives can readily interact with nucleic acids [80]. Such interactions may partially account for the broad spectrum and high potency of the biological activities commonly observed for thiadiazole-containing compounds. The ability of thiadiazole derivatives to interact with DNA further suggests their potential application in chemical intervention at the genetic level, highlighting their relevance in the development of novel therapeutic strategies [81].

From an electronic standpoint, thiadiazoles belong to the class of mesoionic compounds, characterized by a five-membered heterocyclic system rich in heteroatoms and extensive π - π conjugation, characterized by clearly defined areas of opposite charge in the same molecule. This unique electronic structure confers high polarizability, enabling thiadiazole derivatives to efficiently penetrate biological membranes and interact with biomolecular targets. In addition, the presence of sulfur

within the heterocycle enhances lipid solubility, which can positively influence both the biological activity and pharmacokinetic behavior of thiadiazole-based compounds [77].

The growing interest in the synthesis of functionalized five-membered 1,3,4-thiadiazole derivatives stems from their exceptionally broad range of applications. For several decades, 1,3,4-thiadiazole derivatives have been recognized as a prominent class of bioactive compounds exhibiting diverse pharmacological properties, including antibacterial, analgesic, muscle relaxant, anticonvulsant, antimitotic, anti-inflammatory, diuretic, and anticancer activities. Moreover, these compounds have found important non-therapeutic applications as metal-chelating agents [82], as well as oxidation and corrosion inhibitors, further highlighting the versatility and scientific significance of this heterocyclic scaffold [83].

II.3. Preparation methods synthesis of 1,3,4-thiadiazole:

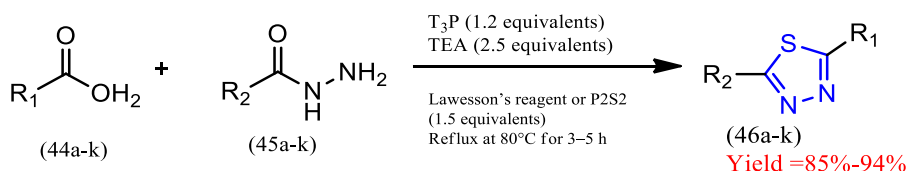
Heterocyclic systems containing the 1,3,4-thiadiazole nucleus have attracted significant scientific interest due to their wide range of biological and functional activities. Recent studies have highlighted the antimicrobial potential of 1,3,4-thiadiazole derivatives, which exhibit pronounced antibacterial and antifungal activities in vitro [84]. Furthermore, the structural diversity of 1,3,4-thiadiazole derivatives has been associated with inhibitory activity against a variety of enzymes, as demonstrated in numerous biological studies. In addition, these compounds have shown promising corrosion-inhibitory properties. Consequently, a wide range of synthetic methodologies has been developed for the preparation of 1,3,4-thiadiazole derivatives, reflecting their remarkable versatility and broad practical importance [85].

II.3.1. Synthesis from acylhydrazides:

II.3.1.1. From the condensation of hydrazide acids with carboxylic acids:

Augustin et al. reported an efficient, single-pot method for synthesizing 1,3,4-thiadiazole derivatives (46a-k), starting from the corresponding carboxylic acids (44a-k) and acid hydrazides (45a-k). The reaction was carried out in the presence of propylphosphonic anhydride (T3P), which acted as a binding agent and cyclic desiccant [86].

The process was conducted using ethyl acetate as the solvent, triethylamine as the base, and phosphorus pentasulfide (P_2S_5), and was carried out under reflux for 3–5 hours in an inert atmosphere. Following completion of the reaction and subsequent purification, The target 1,3,4-thiadiazole derivatives were isolated in excellent yields of 85–94% (see figure 28).



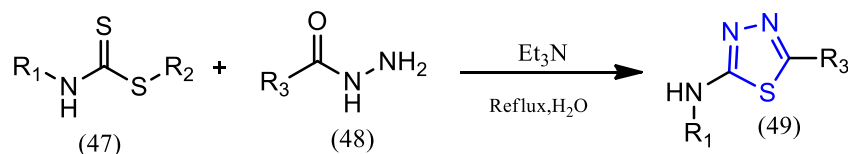
R_1 = a) 4-Me-Ph, b) 4-CN-Ph, c) Cyclopentyl, d) Cyclobutyl, e) 2-Me-propyl, f) 4- NO_2 -Ph, g) 5-Br-furan-2-yl, h) N-Boc-piperidin-4-yl, i) 4- CF_3 -b-styryl, j) 4-Cl-2-Me-Ph

R_2 = a) 3-F-Ph, b) 5-Br-pyridin-3-yl, c) 3-Me-4- NO_2 -Ph, d) tBu, e) 3-F-Ph, f) tBu, g) Me, h) 3-Me-4- NO_2 -Ph, i) H, j) Me

Figure 28 . the condensation of hydrazide acids with carboxylic acids

II.3.1.2. From the condensation of hydrazide acids with dithiocarbamates:

In 2010, Arianasap and colleagues reported the synthesis of novel 1,3,4-thiadiazole derivatives (49) by the condensation reaction of hydrazide acids (48) with dithiocarbamate (47), which allowed for the formation of an inner ring. The reaction was carried out in an aqueous medium under reflux conditions using triethylamine as a catalyst, for 18 hours. After cooling the mixture to room temperature, the target compounds were isolated by filtration, achieving yields ranging from 51% to 97% [87] (figure 29).



R_1 = Ph, 3,4- $Cl_2C_6H_3$, n-Bu, $PhCH_2$, (R)-1-Phenylethyl, Adamantyl

R_2 = $-CH_2CH_2CN$, Et

R_3 = Ph, $PhCH_2$, 4-Me C_6H_4 , 4-Pyridine, 2-HOC $_6H_4$

Figure 29 . the condensation of hydrazide acids with dithiocarbamates

II.3.2. From diacylhydrazides:

N,N'-Diacylhydrazides are commonly used as key precursors in synthesising 1,3,4-thiadiazole derivatives. Several research groups have used this approach with phosphorus-sulfur reagents, particularly Lawesson's reagent, in various organic solvents, including dimethylformamide (DMF), dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), dioxane and toluene.

In this context, Kumar et al. reported the synthesis of a series of 1,3,4-thiadiazole derivatives (51a–m) from the corresponding diacylhydrazides (50a–m) via cyclisation using Lawesson's reagent. The reaction was conducted under reflux in THF at 80 °C for approximately five days. After completion, The targeted 1,3,4-thiadiazole products were isolated and purified using column chromatography with a 7:3 (v/v) ethyl acetate/hexane solvent system. This process yielded compounds with yields ranging from 55% to 75%, classifying them as medium to good [88] (figure 30).

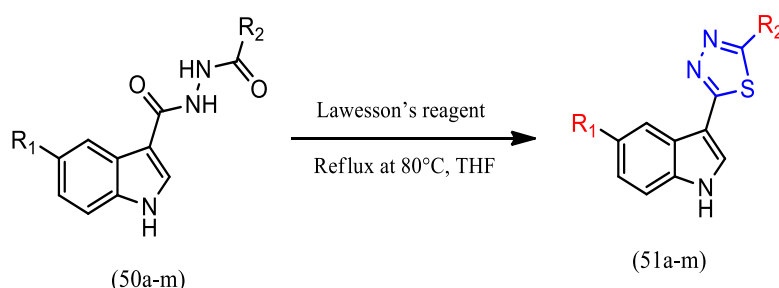


Figure 30 . Synthesis of 1,3,4-thiadiazoles from diacylhydrazides

II.3.3. From Thiohydrazines:

II.3.3.1. From Thiosemicarbazides:

A wide range of 1,3,4-aminothiadiazoles has been synthesized in the presence of phosphoryl chloride (POCl_3) [89]. For instance, Jadhav et al. prepared 2-amino-1,3,4-thiadiazole through the condensation of a carboxylic acid with thiosemicarbazide, leading to intramolecular cyclization. The reaction mixture was refluxed for 30 minutes in 30 mL of POCl_3 , and the resulting product was recrystallized from a DMF/ethanol solvent mixture, yielding 65% [90](figure 31).

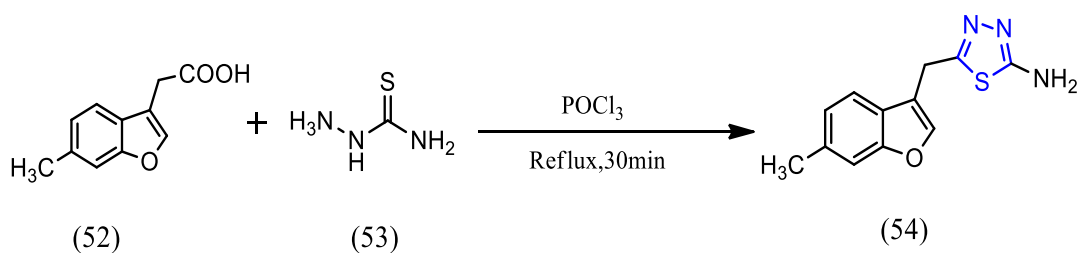
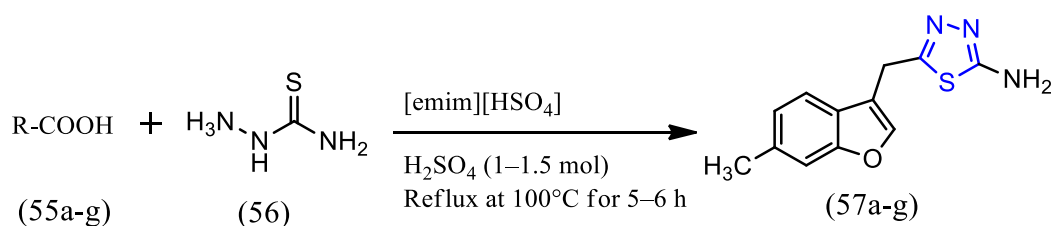


Figure 31 . Synthesis of 1,3,4-thiadiazoles from Thiosemicarbazides

In 2011, Epishina et al. reported an alternative method for the synthesis of 2-amino-1,3,4-thiadiazoles (**57a-g**) using ionic liquids, such as 1-ethyl-3-methylimidazolium hydrosulfate ([emim][HSO₄]). The procedure involves mixing a carboxylic acid (**55a-g**) with thiosemicarbazide (**56**) in [emim][HSO₄], followed by refluxing the mixture at 100 °C under magnetic stirring for 5–6 hours. The desired 2-amino-1,3,4-thiadiazoles are obtained in good to excellent yields, ranging from 54% to 96% [91] (figure 32).



R = PhCH₂, Pr, iPr, CH₂NHBz, MeS(CH₂)₂CH(NHBz), MeCH(NHBz), CH₂(NHTs)

Figure 32. Synthesis of 1,3,4-thiadiazoles from Thiosemicarbazides

II.3.3.2. From Thiosemicarbazones:

A series of substituted 1,3,4-thiadiazoles (**60**) was synthesized using thiosemicarbazides (**58**) and 2-chloroquinoline-3-carbaldehyde derivatives (**59**) as starting materials. The reaction proceeded in the presence of acetic anhydride, with the mixture heated to a temperature between 90 and 100 °C [92]. At the end of the process, the target 1,3,4-thiadiazole compounds were obtained with moderate yields, ranging from 54% to 71%, as illustrated in (figure 33).

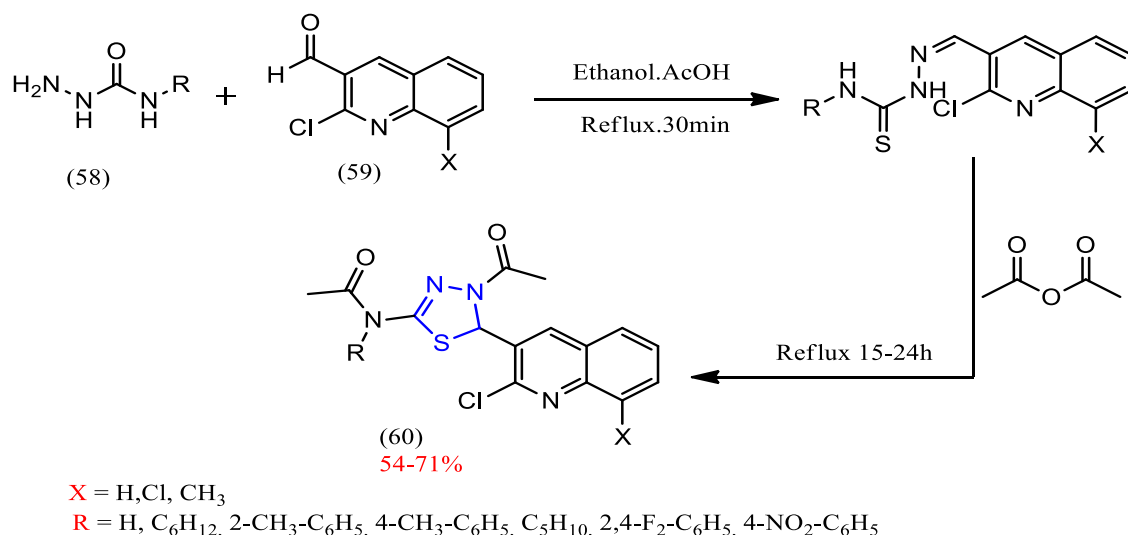


Figure 33 . Synthesis of 1,3,4-thiadiazoles via thiosemicarbazide intermediates

In the following example, 1,3,4-thiadiazole derivatives (62) are synthesized via intramolecular cyclization of thiosemicarbazones (61) using iodine (I_2) and potassium carbonate (K_2CO_3). The reaction mixture is refluxed in 1,4-dioxane [93] (figure 34).

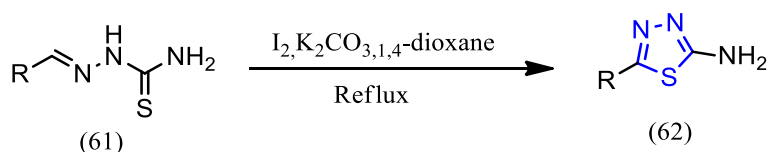


Figure 34. Synthesis of 1,3,4-thiadiazoles from Thiosemicarbazones

II.3.4. From Tosylhydrazones:

The reaction of tosylhydrazones (63) with potassium thiocyanate (KSCN) in the presence of boron trifluoride diethyl etherate ($\text{BF}_3 \cdot \text{OEt}_2$) and N-chlorosuccinimide (NCS), using tert-butanol as the solvent under reflux, affords 2-aminothiadiazole derivatives (64) in yields ranging from 47% to 83% [94] (figure 35).

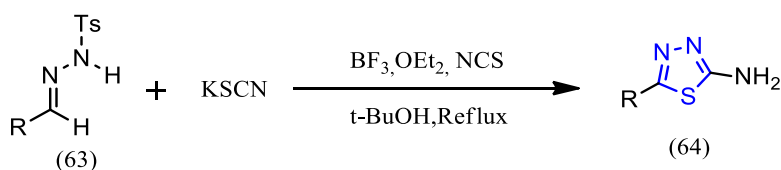


Figure 35. Synthesis of 1,3,4-thiadiazoles from Tosylhydrazones

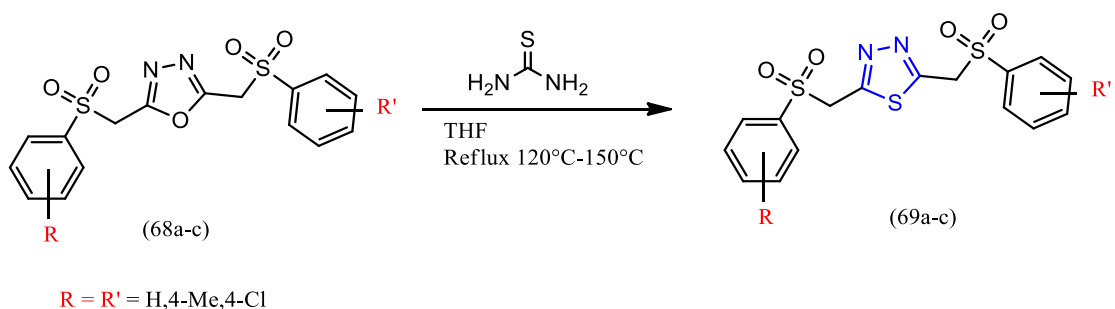


Figure 37. Synthesis of 1,3,4-thiadiazoles from 1,3,4-Oxadiazoles

II.3.7. From Chabrier et al. Reaction:

The reaction is carried out in an aqueous medium using thionicotinamide or thiobenzamide, with dichlorocarbamate serving as the oxidant. During the course of the reaction, elemental sulfur precipitates and is removed by filtration [97] (figure 38).

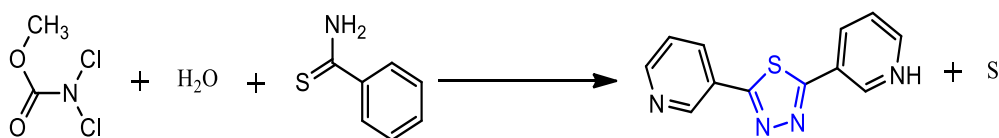


Figure 38 . Synthesis of 1,3,4-thiadiazoles from Chabrier reaction

II.4. Biological Activities of 1,3,4-Thiadiazoles and Their Derivatives:

The biological significance of 1,3,4-thiadiazole derivatives has been extensively studied and recognised for several decades. These compounds are notable for their structural versatility, and their pharmacological performance is often enhanced when they are combined with other heterocyclic systems or coordinated with metal ions [98]. The activity of these derivatives is strongly influenced by both the type and position of the substituents on the thiadiazole ring, as these can alter their chemical and biological properties. A wide range of pharmacological effects has been reported for 1,3,4-thiadiazole derivatives, demonstrating their potential as therapeutic agents. Notably, antiviral, antiparasitic and antituberculosis activities have been observed alongside strong antimicrobial, anticonvulsant, anti-inflammatory and analgesic effects [99]. This broad spectrum of biological activities underscores the importance of 1,3,4-thiadiazoles as privileged scaffolds in drug design and medicinal chemistry [100].

II.4.1. Anti-inflammatory Activity:

The anti-inflammatory potential of 1,3,4-thiadiazole derivatives has been extensively explored due to their structural versatility and pharmacological relevance. In a recent investigation, a series of molecules incorporating the 1,3,4-thiadiazole scaffold was synthesized and evaluated using the rat paw edema model, with diclofenac serving as the reference drug. The experimental results demonstrated that certain derivatives, specifically compounds 75 and 76, exhibited a higher degree of efficacy in reducing paw edema compared to diclofenac at 2, 3, and 4 hours post-administration. The inhibition percentages for these compounds ranged from 30% to 35% for compound 75 and 38% to 48% for compound 76, indicating significant anti-inflammatory activity [97].

These findings underscore the relevance of the 1,3,4-thiadiazole ring as a privileged pharmacophore in the design of anti-inflammatory agents. The study further revealed that the thiadiazole isosteres maintained consistent efficacy across the tested derivatives, suggesting that subtle structural variations in the substituents did not markedly influence their anti-inflammatory properties. Collectively, these results highlight the promising role of 1,3,4-thiadiazole derivatives as potential therapeutic candidates for managing inflammatory conditions, demonstrating advantages in therapeutic activity and possibly a better safety profile versus traditional (NSAIDs) [97] (Figure 39).

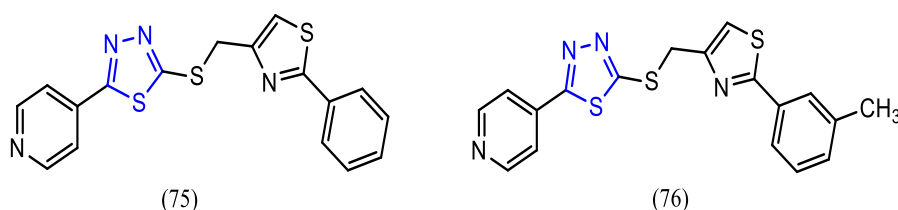


Figure 39 . the chemical structures of the synthesized compounds

II.4.2. Anticancer Activity:

Rajak et al. synthesised a series of 1,3,4-thiadiazole derivatives incorporating hydroxamic acid moieties, evaluating their antitumour potential in murine cancer models. The investigation revealed that these compounds exhibited varying degrees of histone deacetylase (HDAC) inhibitory activity, which is a key target in cancer therapy.

Of the tested derivatives, compound (77) exhibited the highest potency, demonstrating maximal inhibition of HDAC activity. Specifically, compound (77) showed an IC_{50} value of 0.018 μ M against HDAC-1 and an IC_{50} value of 0.31 μ M in HCT-116 colon cancer cells, indicating strong cytotoxic and anti-tumour potential (see Figure 40). These results suggest that hydroxamic acid-linked thiadiazoles have great potential as HDAC inhibitors and as anticancer agents [101].

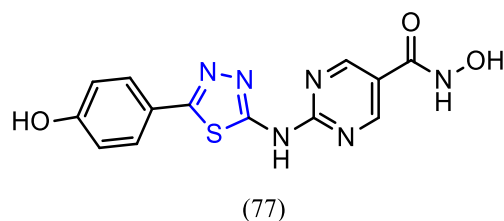


Figure 40. the chemical structure of the synthesized compound 77

Novel 1,3,4-Thiadiazole Derivatives

In a separate study, Janowska et al. synthesised a new series of 1,3,4-thiadiazole derivatives to evaluate their in vitro anticancer activity across different breast cancer cell lines. Within the series compound 78 showed the greatest efficacy against MCF-7 breast cancer cells, reducing cell viability to $74\% \pm 3$ at a concentration of 100 μ M. Meanwhile, compound 79 was identified as the most effective derivative toward MDA-MB-231 breast cancer cells, decreasing cell viability to $75\% \pm 2$ at the same concentration (see Figure 41). These outcomes suggest that slight variations in the thiadiazole scaffold and the position of substituents can greatly affect the efficacy of cytotoxicity, emphasising the importance of structural optimisation in designing anticancer thiadiazole derivative [102].

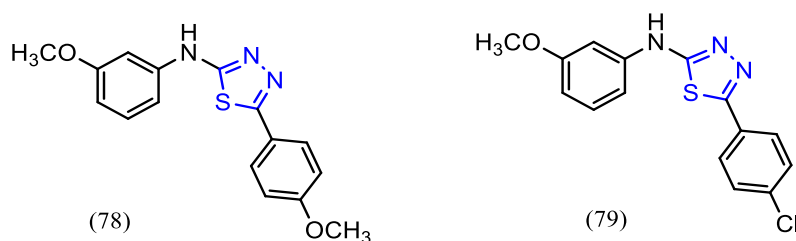


Figure 41. the chemical structures of the synthesized compounds 78 and 79

II.4.3. Antiviral Activity:

In late December 2019, a novel infectious disease, which was later designated as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, and rapidly disseminated to multiple countries worldwide. In view of the serious consequences of the virus on human health, the World Health Organization (WHO) formally designated the outbreak as a global pandemic in March 2020. At that time, no commercially available treatment existed, making the search for effective therapeutic agents an urgent priority for the scientific community.

In response to this urgent need, a research group in 2020 synthesised a series of eight novel 1,3,4-thiadiazole derivatives and evaluated their potential antiviral activity using computational methods. Specifically, *in silico* studies were performed to investigate the interaction of these compounds with the TMPRSS2 enzyme, a key protein involved in viral entry, using the PyRx docking software. In order to carry out a more in-depth assessment of the drug-likeness and pharmacokinetic profiles of the compounds, additional ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) studies were conducted using open-source software tools. These tools provided insight into the absorption, distribution, excretion, metabolism and toxicity parameters [103].

In the course of synthesising eight derivatives, it was found that compound **(80)** exhibited the strongest binding affinity for TMPRSS2, with a calculated docking score of -9.1 kcal/mol. This finding indicates a robust and stable interaction with the target enzyme. Compound **(80)** may represent a viable candidate for further investigation as a treatment for SARS-CoV-2, according to these findings. This study underscores the promise of 1,3,4-thiadiazole scaffolds in the realm of antiviral drug design, particularly in the context of emergent viral diseases where expeditious identification of efficacious compounds is paramount [104] (see Figure 41).

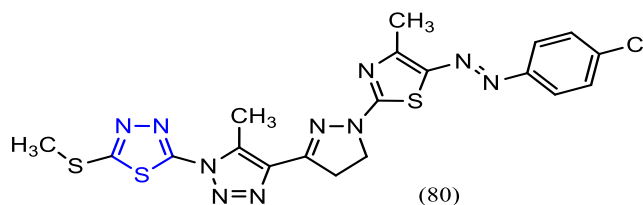


Figure 42. the chemical structure of the synthesized compound 80

II.4.4. Antituberculosis Activity:

In the study by Patel et al. a series of substituted 1,3,4-thiadiazole derivatives were synthesised and their in vitro antituberculosis activity evaluated. This activity was assessed against both the standard *Mycobacterium tuberculosis* H37Rv strain and multidrug-resistant tuberculosis (MDR-TB) strains. The objective of the study was to identify compounds capable of inhibiting the growth of *M. tuberculosis* while maintaining low toxicity towards mammalian cells [105].

Among the derivatives that were tested, compound **(81)** showed notable inhibition, displaying a MIC value of 9.87 μM against both the H37Rv and MDR-TB strains. For the purpose of comparison, the standard antituberculosis drugs were examined, with isoniazid exhibiting an MIC of 3.64 μM against H37Rv and >200 μM against MDR-TB, while rifampicin displayed an MIC of 0.152 μM against H37Rv and 128 μM against MDR-TB. These results indicate that compound **(81)** possesses significant antituberculosis activity, particularly against drug-resistant strains, thus highlighting its potential as a lead compound for further drug development [105].

Furthermore, the in vitro mammalian Vero cell line was utilised to assess the cytotoxic potential of these thiadiazole derivatives, thereby confirming that the active compounds exert their antituberculosis effects at non-toxic concentrations. This demonstrates a favourable safety profile and supports the potential therapeutic relevance of 1,3,4-thiadiazole scaffolds in the design of novel antituberculosis agents [105] (Figure 42).

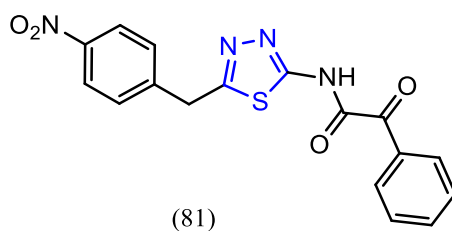


Figure 43 . the chemical structure of the synthesized compound 81

II.4.5. Antioxidant activity:

Khan et al. (2010) demonstrated that the incorporation of an electron-withdrawing nitro substituent significantly improves the antioxidant performance of 1,3,4-thiadiazole

derivatives. This class of compounds exhibits pronounced antioxidant properties, particularly in scavenging superoxide radicals. A newly synthesised series of derivatives (9a–9h) was evaluated for free radical scavenging activity, among which compound 9h showed the highest antioxidant efficacy, with an IC_{50} value of 94.84 ± 0.34 , exceeding that of the reference antioxidant propyl gallate [106].

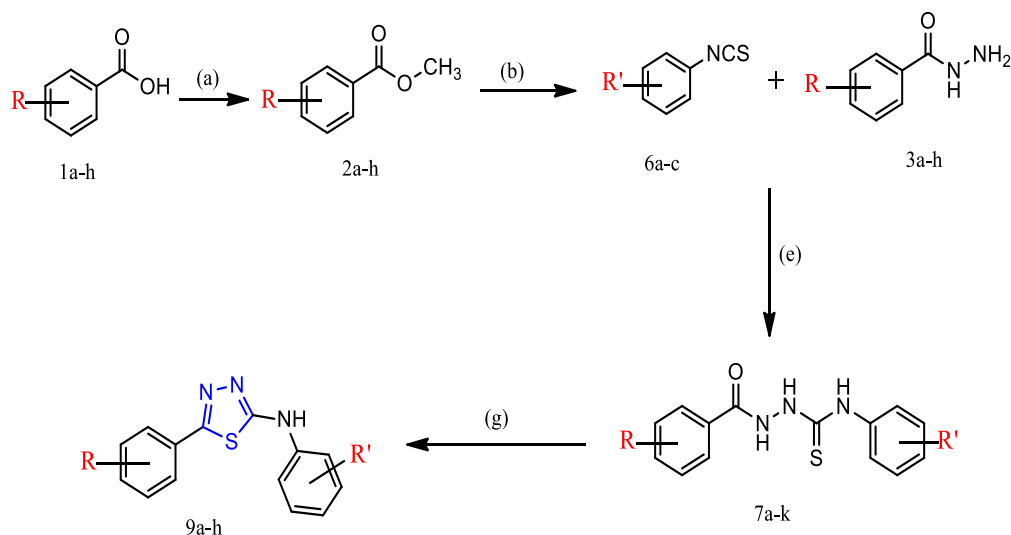


Figure 44 . Synthesis of 1,3,4-thiadiazole derivatives. (a) Dry methanol, concentrated H_2SO_4 , reflux; (b) Dry methanol, hydrazine hydrate (80%), reflux; (e) Dry methanol, reflux; and (g) Polyphosphoric acid (PPA), stirring

The superior activity of compound 9h is mainly attributed to the strong electron-withdrawing nature of the nitro group, which effectively stabilises the negatively charged intermediate formed during the superoxide radical scavenging process. In addition, compounds 9c, 9d, and 9g exhibited antioxidant activities comparable to the standard, whereas compound 9f displayed moderate activity. These findings highlight the critical influence of electronic factors on the antioxidant behavior of 1,3,4-thiadiazole derivatives and reinforce their potential as promising therapeutic agents for managing oxidative stress-related disorders [106].

II.4.6. Antimicrobial Activity:

Bath et al. synthesised a series of 1,3,4-thiadiazole derivatives containing a quinoline moiety and assessed their antimicrobial activity against a range of bacterial strains, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium*,

and *Escherichia coli*. The objective of the present study was to investigate the efficacy of these compounds in inhibiting bacterial growth and to compare their activity with that of standard antibiotics [92].

Compounds **70** and **71** showed the highest potency among the series of synthesized derivatives antibacterial activity against *Staphylococcus aureus*, achieving a minimum inhibitory concentration (MIC) of 16 µg/mL, which is notably more potent than amoxicillin, which showed an MIC of 32 µg/mL against the same strain. In addition, compounds **72** and **73** exhibited superior inhibition of *Streptococcus pyogenes* relative to the reference drug. The findings indicate that the 1,3,4-thiadiazole scaffold, particularly when combined with a quinoline moiety, can enhance antibacterial efficacy, highlighting these derivatives as potential candidates for the development of novel antimicrobial agents [92] (Figure 45).

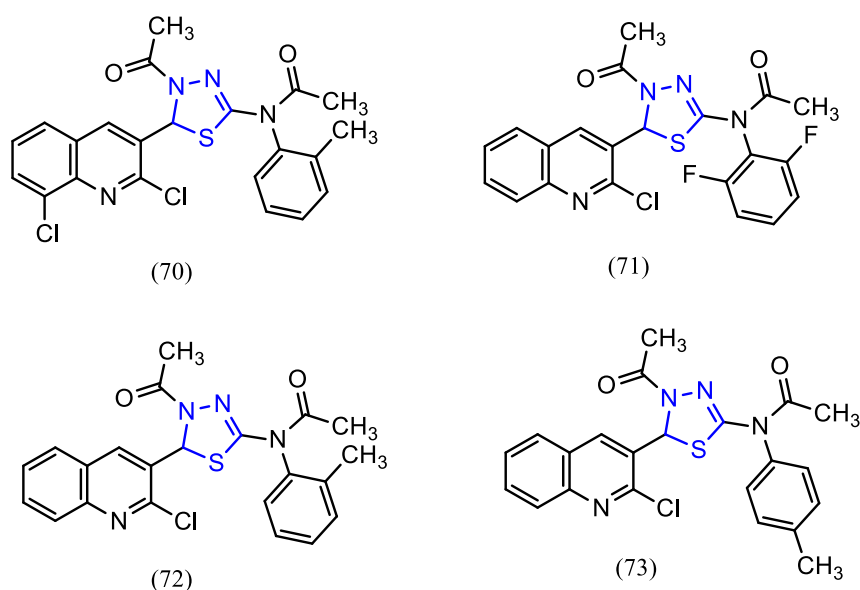


Figure 45. the chemical structures of the synthesized compounds

Conversely, Kamel and colleagues synthesised a series of 1,3,4-thiadiazole derivatives and evaluated their antimicrobial potential in vitro against a variety of bacterial and fungal strains. Among the compounds that were examined, compound **74** exhibited significant inhibitory activity, demonstrating notable efficacy against *Bacillus mycoides*, *Candida albicans*, and *Escherichia coli*. Its performance was comparable to, and in some cases surpassed, that of streptomycin, which was used as the reference standard. These findings emphasise the versatility of the 1,3,4-thiadiazole scaffold in

conferring potent antimicrobial properties and underscore its potential for the creating of new broad-spectrum antibacterial and antifungal agents [107] (Figure 46).

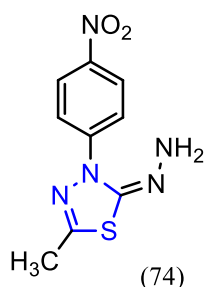


Figure 46. the chemical structure of the synthesized compound

II.4.7. Anticonvulsant Activity:

Stillings et al. investigated the anticonvulsant properties of a series of substituted 2-hydrazino-1,3,4-thiadiazole derivatives. The study demonstrated that these compounds exhibit significant activity in suppressing seizure activity in experimental models. In the course of the present study, a range of derivatives were subjected to rigorous testing. Among these, 2-(aminomethyl)-5-(2-biphenyl)-1,3,4-thiadiazole (32) was recognized for its potent anticonvulsant properties in both rodent models [108].

The efficacy of this compound was found to be similar to that of standard anticonvulsant drugs, including phenytoin, phenobarbital, and carbamazepine, across multiple experimental seizure models. These results suggest that the 1,3,4-thiadiazole scaffold, when appropriately substituted, can serve as a promising framework for the development of novel anticonvulsant agents. The study under discussion highlights the potential of such derivatives to provide therapeutic benefits, possibly with improved pharmacological profiles, offering an alternative to conventional anticonvulsant medications [108].

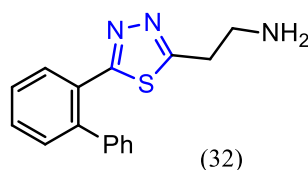


Figure 47 . the chemical structure of the synthesized compound 32

Dogan et al . prepared a series of 1,3,4-thiadiazole derivatives and observed that compounds 33a and 33b exhibited significant anticonvulsant activity. These results suggest that these two derivatives could serve as promising lead compounds for the development of novel anticonvulsant agents, highlighting the potential of the thiadiazole scaffold in neuropharmacological applications [109].

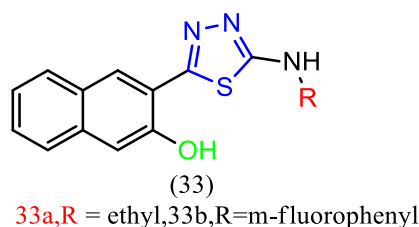


Figure 48. the chemical structure of the synthesized compound 33a

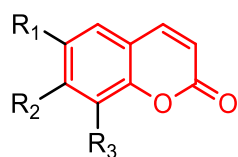
II.5. Coumarin 1, 3,4-Thiadiazole Derivatives:

II.5.1. Generality:

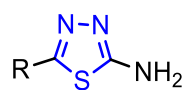
The synthesis of two or more pharmacologically active molecules into a single hybrid molecule has become a crucial strategy in modern drug design. This approach facilitates the development of new derivatives that often exhibit superior biological activity compared to their parent compounds, while minimising toxicity and undesirable side effects. This tendency is especially pronounced in the field of coumarin chemistry, where there has been a notable surge in the number of hybrid molecules derived from the coumarin structure that have been documented, particularly with regard to their inhibitory effects on a multitude of biological enzymes. A comparable progression has been witnessed in 1,3,4-thiadiazole chemistry, where many hybrid and conjugated derivatives have revealed extensive and pronounced biological activity [110].

Coumarins and 1,3,4-thiadiazoles are highly important heterocyclic compounds in pharmaceutical chemistry due to their presence in a wide range of biologically active compounds, both natural and synthetic and their unique physical and chemical properties that facilitate structural modification and their use in designing novel therapeutic and functional molecules [111]. Hybrid coumarin derivatives have demonstrated remarkable efficacy, particularly in antimicrobial activity often surpassing that of some standard drugs. 1,3,4-Thiadiazole derivatives are valuable structural

frameworks for developing antimicrobial agents, particularly given the increasing prevalence of drug resistance [112].



Coumarin



1,3,4-thiadiazole

Recent studies have shown that several coumarin and thiadiazole derivatives act as potent cholinesterase inhibitors, making them promising candidates for developing new drugs to treat neurodegenerative diseases such as Alzheimer's and Parkinson's. The presence of electron-donating heteroatoms in these compounds also gives them a high chelating capacity with transition metal ions, a crucial property given the pathological role of metals in the development of neurodegenerative disorders [113].

II.5.2 . Some methods for synthesizing 1,3,4-thiadiazole coumarin derivatives:

procedure (Schiff base synthesis)

In the study by Rufino VC et al. a general method for the preparation of coumarin–1,3,4-thiadiazole hybrids was reported. This method involved the condensation of coumarin-3-carbaldehydes (3A–3C, 2 mmol) with 2-amino-5-phenyl-1,3,4-thiadiazole derivatives (4A–4C, 2 mmol). In this approach, the reactants were combined in absolute ethanol as the solvent, with a small amount of acetic acid serving as a catalyst (Scheme 2). The reaction was carried out under reflux conditions to encourage Schiff base formation, after which the resulting compounds were allowed to precipitate. The solid products were then isolated by filtration and purified through crystallisation from ethanol [114].

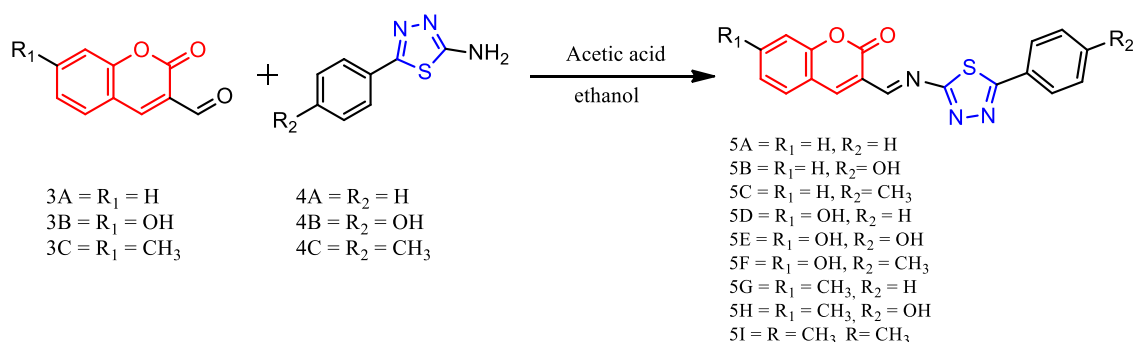


Figure 49. General procedure for the synthesis of 1,3,4-thiadiazole-coumarin hybrid derivatives

Structural confirmation of the coumarin–thiadiazole hybrids was achieved through IR and NMR spectroscopic analyses, ensuring both the correct functional groups and the molecular framework were intact. Efficient strategies for constructing these derivatives often involve stepwise condensation reactions, in which imine formation can serve as a key intermediate [115].

Hu et al. applied analogous synthetic principles in their development of a series of coumarin derivatives, incorporating 1,3,4-thiadiazole/1,3,4-oxadiazole moieties through stepwise condensation and heterocyclization reactions. The work of the present authors thus confirms the versatility of coumarin scaffolds as key building blocks for the construction of sulfur- and nitrogen-containing heterocycles, particularly 1,3,4-thiadiazole, which is frequently introduced via thiosemicarbazide-based cyclization protocols [116].

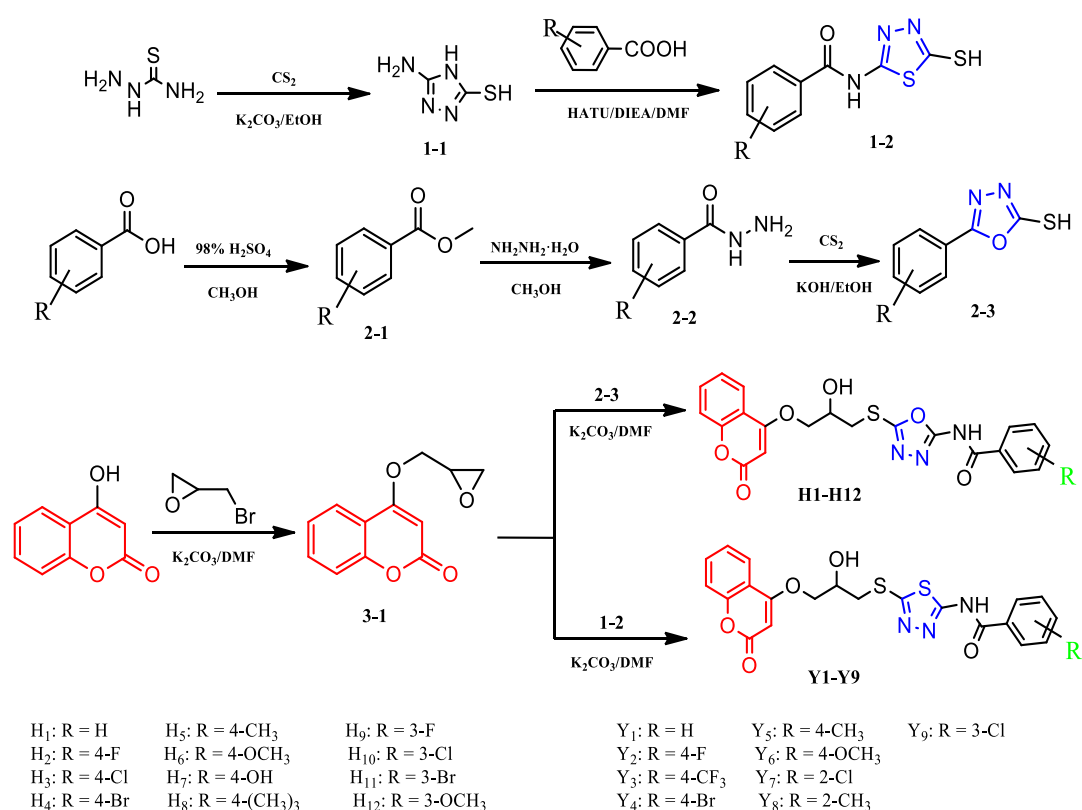


Figure 50. Synthetic route of the target compounds H1–H12 and Y1–Y9

In addition to these conventional stepwise strategies, several studies have demonstrated the feasibility of one-pot synthetic approaches for the construction of 1,3,4-thiadiazole derivatives. These approaches offer attractive alternatives that minimise purification steps, reduce reaction time, and improve overall synthetic efficiency. Despite the

preponderance of documented one-pot protocols centring upon thiadiazole frameworks devoid of coumarin units, these methodologies furnish a seminal conceptual framework for the prospective advancement of one-pot syntheses of coumarin–1,3,4-thiadiazole hybrids. This enhancement holds the potential to augment their synthetic accessibility and scalability [117].

II.6. Conclusion:

In summary, the 1,3,4-thiadiazole nucleus represents a highly valuable heterocyclic scaffold in medicinal chemistry due to its unique electronic structure, chemical stability and remarkable pharmacological versatility. The presence of both sulfur and nitrogen heteroatoms within a compact aromatic ring system confers distinctive physicochemical properties that strongly influence molecular recognition, binding affinity and biological performance.

Exhibiting activities such as antimicrobial, anticancer, antioxidant, anti-inflammatory, anticonvulsant, and antiviral effects, 1,3,4-thiadiazole derivatives exemplify the significant therapeutic promise of this heterocycle. Extensive SAR studies indicate that slight structural modifications on the thiadiazole ring can substantially modulate biological responses, allowing for targeted optimization of potential therapeutics.

Furthermore, the molecular hybridization of the 1,3,4-thiadiazole pharmacophore with the coumarin scaffold emerges as a particularly promising strategy in contemporary drug design. This approach combines the well-established bioactivity of coumarins with the pharmacological richness of the thiadiazole moiety, generating hybrid systems capable of producing synergistic biological effects and improved therapeutic profiles.

Consequently, the integration of the 1,3,4-thiadiazole framework into coumarin-based structures constitutes a rational and scientifically justified pathway toward the development of innovative bioactive compounds. This concept provides a strong conceptual foundation for the experimental work presented in the following chapters, which focuses on the synthesis and biological evaluation of new coumarin 1,3,4 – thiadiazole hybrids.

CHAPTER III:
Results and Discussion

III.1. Introduction:

In previous studies, several alkylated coumarin derivatives were synthesized and characterized, allowing for important insights into their chemical structures and behaviors. Building on this foundation, the present work focuses on the design and synthesis of novel asymmetric coumarin–1,3,4-thiadiazole hybrids by incorporating thiosemicarbazide into the coumarin framework. This structural modification was undertaken to introduce new functionalities and explore potential biological activities, particularly their antioxidant effects and inhibition of acetylcholinesterase. The following sections will detail the synthesis, structural characterization, and comprehensive biological evaluation of these newly prepared derivatives.

III.2. Chemical synthesis of alkylated coumarin derivatives:

The synthetic route for preparing coumarin–1,3,4-thiadiazole derivatives was accomplished through a three-step strategy involving the synthesis of substituted starting coumarin scaffolds, their functionalization via a MALDI cascade reaction, and the final cyclization with thiosemicarbazide to afford the desired heterocyclic hybrids. This sequential approach enabled efficient modification of the coumarin core while maintaining good reaction selectivity and product purity.

The starting coumarin scaffolds (C1–C3) were synthesized through a classical Knoevenagel condensation [118] between dimethyl malonate and various substituted salicylaldehydes (salicylaldehyde, o-vanillin, and 4-(diethylamino)-2-hydroxybenzaldehyde) in ethanol using piperidine as a mild base catalyst. Mechanistically, piperidine first deprotonates the activated methylene group of dimethyl malonate to generate a stabilized enolate, which subsequently attacks the electrophilic carbonyl carbon of the aldehyde. The resulting intermediate undergoes dehydration followed by intramolecular lactonization, leading to the formation of the coumarin ring system under relatively mild reaction conditions [119], [120], [121].

The proposed reaction mechanism for the synthesis of the starting coumarin scaffolds is illustrated below.

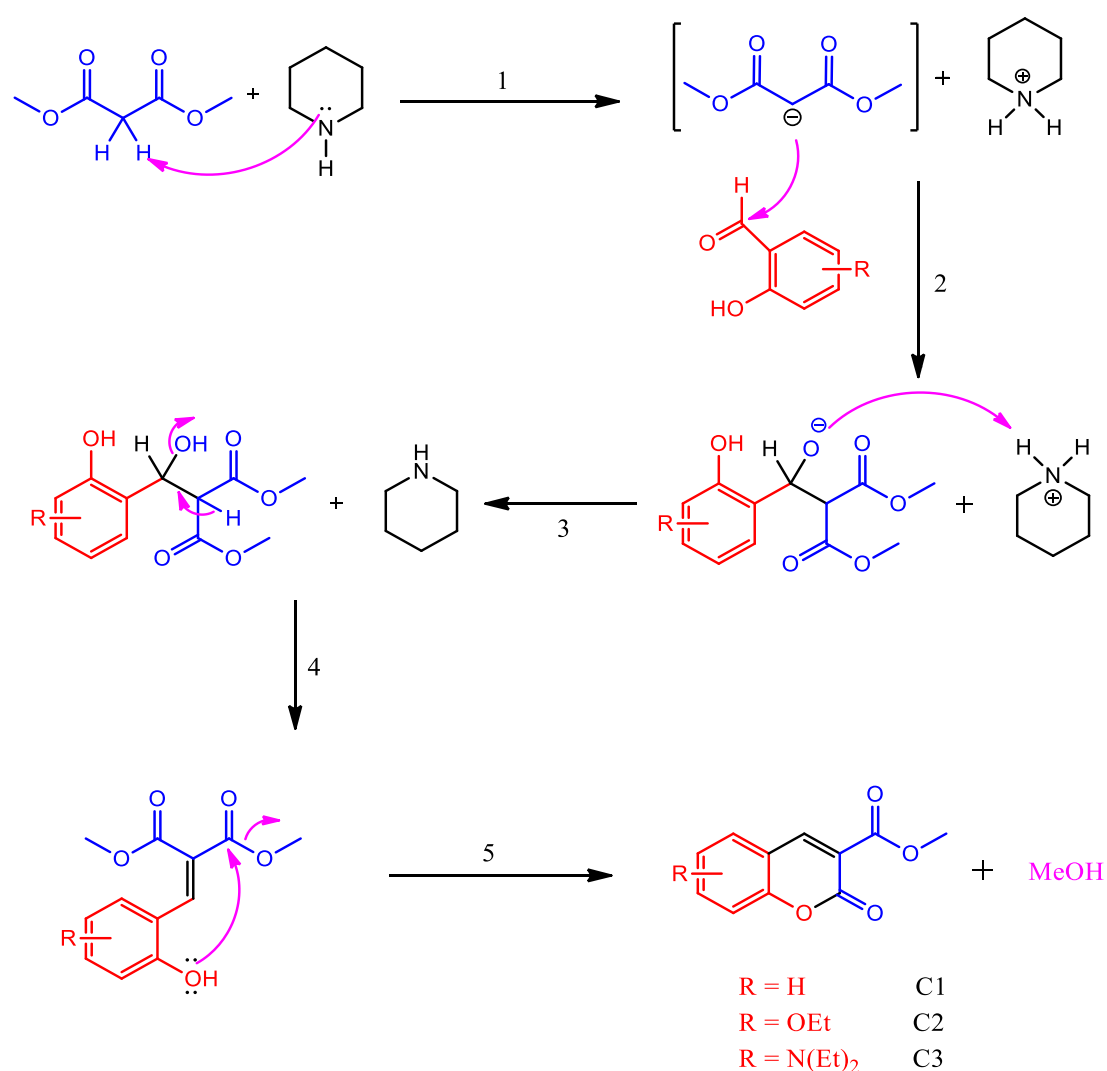
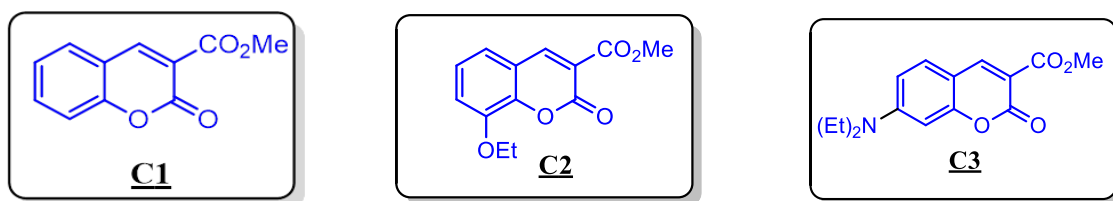
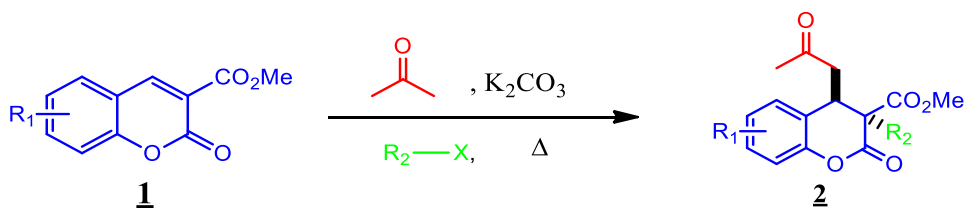
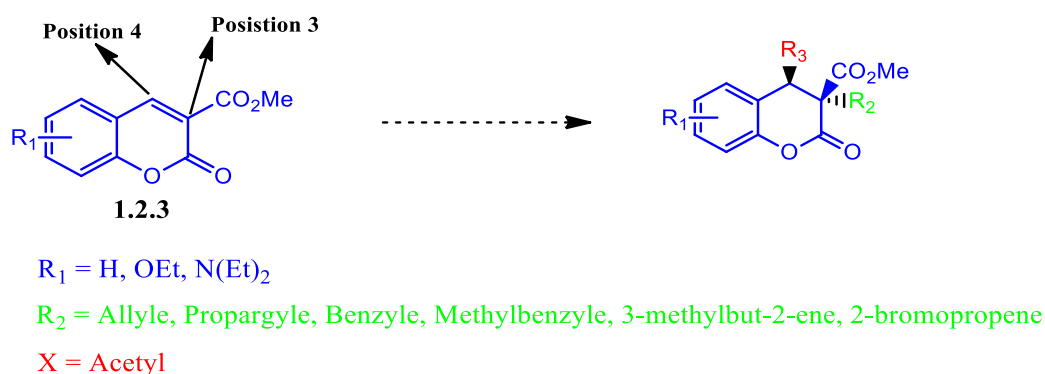


Figure 51. Schematic representation of the synthesis of coumarin derivatives (C1–C3)

Three coumarin scaffolds (C1–C3) were successfully obtained using this protocol. The synthesized compounds differ in the aromatic substituent ($R = \text{H}$, OEt , and $\text{N}(\text{Et})_2$), which influences the electronic distribution within the coumarin nucleus. In particular, the electron-donating ethoxy (C2) and diethylamino (C3) substituents enhance electron density and π -conjugation of the aromatic ring, thereby affecting the subsequent chemical reactivity of the coumarin scaffold (Wang et al., 2020).

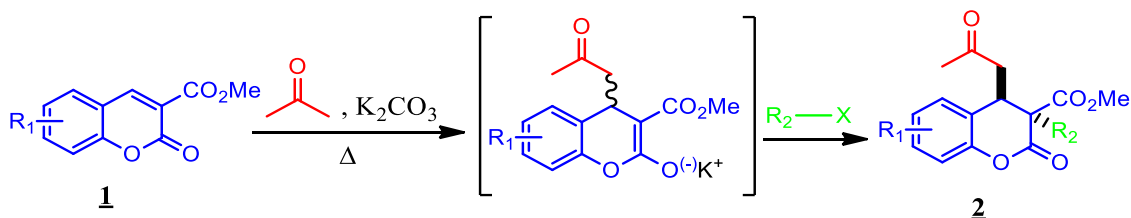


Following the successful synthesis of the starting coumarins, structural diversification was achieved through a MALDI cascade reaction involving an initial Michael addition followed by selective alkylation at the C3 position under basic conditions [124]. The general synthetic pathway is illustrated in Figure 52.



Step 1 : Maldi cascade reaction

Michael reaction followed by an alkylation reaction.



Step 2 : Mechanism of the MALDI cascade reaction.

Figure 52 . The general preparation of asymmetric coumarins and their subsequent derivatization. Coumarin precursors (C1–C3) undergo a cascade addition followed by alkylation to afford diverse asymmetric derivatives

The nature of the introduced alkyl substituent had a significant influence on the reaction efficiency, with isolated yields ranging from 80% to 90% (Table 4). Allyl- and propargyl-substituted derivatives afforded the highest yields (90%), mainly owing to their relatively low steric hindrance and higher reactivity toward nucleophilic substitution (Adimule et al., 2025; Lončarić et al., 2020; Salem et al., 2016). In contrast, bulkier substituents, such as benzyl and methylbenzyl groups, resulted in slightly lower yields (approximately 80%), most likely because of increased steric congestion around the electrophilic center, which reduces the efficiency of the substitution process. These observations are consistent with the well-established steric and electronic factors governing substitution reactions in activated coumarin systems.

To provide a mechanistic interpretation of this transformation, a plausible reaction pathway is proposed using the allyl-substituted derivative as a representative example. As shown in Figure 53, the cascade process begins with the Michael addition of the acetone-derived enolate to the activated coumarin double bond, followed by the generation of a new nucleophilic intermediate that undergoes an S_N2 alkylation with the corresponding alkyl halide to afford the final functionalized coumarin derivative.

Conversely, bulkier substituents, such as benzyl and methylbenzyl groups, led to slightly diminished yields (80%), likely as a result of increased steric hindrance that impeded efficient approach to the reactive centre. These trends are consistent with well-established steric and electronic effects governing electrophilic and nucleophilic substitutions in activated aromatic systems.

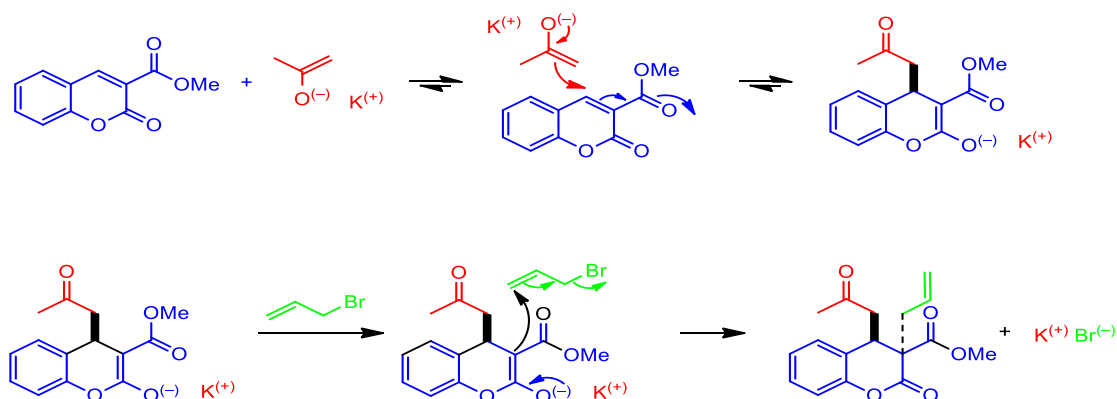
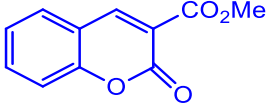
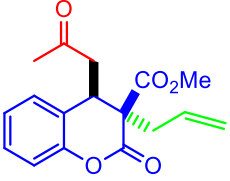
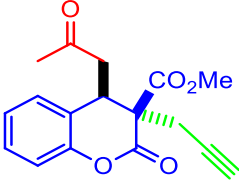
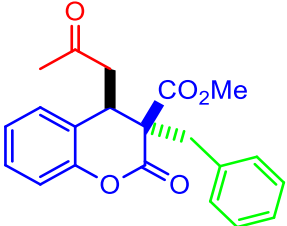
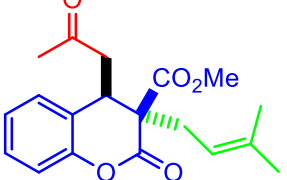
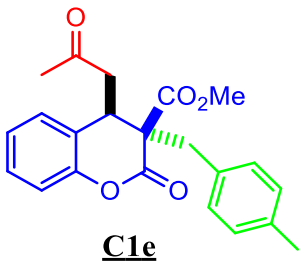
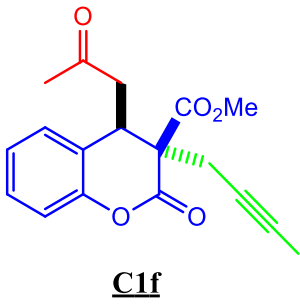
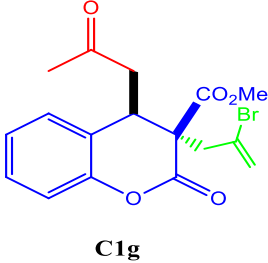
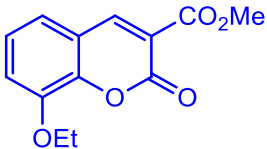
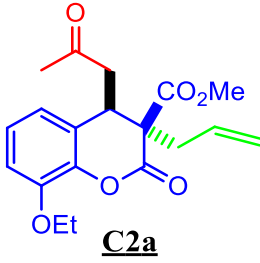
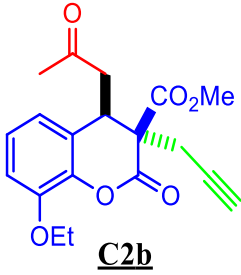


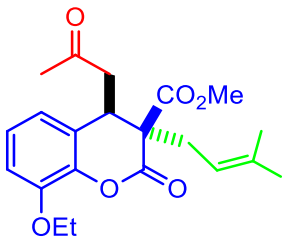
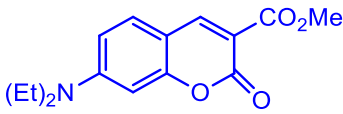
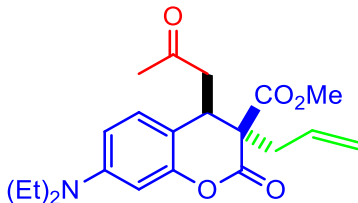
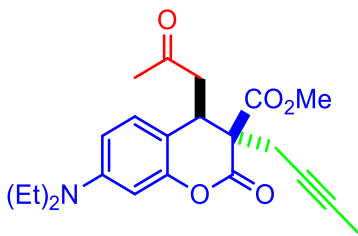
Figure 53. Mechanism of the MALDI cascade reaction

Using this synthetic strategy, a library of twelve asymmetric alkylated coumarin derivatives was successfully obtained. Their structures and isolated yields are summarized in Table 3.

Table 3. Characteristics and yields of synthesized coumarin derivatives C1a to C3b.

Starting Coumarins	Reagent (R ₂ -X)	Products	Yields (%)
 C1	Allyl Bromide	 C1a	90
	Propargyl bromide	 C1b	91
	Benzyl chloride	 C1c	85
	1-Bromo-3-methylbut-2-ene	 C1d	88

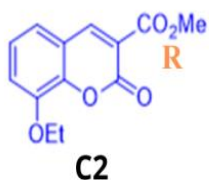
	Methylbenzyl chloride	 C1e	83
	1-Chloro-2-butyne	 C1f	89
	2-Bromo-3-chloropropene	 C1g	87
 C2	Allyl Bromide	 C2a	91
	Propargyl bromide	 C2b	90

	Methylbenzyl chloride	 C2c	82
 C3	Allyl Bromide	 C3a	89
	1-Chloro-2-butyne	 C3b	84

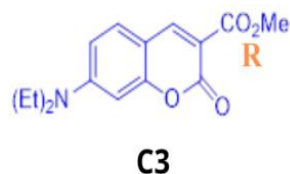
- High yield (>90%)
- Moderate (85–89%)
- Lower (<85%)



R = Methylbenzyl → 83%
 R = Benzyl → 85%
 R = 2-Bromo-3-chloroprop-1-enyl (halogenated allylic) → 87%
 R = 1-Bromo-3-methylbut-2-enyl (branched allylic) → 88%
 R = 1-Chloro-2-butyne (halogenated alkynyl) → 89%
 R = Allyl → 90%
 R = Propargyl → 91%



R = Methylbenzyl → 82%
 R = Propargyl → 90%
 R = Allyl → 91%



R = Allyl → 89%
 R = 1-Chloro-2-butyne (halogenated alkynyl) → 84%

Bulky substituents → Small linear substituents
 Lower yield → Higher yield

Reaction yield increases with decreasing steric bulk and increasing linearity of the substituent at the C-3 position

Figure 53. Structure–yield relationship (SAR) illustrating the effect of substituent nature at the C-3 position of coumarin scaffolds (C1–C3) on synthetic yield

As demonstrated in Figure 53, A clear correlation between structure and yield is evident for substitutions at the C-3 position of the coumarin scaffold. Linear substituents with minimal steric bulk, such as allyl and propargyl groups, have been shown to consistently afford superior synthetic yields [129]. This can be attributed to their favourable spatial orientation and reduced steric congestion during the substitution process. Conversely, bulkier or more branched substituents were associated with moderate reductions in yield, which can be attributed to increased steric hindrance that limits efficient access to the reactive centre and compromises productive bond formation [130].

These observations suggest that steric factors, particularly substituent size, shape, and linearity, are the predominant parameters influencing reaction efficiency substituted at the C-3 site of the coumarin core. Electronic contributions, in contrast, appear to be of secondary importance. The data therefore underscore the significance of steric accessibility in optimising functionalisation at this site, thus providing valuable guidance for the rational design of future coumarin-based derivatives with improved synthetic performance.

III.3. Chemical synthesis of coumarin 1,3,4-thiadiazole derivatives:

The condensation reaction of the corresponding alkylated coumarin precursors with thiosemicarbazide was utilized to synthesize the novel coumarin–1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, and T3a–T3b), employing piperidine as an efficient catalytic promoter in an acetic acid medium (see Figure 4). The efficacy of this synthetic pathway is primarily attributable to the intrinsic electrophilicity of the carbonyl functionality, which is highly activated under the applied reaction conditions to facilitate nucleophilic attack. Specifically, the terminal amino group ($-NH_2$) of thiosemicarbazide attacks the activated carbonyl carbon, generating a key hydrazone-like intermediate. This intermediate subsequently undergoes intramolecular cyclization and dehydration processes, ultimately resulting in the construction of the heteroaromatic

1,3,4-thiadiazole ring system [131]. This stage of the synthesis proved to be highly robust and reproducible, as demonstrated by the consistently high isolated yields obtained for most of the target hybrids, which typically surpassed 82% (see Table 2) [132]. Such results confirm the efficiency of the optimized reaction protocol and highlight its suitability for the scalable preparation of these hybrid molecules with minimal material loss during purification. Consequently, the described synthetic approach is both reliable and versatile, offering a highly efficient method for producing structurally diverse coumarin–thiadiazole hybrids in excellent yields. From a mechanistic viewpoint, the high reactivity of the carbonyl functionality originates from the strong polarization of the C=O bond, which confers a marked electrophilic character upon the carbonyl carbon atom [133]. This electrophilicity is further intensified through the synergistic effect of the acetic acid-piperidine catalytic system, wherein the base promotes the formation of a highly reactive iminium-type species, thereby lowering the activation energy for the nucleophilic attack by the terminal amino group of thiosemicarbazide. The subsequent intramolecular ring-closure process is promoted by favorable proximity and conformational effects, which reduce the energetic barrier for cyclization and drive the reaction toward the formation of the thermodynamically stable 1,3,4-thiadiazole heterocycle. This sequential condensation–cyclization pathway ultimately leads to enhanced stabilization of the final hybrid architectures through extended π -conjugation across the newly formed heteroaromatic system [134], thereby significantly contributing to their structural integrity and chemical stability.

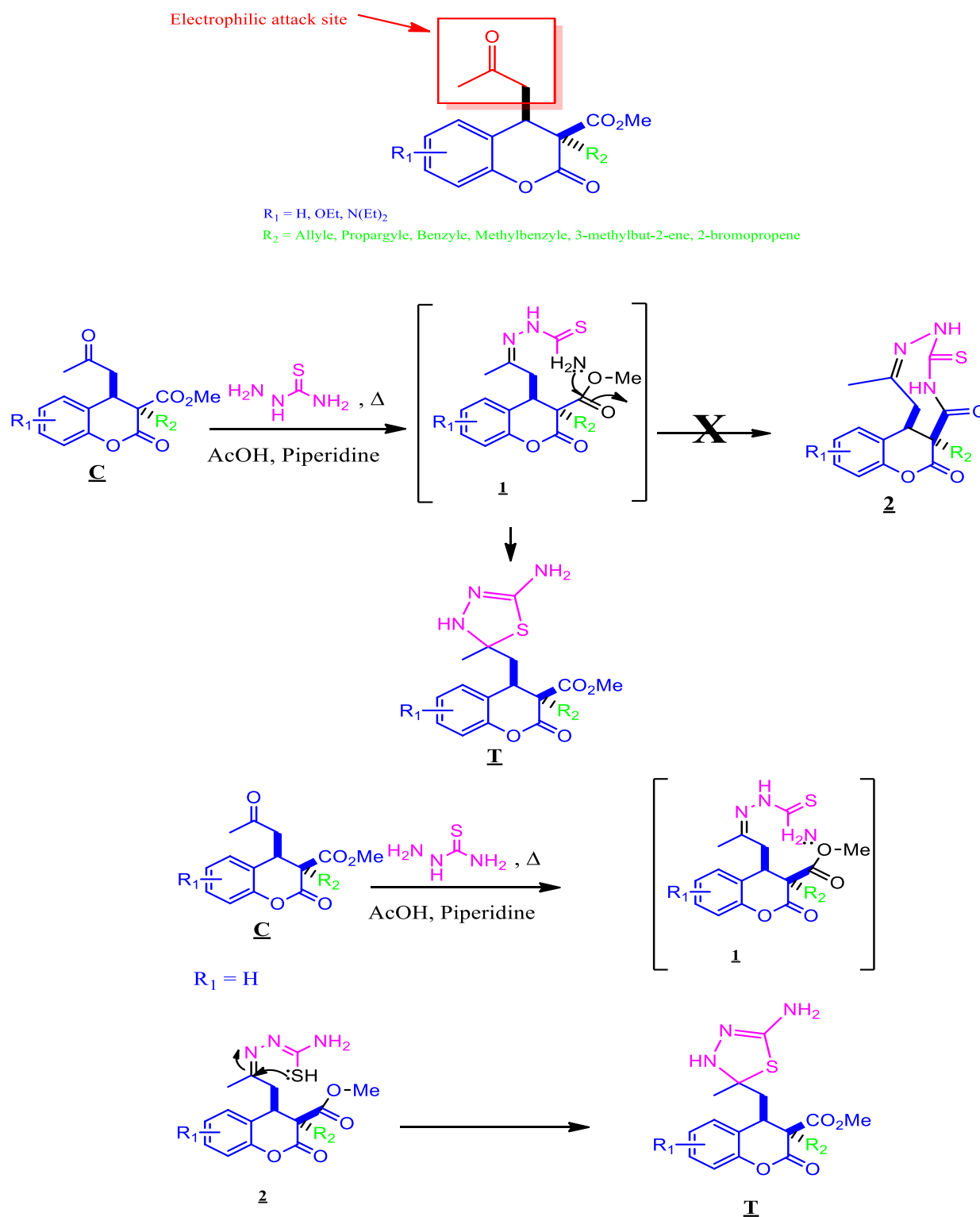


Figure 54. Carbonyl Reactivity and Intramolecular Cyclization in Coumarin Derivatives

The scheme illustrates the reactivity of the carbonyl group in coumarin derivatives, the intramolecular cyclization reaction, and its detailed mechanism. Key intermediates, bond-forming steps, and electron movements are shown to provide a comprehensive overview of the reaction pathway

The synthesis of seven derivatives (T1a, T1b, T1c, T1d, T1e, T1f, and T1g) originating from coumarin C1 was achieved through the introduction of structurally diverse substituents at the 3-position of the coumarin nucleus, followed by a comparative evaluation of their isolated yields. This systematic variation in substituent type enabled a detailed assessment of the influence of steric and electronic factors on reaction efficiency and product formation.

- **Compound T1a**, obtained from coumarin C1 and bearing an allyl substituent at the 3-position, was isolated in an 87% yield. This high yield can be rationalized by the favorable electronic properties and relatively low steric demand of the allyl group, which facilitate nucleophilic substitution and promote smooth progression of the reaction pathway when compared with more sterically congested substituents.
- **Compound T1b**, also derived from coumarin C1 and functionalized with a propargyl group at the 3-position, afforded a yield of 88%. The presence of a highly reactive alkyne moiety contributes to enhanced electrophilic activation and efficient nucleophilic substitution, while the linear geometry of the substituent minimizes steric hindrance. Although alkynes may sometimes participate in competing side reactions, the transformation proceeded efficiently, providing a yield comparable to that obtained for T1a.
- **Compound T1c**, synthesized from coumarin C1 and incorporating a benzyl substituent at the 3-position, was isolated in an 85% yield. The benzyl group is capable of stabilizing reaction intermediates through resonance effects; however, its relatively bulky nature introduces steric congestion in the vicinity of the reactive center, thereby slowing nucleophilic substitution and resulting in a slightly reduced yield relative to the allyl and propargyl analogues.
- **Compound T1d**, obtained from coumarin C1 and bearing a 3-methylbut-2-yn-1-yl substituent, afforded a yield of 87%. The unsaturated alkenyl functionality contributes to enhanced electronic activation of the reaction center, thereby facilitating substitution, although subtle steric and electronic effects associated with this substituent moderately influence the overall efficiency of the transformation.

- **Compound T1e**, derived from coumarin C1 and substituted with a methylbenzyl group at the 3-position, was isolated in an 82% yield. While the aromatic substituent provides resonance stabilization to the developing intermediates, its increased steric bulk creates significant congestion around the reactive site, leading to diminished nucleophilic accessibility and, consequently, a lower yield compared to less bulky derivatives.
- **Compound T1f**, synthesized from coumarin C1 and characterized by the presence of a triple bond at the 3-position, exhibited a yield of 85%. The unsaturated substituent enhances the electrophilic character of the reaction center and promotes efficient nucleophilic substitution. Despite the possibility of competing side reactions associated with unsaturated systems, the reaction proceeded smoothly, affording the desired product in consistently high yield.
- **Compound T1g**, obtained from coumarin C1 and featuring an (E)-2-bromobut-2-yne substituent at the 3-position, afforded a yield of 84%. The presence of the bromine atom introduces both steric and electronic effects that slightly impede nucleophilic substitution, resulting in a marginally lower yield when compared with the less substituted analogues, although the reaction remains overall efficient and reliable.

The synthesis of three derivatives (T2a, T2b, and T2c) originating from coumarin C2 was achieved through the introduction of structurally diverse functional groups at different positions on the coumarin scaffold, followed by a systematic evaluation of their isolated yields. This comparative approach enabled an assessment of how steric and electronic factors associated with each substituent influence reaction efficiency and overall synthetic performance within this series.

- **Compound T2a**, derived from coumarin C2 and bearing an ethoxy substituent at the 8-position, was isolated in an 87% yield. The presence of the ethoxy group contributes to enhanced solubility and introduces only minimal steric hindrance at the reaction site, thereby facilitating efficient nucleophilic substitution. This relatively high yield can therefore be regarded as a reference point for this series, reflecting the favorable balance between electronic donation and moderate steric demand imparted by the ethoxy substituent.

- **Compound T2b**, also derived from coumarin C2 and functionalized with a propargyl substituent at the 3-position, afforded an 88% yield. The propargyl moiety is known to promote efficient nucleophilic substitution owing to its unsaturated and electronically activating character. However, its intrinsic reactivity and potential susceptibility to competing side reactions may account for the slight variation in yield compared to T2a. Nevertheless, the alkyne-based substitution proceeds smoothly overall, resulting in a high isolated yield.
- **Compound T2c**, obtained from coumarin C2 and bearing a 3-methylbut-2-en-1-yl substituent, was isolated in an 82% yield. The alkenyl group can enhance the reactivity of intermediate species, thereby facilitating nucleophilic substitution; however, the combined steric bulk and electronic influence of this substituent appear to slightly diminish reaction efficiency relative to T2a. Despite this modest reduction, the synthesis of T2c remains effective and reproducible, further illustrating the versatility of the applied methodology.

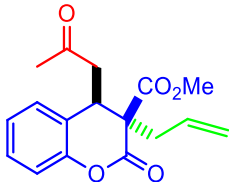
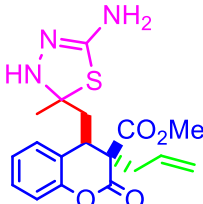
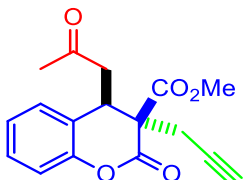
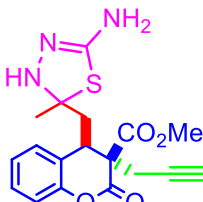
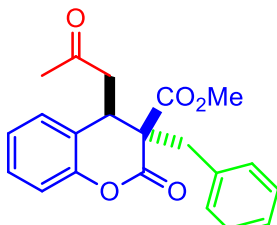
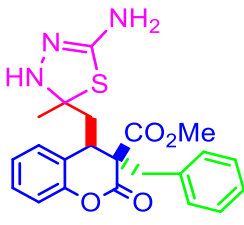
The synthesis of two derivatives (T3a and T3b) originating from coumarin C3 was achieved through the selective introduction of structurally distinct functional groups at the 3-position of the coumarin framework, followed by a comparative evaluation of their isolated yields. This targeted functionalization strategy enabled an assessment of how subtle variations in substituent structure influence reaction efficiency and overall synthetic performance within this series.

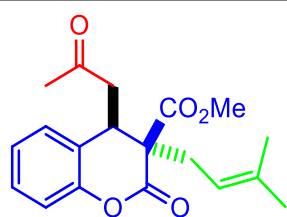
- **Compound T3a**, derived from coumarin C3 and bearing an allyl substituent at the 3-position, was obtained in a high yield of 86%. The allyl group effectively promotes nucleophilic substitution due to its favorable electronic properties and minimal steric bulk, which facilitate unhindered access to the reactive center and ensure smooth progression of the reaction pathway. Consequently, the elevated yield reflects the compatibility of this substituent with the applied reaction conditions.
- **Compound T3b**, also derived from coumarin C3 and functionalized with a propargyl substituent at the 3-position, afforded an isolated yield of 87%. The highly reactive alkyne moiety enhances the rate and efficiency of nucleophilic

substitution, leading to effective conversion under the applied conditions. This high yield can be attributed to the favorable electronic characteristics and linear geometry associated with the propargyl group, which together promote efficient bond formation and minimize steric interference during the reaction process.

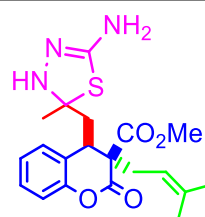
Using this synthetic approach, twelve coumarin-1,3,4-thiadiazole derivatives were successfully obtained from the corresponding alkylated coumarin precursors. The structures of the synthesized compounds together with their isolated yields are summarized in Table 4.

Table 4. Characteristics and yields of synthesized coumarin derivatives T3a to 13b.

Starting Coumarins	Reagent (R ₂ -X)	Products	Yields (%)
 C1a	Thiosemicarbazide	 T1a	87
 C1b		 T1b	88
 C1c		 T1c	85

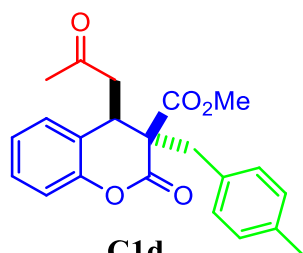


C1d

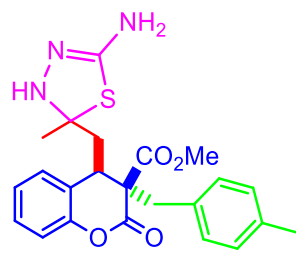


T1d

87

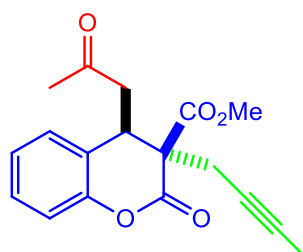


C1d

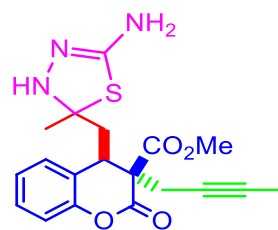


T1e

82

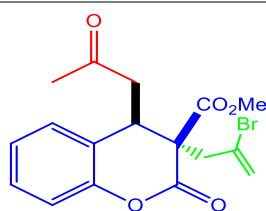


C1f

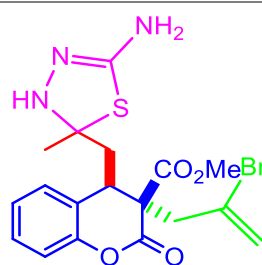


T1f

85

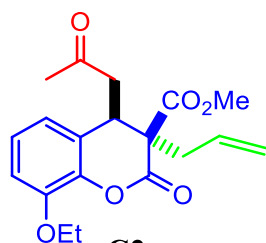


C1g

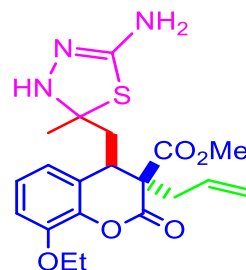


T1g

84

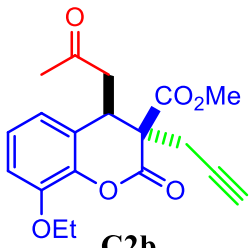
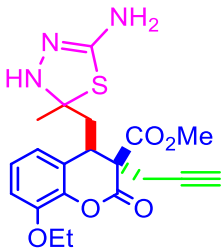
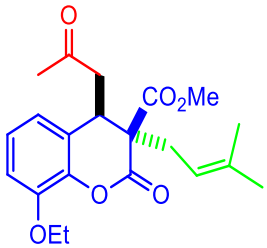
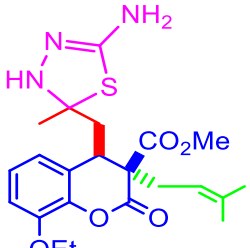
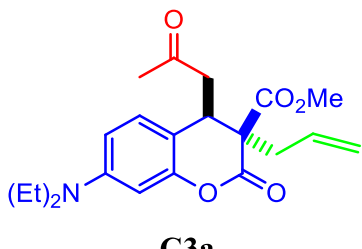
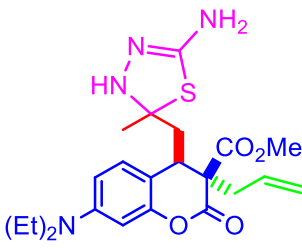
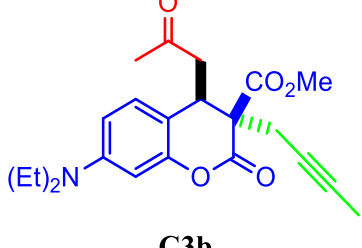
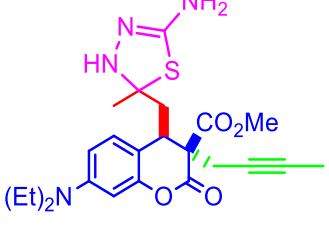


C2a



T2a

87

 C2b	 T2b	88
 C2c	 T2c	82
 C3a	 T3a	86
 C3b	 T3b	87

III.2.3. Spectroscopic study

The FTIR, ^1H NMR, ^{13}C NMR and DEPT techniques were used to identify and characterise the synthesised compounds, enabling functional groups and the nature of carbons to be determined and the proposed structures to be confirmed.

- FTIR spectroscopy analysis

The FTIR spectra of all synthesised coumarin 1,3,4-thiadiazole derivatives exhibited characteristic absorption bands that confirmed the successful formation of the targeted structures. A strong band was observed at approximately 1710 cm⁻¹ and was assigned to the lactone carbonyl (C=O) group of the coumarin moiety. This indicated that the coumarin core was preserved throughout the synthetic sequence. Furthermore, a medium-intensity band near 1620 cm⁻¹ corresponded to the cyclic imine (C=N) stretching vibration of the 1,3,4-thiadiazole ring, providing clear evidence of heterocycle formation.

Further structural confirmation was obtained from weak to moderate absorption bands around 740 cm⁻¹, attributed to C-S-C stretching vibrations within the thiadiazole framework. The consistent appearance of these diagnostic bands across all compounds supports the proposed molecular structures and demonstrates the effectiveness and reliability of the applied synthetic and characterisation methods.

- **¹H proton NMR analysis**

The ¹H NMR spectra of the synthesised coumarin-1,3,4-thiadiazole derivatives provided spectral evidence consistent with the proposed structures. The disappearance of the signal at $\delta = 2.13$ ppm, corresponding to the methyl protons of the coumarin precursor, together with the appearance of a new singlet at $\delta = 1.55$ ppm, assigned to the methyl group (-CH₃) attached to the 1,3,4-thiadiazole ring, indicates that the carbonyl group of the starting coumarin derivative reacted with thiosemicarbazide during the cyclisation process. Furthermore, a singlet at $\delta = 8.56$ ppm was assigned to the NH₂ protons present in the final product, providing additional support for the proposed 1,3,4-thiadiazole structure. Collectively, these spectral changes are consistent with the proposed structural transformation and support the assigned structures of the synthesised derivatives.

- **¹³C carbon NMR analysis**

¹³C NMR analysis of the synthesised coumarin-1,3,4-thiadiazole derivatives allowed the identification of the characteristic carbon resonances associated with the proposed structures.

- The disappearance of the signal at δ 206 ppm, assigned to the ketone carbonyl carbon (C=O) of the starting coumarin derivative, indicates the consumption of the carbonyl group during the reaction with thiosemicarbazide.
- The signal at δ 52.4 ppm is assigned to the saturated carbon of the 1,3,4-thiadiazole ring.
- The signal at δ 148.5 ppm is attributed to the imine carbon (C=N) within the same ring.
- The signal at δ 31.1 ppm corresponds to the methyl carbon ($-\text{CH}_3$) attached to the thiadiazole ring.

The ^1H NMR, ^{13}C NMR, and DEPT spectra of the synthesized coumarin 1,3,4-thiadiazole derivatives are presented in Appendix , together with their corresponding chemical structures.

Following the successful synthesis and structural characterisation of the coumarin 1,3,4-thiadiazole derivatives, the focus now shifts to evaluating their biological properties. This section explores the potential antioxidant activity and inhibitory effects on selected enzymes of the structural modifications introduced during synthesis, thereby providing insight into their functional implications.

III.4. Biological Activity:

The biological activities of the synthesised coumarin–1,3,4-thiadiazole derivatives were evaluated in order to ascertain their potential as multifunctional pharmacological agents. The present study specifically investigated their antioxidant capacity and enzyme inhibitory activity, due to their importance in addressing oxidative stress and neurodegenerative disorders. A series of complementary assays were conducted in order to provide a comprehensive assessment of the biological properties of the compounds under investigation. The assays included ABTS radical scavenging, o-phenanthroline reducing power, FRAP, and acetylcholinesterase (AChE) inhibition. The results were correlated with structural characteristics and *in silico* predictions to clarify the structure-activity relationships.

III.4.1. Antioxidant Activities:

III.4.1.1. ABTS radical Activity of coumarins 1.3.4-thiadiazols derivatives:

The antioxidant potential of the synthesized asymmetric coumarin 1,3,4-thiadiazole derivatives was systematically evaluated using two complementary *in vitro* assays: the ABTS radical scavenging assay and the phenanthroline reduction assay. The radical-scavenging and reducing activities of the derivatives were systematically evaluated by applying these techniques, thereby providing a comprehensive understanding of their antioxidant potential. The results of the study have been summarised and are set out in Table 5, are reported in terms of IC_{50} and $A_{0.5}$ values, and are compared with well-established synthetic antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which served as standard references.

In the ABTS radical scavenging assay, compounds T1c and T1f demonstrated the most pronounced antioxidant activities, with IC_{50} values of $24.32 \pm 0.36 \mu\text{M}$ and $35.41 \pm 1.35 \mu\text{M}$, respectively. Remarkably, these values surpass those recorded for BHA ($42.85 \pm 0.82 \mu\text{M}$) and BHT ($47.84 \pm 0.22 \mu\text{M}$), indicating a superior radical-neutralizing efficiency. The high antioxidant performance of these particular derivatives can be rationalized by the presence of conjugated alkenyl and alkynyl substituents, which facilitate extended π -electron delocalization across the molecular scaffold. This delocalization stabilizes the radicals generated during the scavenging process and enhances the overall reactivity of the molecules. Furthermore, the allylic and propargylic linkages present in these compounds are conducive to both hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms, enabling rapid and efficient neutralization of ABTS radicals.

Other derivatives, including T1d, T1b, T1a, and T2b, exhibited moderate to strong radical scavenging capacities, with IC_{50} values ranging from 43 to 50 μM . The observed activities of these compounds can be largely attributed to the electronic influence of substituents at the 3-position, which modulate electron density within the coumarin–thiadiazole system and facilitate stabilization of reactive radical species. These findings indicate that even in the absence of extended conjugation seen in T1c and T1f, strategically positioned substituents can still significantly contribute to antioxidant performance.

In contrast, derivatives such as T1e, T1g, and T3b displayed comparatively lower radical scavenging activity. This diminished performance is likely the result of steric hindrance or the presence of electron-withdrawing substituents, which can impede effective electron delocalization and reduce the accessibility of reactive sites to radical species. Such structural limitations underscore the importance of substituent size, electronic properties, and molecular geometry in modulating the antioxidant behavior of coumarin–thiadiazole derivatives.

Overall, the results demonstrate that the antioxidant activity of these molecules is strongly dependent on the nature and position of substituents on the coumarin–thiadiazole framework. Compounds with conjugated unsaturated substituents and minimal steric hindrance tend to exhibit superior radical scavenging capabilities, whereas bulky or electron-withdrawing groups may compromise activity. These insights provide valuable guidance for the rational design of future derivatives with optimized

antioxidant properties and highlight the critical role of molecular architecture in determining bioactivity.

III.4.1.2. Antioxidant Activity by the 1,10-Phenanthroline Reducing Power Assay:

Within the phenanthroline reduction assay, T3a and T1f exhibited the most pronounced reducing activity among the synthesised coumarin 1,3,4-thiadiazole derivatives, as indicated by their minimal $A_{0.5}$ values of $11.38 \pm 1.38 \mu\text{M}$. These values are closely comparable to that of the reference antioxidant BHA ($7.32 \pm 0.83 \mu\text{M}$) and substantially surpass the performance of BHT ($190.32 \pm 0.83 \mu\text{M}$), highlighting the exceptional electron-donating potential of these molecules within the Fe^{3+} /phenanthroline redox system. The remarkable reducing activity of compound T3a can be rationalized by the presence of an electron-rich allyl substituent, which increases electron density across the molecular framework. The enhanced electron density thus facilitates efficient electron transfer to the Fe^{3+} ions, promoting their rapid reduction to Fe^{2+} and subsequent formation of the Fe^{2+} -phenanthroline chromophore.

In a similar manner, compound T1f exhibited substantial reducing activity, suggesting a synergistic equilibrium between electronic effects and steric accessibility that promotes effective electron donation. Furthermore, compound T1d demonstrated a significant reducing capacity ($A_{0.5} = 31.90 \pm 3.61 \mu\text{M}$), indicating that its specific substitution pattern and molecular conformation may facilitate electron transfer processes. The findings emphasise the pivotal role of substituent type, position and molecular geometry on the redox behaviour of the coumarin-thiadiazole scaffold.

In contrast, compounds such as T2c, T1b, and T2a displayed moderate reducing power, reflecting the sensitivity of the electron-donating process to subtle variations in electronic distribution and steric constraints. It was hypothesised that minor differences in substituent electronics, molecular planarity, and conjugation would exert a measurable effect on the efficiency of Fe^{3+} reduction. This would in turn influence the overall antioxidant potential of these derivatives. The findings of this study, when considered collectively, underscore the pivotal influence of molecular structure on redox behaviour. Furthermore, they suggest that deliberate modifications of the coumarin-thiadiazole scaffold can significantly enhance electron-transfer-mediated antioxidant activity.

Across both antioxidant assays, compounds T1c, T1d, and T1f consistently emerged as the most potent antioxidants, as evidenced by their low IC_{50} values in the ABTS radical scavenging assay and correspondingly low $A_{0.5}$ values in the phenanthroline reduction assay. The enhanced activity exhibited by these derivatives can be attributed to a multitude of interconnected structural and electronic factors. Firstly, the presence of extended π -conjugation within the coumarin–thiadiazole framework facilitates effective delocalization of electrons, thereby stabilizing radical intermediates generated during the scavenging process. Secondly, the incorporation of electron-donating substituents, such as alkenyl and alkynyl groups, enhances electron density across the molecular scaffold. These modifications have been shown to enhance both hydrogen atom transfer (HAT) and single-electron transfer (SET) pathways. Furthermore, the frontier molecular orbitals (HOMO–LUMO) are adjusted, leading to a reduction in the ionisation potential and an enhancement in the electron donation towards reactive species.

These observations are in agreement with previous studies, which have demonstrated that electron-donating groups, including $-OH$, $-OCH_3$, and $-CH=CH_2$, increase antioxidant potency by enhancing π -electron density and stabilising radical species through conjugation [135] [136]. Furthermore, density functional theory (DFT) investigations have confirmed that extended π -systems play a crucial role in stabilising radicals by efficient electron delocalization [137]. Consequently, the high activity of T1c, T1d, and T1f can be attributed to the synergistic effects of their conjugated substituents, which collectively enhance electron-donating capacity, reduce ionisation energy, and stabilise radical intermediates generated during both ABTS and phenanthroline assays.

In conclusion, the results obtained demonstrate that the coumarin–thiadiazole scaffold is a structural platform with considerable potential for the design of novel antioxidant agents. Table 5.

Table 5. Antioxidant activity of asymmetric coumarin–thiadiazole derivatives.

Compound	ABTS IC ₅₀ (MM)	PHEN A _{0.5} (MM)
T2c	194.31 ± 0.72	94.48 ± 3.58
T1d	45.56 ± 0.89	31.90 ± 3.61
T1b	43.73 ± 0.70	92.00 ± 6.39
T2a	222.95 ± 2.59	85.90 ± 0.52
T3a	69.40 ± 3.89	11.38 ± 1.38
T1g	72.28 ± 2.11	>200
T1e	>800	>200
T3b	63.02 ± 0.36	>200
T1f	35.41 ± 1.35	11.38 ± 1.38
T1a	43.02 ± 1.16	>200
T2b	50.14 ± 0.17	>200
T1c	24.32 ± 0.36	>200
BHA	42.85 ± 0.82	7.32 ± 0.83
BHT	47.84 ± 0.22	190.32 ± 0.83
<i>BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene. both were tested as reference antioxidants under the same experimental conditions</i>		

III.4.1.3. Activity by the Ferric Reducing Antioxidant Power (FRAP) Assay:

The FRAP assay was used to evaluate the electron-donating ability of the synthesized coumarin–1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, T3a–T3b). The approach adopted in this study utilises the reduction potential of the compounds, as measured by the conversion of Fe³⁺ to Fe²⁺ ions. Oyaizu, M. (1986). offering insights into their redox properties and overall antioxidant potential. The results, presented as A_{0.5} values, are summarized in Table 6.

Table 6. FRAP values ($A_{0.5}$, μM) of the synthesized coumarin–1,3,4-thiadiazole derivatives

Compound	$A_{0.5}$ (μM)
T1a	>200
T1b	>200
T1c	>200
T1d	>200
T1e	>200
T1f	>200
T1g	>200
T2a	>200
T2b	>200
T2c	>200
T3a	>200
T3b	>200

Note: Values above 200 μM indicate limited ferric reducing activity under the applied conditions. The complete dataset for all tested concentrations is provided in the Annex.

The results presented in Table X reveal that all synthesized derivatives exhibit only weak ferric reducing activity within the tested concentration range. However, the ABTS and o-phenanthroline assays showcase a remarkable moderate to strong antioxidant effect. This contrast highlights an important distinction in the mechanisms of these assays. The FRAP method requires more robust electron-donating substituents to effectively reduce Fe^{3+} ions, which are unfortunately lacking in the current derivatives. Consequently, while these compounds demonstrate substantial ability to scavenge radicals, their capacity for metal reduction under the FRAP conditions is notably limited. This underscores the powerful potential of these compounds as antioxidants, despite their weaknesses in specific assays.

III.4.2. Enzymatic activity:

AChE inhibitory activities of coumarins 1.3.4-thiadiazols derivatives:

The coumarin 1,3,4-thiadiazole derivatives were evaluated for their (AChE) acetylcholinesterase inhibitory activity, using galantamine ($IC_{50} = 4.14 \pm 0.07 \mu M$) as a reference compound. Among the compounds that were tested, T1g and T1f exhibited the most pronounced inhibition, with IC_{50} values of $20.35 \pm 0.06 \mu M$ and $38.90 \pm 0.46 \mu M$, respectively. Despite its comparatively diminished effectiveness in comparison to galantamine, it is regarded as the most efficacious in this series with regard to inhibiting acetylcholinesterase activity.

Compounds T1e, T3a, and T2c displayed moderate inhibitory effects, with IC_{50} values ranging from 76.99 to 121.72 μM , indicating a measurable, though reduced, capacity to interfere with enzyme function (Jones et al., 2022). T3b demonstrated comparatively weak activity ($IC_{50} = 143.71 \pm 2.53 \mu M$), whereas the remaining derivatives, including T1a–T1d, T1c, T2a, and T2b, exhibited negligible inhibition, with IC_{50} values exceeding 200 μM , suggesting minimal interaction with the binding pocket of AChE under the tested conditions.

These findings demonstrate that a number of asymmetric coumarin–thiadiazole derivatives possess significant acetylcholinesterase (AChE) inhibitory potential, thus highlighting their relevance in the pursuit of novel therapeutic agents Alzheimer's disease (AD). The inhibition of AChE is a well-established approach for the management of AD, as it elevates acetylcholine levels in the synaptic cleft and can improve cholinergic neurotransmission in individuals with cognitive impairment. Coumarin-based scaffolds have been the focus of extensive research for this purpose, and prior studies have validated their efficacy as AChE inhibitors, providing significant insights into structure–activity relationships (SARs) and directing the development of derivatives that can effectively interact with the AChE active site [138] [139].

The coumarin 1,3,4-thiadiazole derivatives evaluated in this study demonstrate a multifunctional profile that integrates both acetylcholinesterase (AChE) inhibitory activity and antioxidant potential, positioning them as highly relevant candidates for Alzheimer's disease (AD) drug discovery. Research has indicated that oxidative stress is a pivotal factor in the development of Alzheimer's disease (AD), exacerbating neuronal damage and accelerating neurodegenerative processes. Consequently, compounds

capable of enhancing cholinergic neurotransmission through AChE inhibition and mitigating oxidative stress through radical scavenging may offer a therapeutic advantage by addressing two central pathogenic mechanisms associated with the disease. The outcomes of the *in vitro* experiments, incorporating AChE inhibition and complementary antioxidant assessments, corroborated by *in silico* docking studies, furnish mechanistic insight into the dual functional capacity of these derivatives.

Among the series that were tested, T1g and T1f were identified as the most effective AChE inhibitors. The enhanced activity exhibited by these compounds can be rationalised by the presence of extended conjugated π -systems and strategically positioned unsaturated substituents. These features facilitate favourable π - π stacking and hydrogen-bonding interactions with key aromatic residues within the catalytic gorge of AChE, notably Trp84, Phe330, and Tyr121. These interactions facilitate the optimal occupation of the hydrophobic cavity of the enzyme by the compounds, thereby stabilising the enzyme-inhibitor complex. This, in turn, enhances binding affinity and inhibitory efficiency. Conversely, derivatives bearing bulky or non-conjugated substituents, such as T1a, T2a, and T1e, exhibited reduced inhibitory potency. The steric hindrance imposed by these groups is likely to restrict access to the catalytic site and diminish the overall ability of the compound to establish effective interactions with critical residues.

Compounds T3a, bearing an allyl substituent, and T2c, with a 3-alkenyl group, exhibited intermediate inhibitory activity. These observations suggest that moderate electronic conjugation combined with limited steric bulk can support productive binding interactions, although not as effectively as the planar, extensively conjugated π -rich systems of T1g and T1f. In contrast, T3b, despite featuring a diethylamino substituent, exhibited reduced activity. This reduced potency may be attributed to increased steric strain and redistribution of electron density, which compromise the compound's ability to engage effectively with the AChE active site.

The collective findings emphasise the pivotal influence of electronic conjugation, molecular planarity, and the nature and positioning of substituents on the modulation of AChE inhibitory activity among these coumarin-thiadiazole derivatives. These conclusions are consistent with those reported in previous studies: Abu-Aisheh et al. (2019) [140] demonstrated that N1-(coumarin-7-yl) derivatives achieve strong inhibition via the process of π - π stacking with Trp84 and Phe330. In contrast, Sonmez et al.

(2017) [141] and Kang et al. (2018) [142] reported that coumarin–thiazole and coumarin–chalcone hybrids exhibit enhanced potency due to extended conjugation and aromatic interactions. When considered as a whole, T1g and T1f represent the most promising lead compounds, combining optimal electronic density, extensive π -conjugation, and favourable binding characteristics, which render them excellent scaffolds for further optimization. These findings provide a solid foundation for future mechanistic investigations, including detailed molecular docking and density functional theory (DFT) analyses. These investigations will aim to elucidate the precise molecular interactions that govern their inhibitory activity and inform the rational design of more potent derivatives.

Table 7. Acetylcholinesterase (AChE) inhibitory activity of asymmetric coumarin–thiadiazole derivatives

Compound	ACHE IC ₅₀ (MM)
T2c	121.72 ± 3.85
T1d	>200
T1b	>200
T2a	>200
T3a	86.33 ± 3.71
T1g	20.35 ± 0.06
T1e	76.99 ± 1.17
T3b	143.71 ± 2.53
T1f	38.90 ± 0.46
T1a	>200
T2b	>200
T1c	>200
GAL (REF.)	4.14 ± 0.07
<i>Gal = galantamine hydrobromide (reference inhibitor).</i>	

Based on the biological evaluation, compounds T1c, T1f, T1g, and T3a exhibited the most promising inhibitory activities among the synthesized derivatives. Consequently, these compounds were selected for further computational investigations to rationalize their biological performance.

III.4. In Silico Computational Studies:

To gain deeper insight into the molecular basis of the observed biological activities, an integrated computational strategy was employed. Density functional theory (DFT) calculations were first performed to investigate the electronic properties of the selected compounds through analysis of the frontier molecular orbitals (HOMO and LUMO), while molecular electrostatic potential (MEP) maps were generated to identify electron-rich and electron-deficient regions that may influence intermolecular interactions. Molecular docking simulations were subsequently carried out to evaluate the binding orientations of the selected derivatives within the active sites of the target enzymes and to characterize the key interactions responsible for complex stabilization, including hydrogen bonding, π - π stacking, and hydrophobic contacts. Finally, in silico ADMET predictions were performed to assess the pharmacokinetic and drug-likeness profiles of the selected compounds, including their absorption, distribution, metabolism, excretion, and toxicity properties. Collectively, these computational investigations provide a theoretical interpretation of the experimental biological findings and support the observed structure–activity relationships (SAR) [143].

III.4.1. Bioactivity and Electronic Properties:

In studies aimed at assessing coumarin 1,3,4-thiadiazole derivatives, a subset of compounds was identified that demonstrated superior biological performance. The compounds in question were T3a, T1g, T1f and T1c, and they exhibited both acetylcholinesterase (AChE) inhibitory activity and antioxidant potential. These bioactivities were systematically assessed through multiple complementary assays, including ABTS radical scavenging, phenanthroline reduction, and FRAP assays, allowing for a thorough evaluation of free-radical neutralization and reducing capacity. T1g displayed notable acetylcholinesterase inhibition, with an IC_{50} of 20.35 μ M, which is nearly equivalent to the activity of galantamine, used as the reference compound. This

finding suggests that T1g possesses a substantial capacity to modulate cholinergic neurotransmission. In the FRAP assay, T3a demonstrated the most significant reducing ability (11.38 μM), thus indicating its substantial electron-donating potential and efficient radical-scavenging activity. Compound T1f exhibited a balanced profile, exhibiting significant AChE inhibition and FRAP activity comparable to that of T3a. In the ABTS assay, T1c demonstrated remarkable efficacy, with an IC_{50} of 24.32 μM , indicating its potent free-radical scavenging properties. These complementary profiles suggest that the selected derivatives possess distinct yet synergistic bioactive properties, warranting further computational and mechanistic analysis to elucidate the underlying electronic and structural factors.

Quantum chemical evaluations further clarified the relationship between electronic properties and biological activity (see Table 8). T1g, which exhibited the highest HOMO energy level of -4.80 eV, demonstrated a notable electron-donating capacity, favouring interactions with electrophilic sites and thereby rationalising its superior AChE inhibitory potency. In contrast, T1c exhibited the lowest LUMO energy level of -3.51 eV and the smallest HOMO–LUMO gap of 1.65 eV. These observations are indicative of an increased electrophilic character and enhanced chemical reactivity, which likely contributes to its remarkable ABTS radical-scavenging performance. The compounds T3a and T1f exhibited intermediate HOMO–LUMO gaps (2.19 and 1.79 eV, respectively), indicating a balance between electronic stability and controlled reactivity, which is consistent with their dual biological activities.

Table 8. A summary of the quantum parameters and physicochemical properties of compounds T3a, T1g, T1f, and T1c.

Compounds	HOMO	LUMO	Gap	IP	EA	μ	Ω	S	X	H
T3a	-4.93	-2.74	2.19	4.93	2.74	-3.84	6.72	0.46	3.84	1.10
T1g	-4.80	-3.07	1.73	4.80	3.07	-3.94	8.95	0.58	3.94	0.87
T1f	-5.20	-3.41	1.79	5.20	3.41	-4.31	10.35	0.56	4.31	0.90
T1c	-5.16	-3.51	1.65	5.16	3.51	-4.34	11.39	0.61	4.34	0.83

Molecular electrostatic potential (ESP) surface analyses (Fig. 55) corroborated these findings by highlighting electron-rich regions (red) favourable for electrophilic attack and electron-deficient areas (blue) conducive to nucleophilic interactions. The complementary electronic features of T1g and T1c suggest a high degree of versatility in interacting with multiple target sites, thereby enhancing their pharmacological potential. The integration of experimental and computational findings establishes a substantial basis for the systematic design of subsequent coumarin–thiadiazole derivatives. These derivatives are distinguished by enhanced bioactivity and customised therapeutic efficacy.

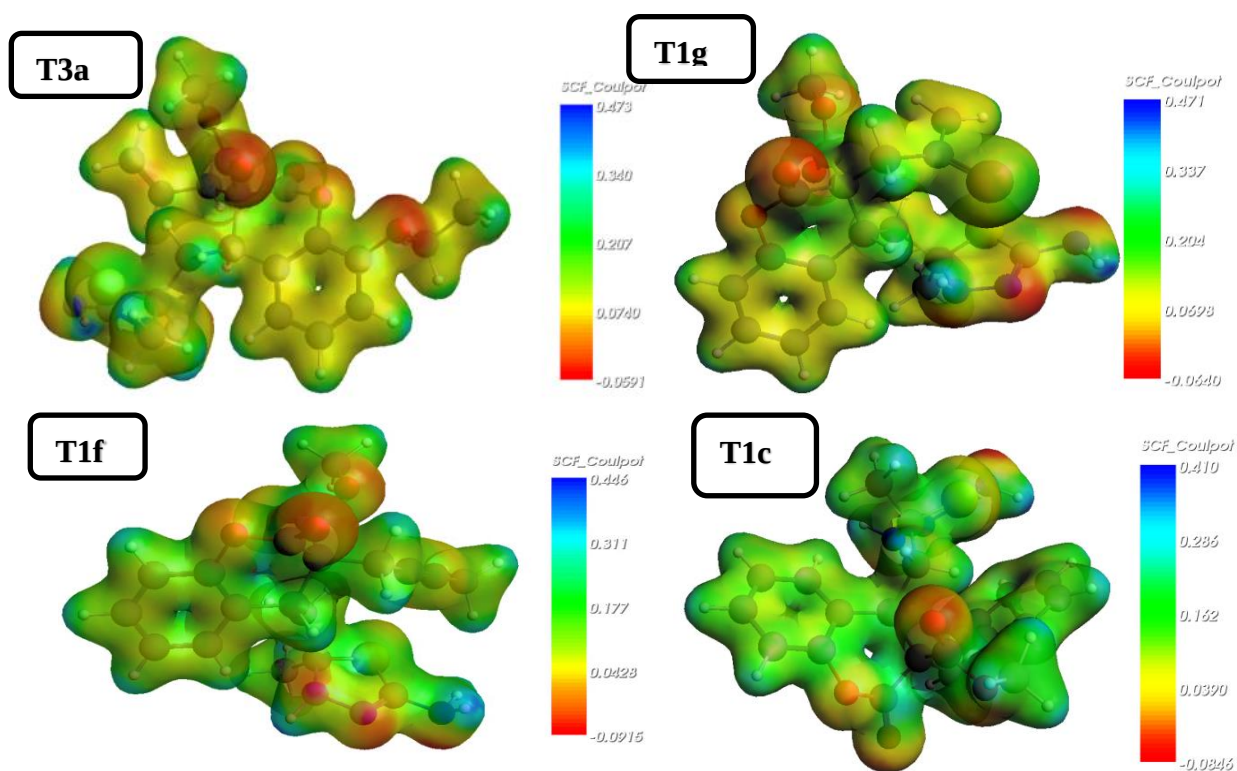


Figure 55. Computational Mapping of Molecular Electrostatic Potential Surfaces for Compounds T3a, T1g, T1f, and T1c

III.5.2. Molecular Docking Analysis:

The heatmap presented in Figure 54 provides a comparative visualisation of the binding affinity scores (ΔG , kcal/mol) for compounds T3a, T1g, T1f, and T1c against three biologically relevant protein targets: 4EY7, 1P0I, and 1DNU. Among the derivatives evaluated, compound T1g exhibited the strongest binding interactions across all targets,

with highly negative ΔG values of -12.1 kcal/mol for 4EY7, -9.7 kcal/mol for 1P0I, and -7.7 kcal/mol for 1DNU. These findings suggest that the ligand–target complexes are highly stable and energetically favourable. The particularly strong affinity for 4EY7 underscores the potential of T1g to effectively engage with this target, suggesting its suitability for further multi-target drug design approaches.

Furthermore, Compound T1f demonstrated notable binding potential, with ΔG values of -10.1 kcal/mol for 4EY7, -8.3 kcal/mol for 1P0I, and -7.0 kcal/mol for 1DNU. While exhibiting a slightly reduced potency in comparison to T1g, these results nevertheless reflect a promising polypharmacological profile. This further demonstrates the ability of T1f to interact favorably with multiple target proteins.. In the evaluation of Compound T3a, the focus was exclusively on 1DNU, resulting in a moderate binding energy of -7.5 kcal/mol. This finding suggests that, in comparison to multi-target active derivatives, there is a fair but more limited interaction. In a similar manner, T1c, which was also tested exclusively against 1DNU, achieved a binding score of -7.3 kcal/mol. This finding suggests that the overall interactions of T1c are weaker in comparison to those of T1g and T1f. Collectively, these findings identify T1g and T1f as the most promising candidates in terms of multi-target engagement, with T1g exhibiting particularly strong affinity for 4EY7. The binding patterns that have been observed emphasise the potential of these derivatives as broad-spectrum bioactive agents and provide a solid rationale for subsequent experimental validation and structure-guided optimisation within a polypharmacological framework. The favourable ligand-target interactions demonstrate the importance of incorporating electronic and structural considerations in the design of coumarin-thiadiazole derivatives intended for targeting multiple biological pathways simultaneously.

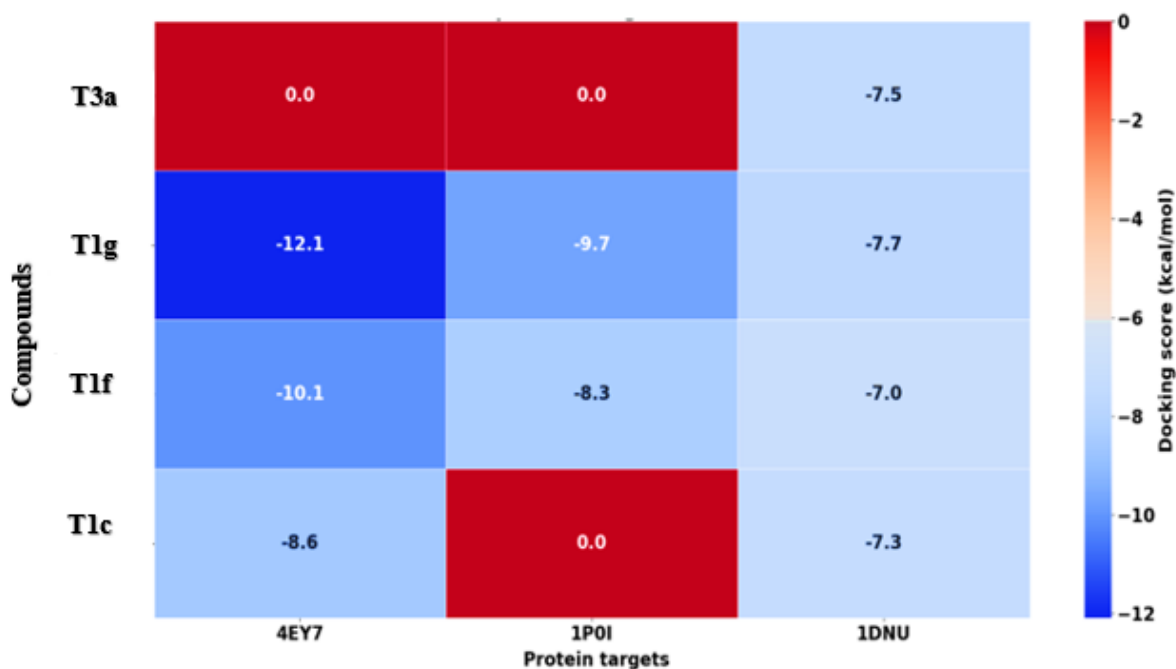


Figure 56. Heatmap illustrating the docking scores (ΔG , kcal/mol) of the compounds against the targets 4EY7, 1P0I, and 1DNU

Although molecular docking was performed against BChE (PDB ID: 1P0I), no in vitro BChE inhibitory assay was carried out in the present study. Therefore, the BChE results are limited to computational predictions.

A thorough evaluation of the docking simulation results reveals a wide range of specific and meaningful interactions between the investigated compounds (T3a, T1g, T1f, and T1c) and three key enzyme targets: 4EY7, 1P0I, and 1DNU (Figure 3, 4 and 5). The visual analysis, where green dashed lines indicate conventional hydrogen bonds and pink dashed lines highlight hydrophobic contacts, provides clear insights into the binding behavior of each ligand.

On the 4EY7 protein, compounds T1g and T1f display significant interactions with both aromatic and polar amino acid residues. Compound T1g forms several conventional hydrogen bonds (e.g., with TYR A:72, TYR A:124, and ASP A:74), as well as carbon–hydrogen bonds (e.g., GLY A:121, HIS A:447), and engages in numerous π -type interactions with key residues such as PHE A:297, PHE A:338, and TRP A:86. These suggest a strong binding affinity supported by aromatic stacking and electrostatic stabilization. Compound T1f demonstrates a comparable interaction profile, establishing robust hydrogen bonding and π -stacking within the same binding region.

In the case of the 1P0I enzyme, compound T1g achieves stable anchoring within the catalytic site, notably through hydrogen bonding (HIS A:438, ASP A:70), supported by multiple hydrophobic interactions with both aromatic (PHE A:329, TYR A:332) and nonpolar residues (ALA A:328, MET A:437). Meanwhile, compound T1f exhibits a diverse interaction network, combining hydrogen bonds (ASN A:83, ASN A:68), π -interactions (TYR A:332, PHE A:329), and carbon–hydrogen contacts that contribute to an energetically favorable binding conformation.

Interactions with 1DNU reveal distinct profiles for each ligand. Compound T1g adopts a versatile binding mode involving π -stacking (with LEU C:398, LEU C:412), hydrogen bonds (e.g., ASP A:94, ASN A:413), and carbon–hydrogen contacts (e.g., HIS C:328), indicating a high-affinity and multifaceted binding pattern. Compound T1f also interacts effectively, particularly with residues central to the active site (e.g., THR C:168, ARG C:239), and is further stabilized by hydrophobic contacts with PHE C:503 and LEU C:506. Compound 5 shows promising anchoring through a combination of hydrogen bonds (ARG C:424, GLU C:242) and hydrophobic interactions (PHE C:146, PHE A:99), while compound T1c primarily relies on a triad of hydrogen bonds and additional hydrophobic stabilization (e.g., ALA C:389, LEU C:338).

Overall, the binding patterns suggest complementary molecular behaviors, with each compound exploiting different interaction mechanisms depending on the structural features of the binding pocket. Notably, π -interactions appear to play a crucial stabilizing role, often overlooked in conventional analyses. The dense network of hydrogen and hydrophobic bonds observed across multiple protein–ligand complexes reflect a high level of steric and electronic compatibility.

Among the compounds tested, compounds T1g and T1f stand out for their binding stability and adaptability across all three targets. Their consistent and multi-target interaction profiles highlight their potential value in polypharmacological drug development strategies aimed at modulating multiple biological pathways. These findings reinforce the utility of molecular docking as a predictive approach for identifying and prioritizing promising bioactive candidates.

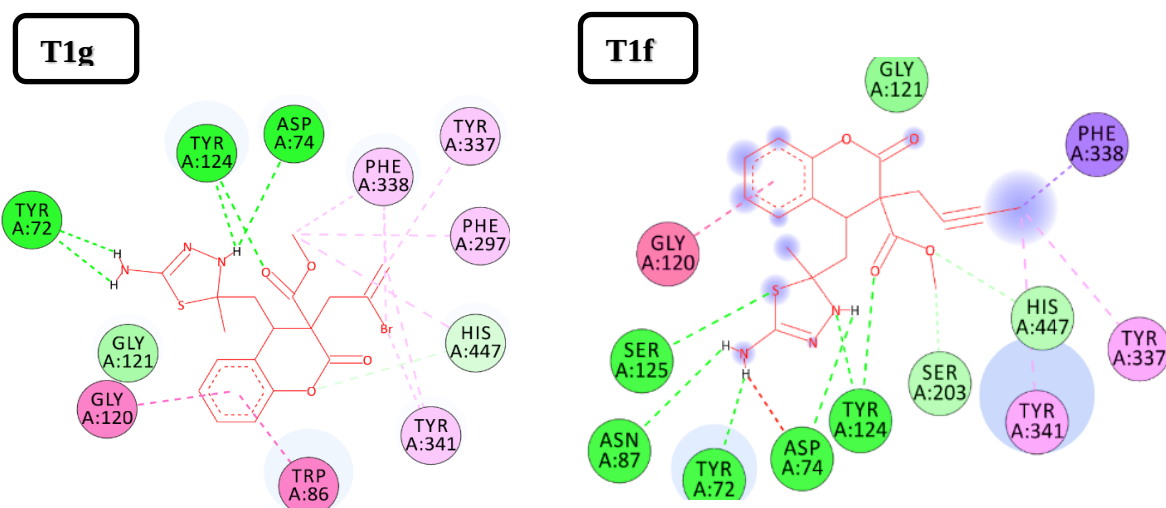


Figure 57. Significant interactions with diverse amino acid residues as shown in 4EY7

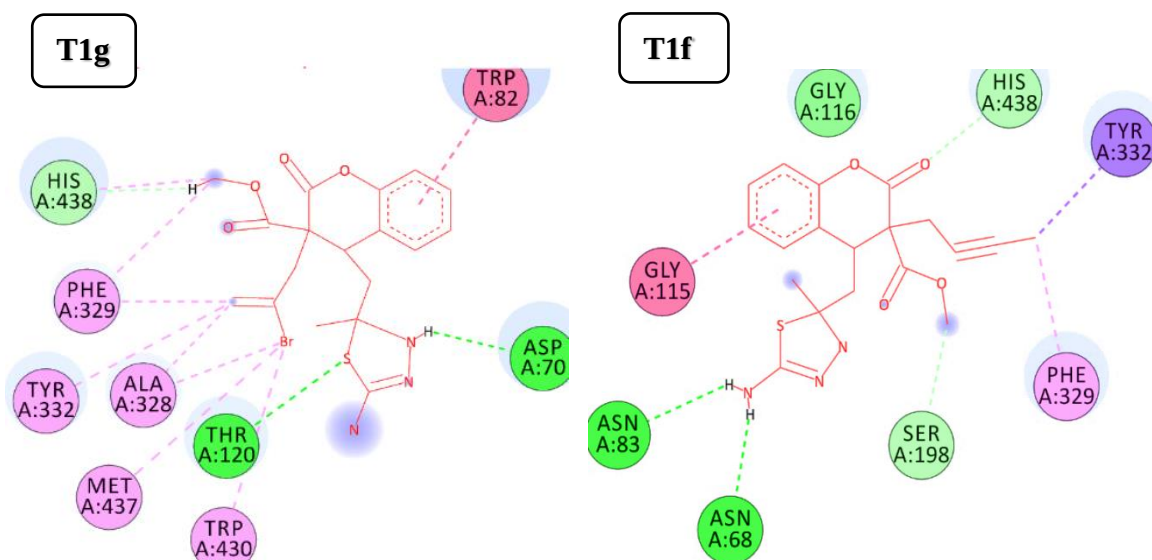


Figure 58. Key ligand–protein interactions of compound T1f within the active site of butyrylcholinesterase (BChE, PDB ID: 1P0I), illustrating important hydrogen bonding interactions (green dashed lines) and hydrophobic contacts (π – π and π –alkyl interactions) with surrounding amino acid residues that stabilize the complex

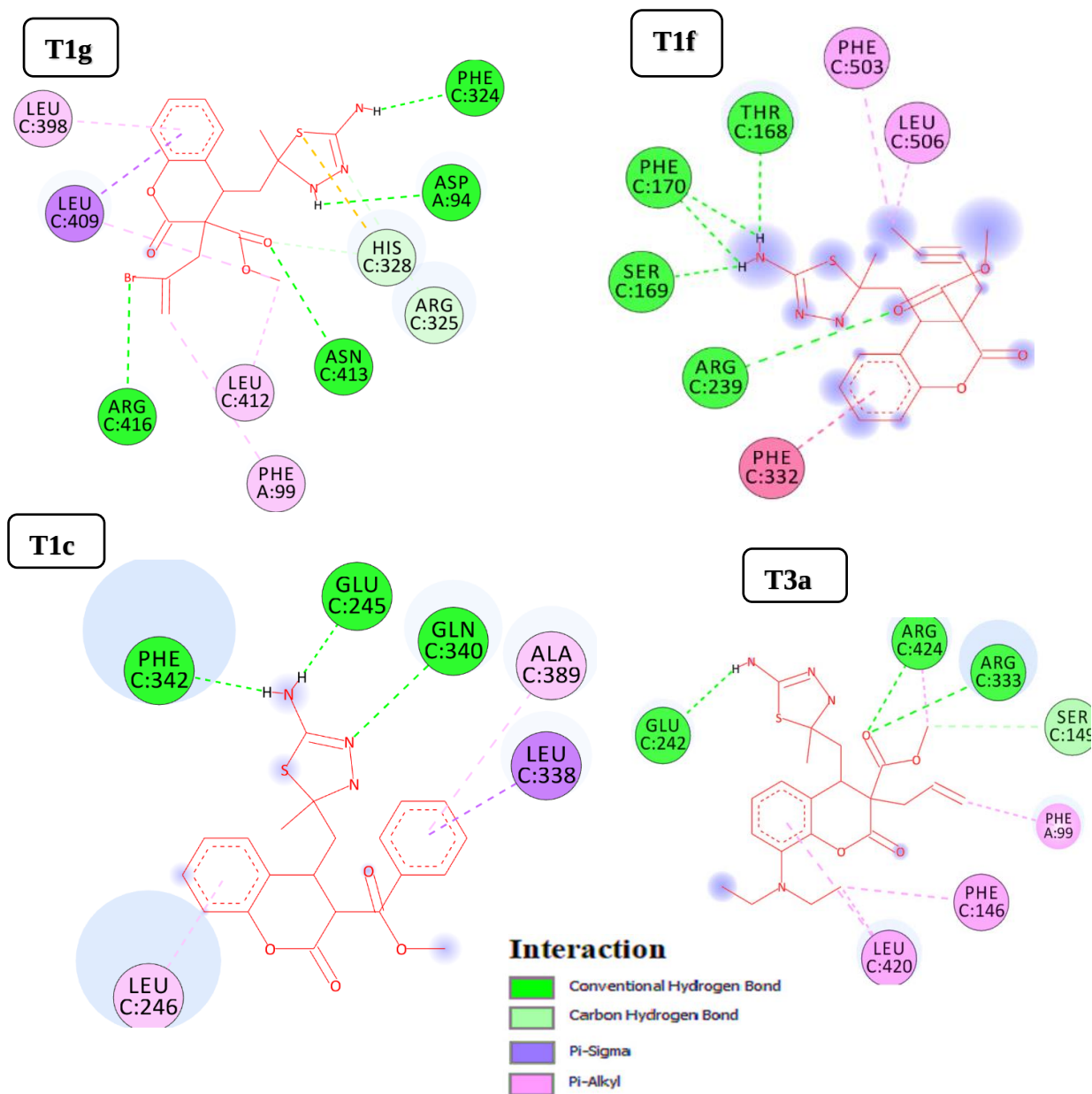


Figure 59. Crucial ligand–protein interactions of compound T3a within the active site of catalase (PDB ID: 1DNU), demonstrating substantial hydrogen bonding and stabilizing hydrophobic contacts with vital active-site residues that enhance binding affinity.

III.5.3. Pharmacochemical and ADMET Properties:

The pharmacochemical and ADMET evaluation of the four selected coumarin–1,3,4-thiadiazole derivatives, as outlined in Table 9, has revealed that the majority of the compounds display favourable drug-like properties. All compounds exhibited high predicted oral absorption, suggesting adequate bioavailability, and were identified as substrates of P-glycoprotein (P-gp), which may modulate intracellular accumulation and distribution. The partial inhibition observed for key metabolic enzymes, including CYP2C19, CYP2C9, and CYP3A4, indicates a potential for drug–drug interactions. Conversely, the lack of inhibitory activity toward CYP1A2 and CYP2D6 represents an advantageous feature with respect to metabolic safety. The predicted blood–brain barrier (BBB) permeability was low, consistent with high topological polar surface area (TPSA) values exceeding 128 Å, implying limited central nervous system penetration. Toxicological profiling further supported the safety of these compounds, with inactivity predicted across hepatic, neural, cardiac, and immune system endpoints. Taken together, these results suggest that T1g and T1f not only exhibit strong multi-target binding and favourable electronic characteristics but also possess pharmacokinetic and toxicity profiles suitable for further drug development. In contrast, T3a and T1c, while exhibiting slight variations in ADMET parameters, contribute complementary electronic and antioxidant properties, thereby reinforcing the overall therapeutic potential and versatility of this series of coumarin–thiadiazole derivatives.

Table 9. The pharmacochemical and toxicological profiles of compounds T3a, T1g, T1f, and T1c

Compounds	T3a	T1g	T1f	T1c
MW	419.49	454.34	387.45	425.5
Fraction Csp3	0.45	0.39	0.42	0.32
NRB	8	6	5	6
NHA	6	5	5	5
NHD	2	2	2	2
TPSA	137.54	128.31	128.31	128.31

MLOGP	2.21	2.62	2.5	3.02
Solubility Class	Moderately	Moderately	Moderately	Moderately
GI absorption	High	High	High	High
BBB permeant	No	No	No	No
Pgp substrate	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	Yes	Yes
Lipinski violations	0	0	0	0
Ghose violations	0	0	0	0
Veber violations	0	0	0	0
Egan violations	1	0	0	0
Muegge violations	0	0	0	0
PAINS alerts	0	0	0	0
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive
Neurotoxicity	Inactive	Inactive	Inactive	Inactive
Cardiotoxicity	Inactive	Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Inactive	Inactive

In conclusion, the in silico analyses of the selected coumarin–1,3,4-thiadiazole derivatives (T3a, T1f, T1g, and T1c) integrated both experimental bioactivity data and computational insights, providing a comprehensive understanding of their pharmacological potential. Compounds T1g and T1f exhibited strong acetylcholinesterase (AChE) inhibition alongside favorable electronic properties, while T3a and T1c contributed complementary antioxidant and electronic characteristics. Quantum chemical evaluations, including HOMO–LUMO energy levels and electrostatic potential mapping, highlighted differences in electron-donating and

electron-accepting capabilities, rationalizing their observed interactions and binding behaviors. Collectively, these results emphasize the multi-target bioactivity, electronic versatility, and therapeutic promise of these derivatives, supporting their prioritization for further experimental studies and structure-based optimization.

CHAPTER IV:
Experimental Part

IV.1. General information:

All chemicals required for the experimental work were sourced from Sigma-Aldrich and, unless otherwise stated, were applied directly without further treatment. The initial substrates consisted of commercially obtained coumarin carboxylates, namely methyl 2-oxo-2H-chromene-3-carboxylate (98% purity), methyl 8-ethoxy-2-oxo-2H-chromene-3-carboxylate (96%), and methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (96%). Additional reagents employed during the study included propargyl bromide (96%), benzyl chloride (98.5%), methyl benzyl chloride (98%), allyl bromide (97%), 1-bromo-3-methylbut-2-ene (96%), acetone (99.9%), acetic acid ($\geq 99.7\%$), thiosemicarbazide (98%), piperidine (99%), and dichloromethane ($\geq 99.5\%$).

Anhydrous potassium carbonate (K_2CO_3 , 99.0%) was used as the base in relevant reactions. Organic layers were washed with sodium chloride (NaCl, 99%), and drying was achieved using magnesium sulfate ($MgSO_4$, 99%) or sodium sulfate (Na_2SO_4 , 99%). Recrystallization was carried out using ethanol (96%) and hexane (98.5%) as solvents. All moisture-sensitive reagents were preserved in tightly sealed amber containers under a dry nitrogen atmosphere at room temperature. Solvents were freshly distilled when necessary; otherwise, analytical-grade commercial solvents were used without further purification.

On silica gel 60 F-254 plates (E. Merck), reaction progress was monitored by thin-layer chromatography (TLC) under UV light at 254 nm. Flash column chromatography on silica gel (60-120 mesh) with a gradient elution from 50:50 to 20:80 (v/v) in pentane/diethyl ether was used to purify the crude products. The reaction mixtures were heated under reflux at 120 °C for 4–6 h. We obtained the necessary products by separating the organic layers, drying them over anhydrous magnesium sulfate, filtering them, and then concentrating them under decreased pressure using a rotary evaporator.

Melting point, thin-layer chromatography (TLC), Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR), including proton (1H) and carbon-13 (^{13}C) NMR, were used to describe the compounds that were produced. Some samples underwent high-performance liquid chromatography (HPLC) testing.

The data presented here are the raw, uncorrected results of melting point measurements taken with a Stuart SMP30 digital melting point equipment. A PerkinElmer Spectrum Two spectrometer was used to record FTIR spectra from KBr disks. We used a Bruker Avance 600 spectrometer (400 MHz for ^1H and 101 MHz for ^{13}C) to acquire NMR spectra at 25 ± 1 °C in deuterated chloroform (CDCl_3) or deuterated dimethyl sulfoxide (DMSO-d_6). The internal reference, with a concentration of 0.00 ppm, was tetramethylsilane (TMS). For ^1H NMR, 64 scans were performed, while for ^{13}C NMR, 1024 scans were performed.

The units of reporting coupling constants (J) are hertz (Hz). The following symbols are used to denote different signal multiplicities: s, d, t, q, m, and br.

According to the results of the TLC and ^1H NMR analyses, the purity of all of the compounds was more than 95%. A C18 column, an Agilent 1200 system, a mobile phase consisting of 70:30 methanol and water, and a flow rate of 1 mL/min were used for the subsequent HPLC analysis of the chosen samples. All compounds were recrystallized from ethanol unless otherwise stated, and the spectral data confirmed the proposed structures.

IV.2. Chemical Synthesis:

IV.2.1. Synthesis of coumarin derivatives (C1.C2.C3):

The coumarin precursors C1–C3 were synthesized according to a general Knoevenagel condensation procedure using the corresponding salicylaldehyde derivative. In a 100 mL round-bottom flask placed in an ice bath under magnetic stirring, dimethyl malonate (14.41 g, 100 mmol, 1.0 equiv.) was dissolved in 30 mL of ethanol. Piperidine (1.0 mL) dissolved in 10 mL of ethanol was then added as a catalyst, and the mixture was stirred for 15 min. Subsequently, the corresponding salicylaldehyde derivative (100 mmol, 1.0 equiv.) dissolved in 20 mL of ethanol was added dropwise. Salicylaldehyde, o-vanillin, and 4-(diethylamino)-2-hydroxybenzaldehyde were used as starting materials for the synthesis of C1, C2, and C3, respectively. The reaction mixtures were stirred at room temperature for 3 h, and the reaction progress was monitored by thin-layer chromatography (TLC) using ethyl acetate/petroleum ether (1:8, v/v) as the developing solvent. Upon completion of the reaction, each mixture was extracted with ethyl acetate (3×15 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude products were purified by recrystallization from an ethyl

acetate/hexane (1:1, v/v) mixture to afford the desired coumarin precursors (C1–C3). TLC analyses were performed on silica gel 60 F-254 plates (E. Merck), and the compounds were visualized under UV light at 254 nm.

IV.2.2. Synthesis of asymmetric alkylated coumarin derivatives:

Twelve asymmetric derivatives of alkylated coumarins (C1a–C1g, C2a–C2c, C3a–C3b) were synthesised from three coumarin precursors (C1–C3) using a sequential addition and alkylation methodology. In a typical procedure, the selected coumarin precursor (10 mmol) was dissolved in anhydrous acetone (20 mL), and anhydrous potassium carbonate (K_2CO_3 , 25 mmol, 3.45 g) was added as a base. The mixture was stirred at room temperature for 10 minutes in order to ensure homogeneity, followed by the dropwise addition of the corresponding alkyl halide (R–Br or R–Cl, 15 mmol) in acetone (10 mL). Subsequently, the reaction mixture was subjected to reflux at a temperature of 56 °C for a duration of 7 hours.

After the reaction mixture was finished, it was allowed to cool to room temperature. Then, 25 mL of water was added. We used three 20 mL portions of ethyl acetate to extract the product. After that, we rinsed the organic layers with brine, dried them over anhydrous magnesium sulfate ($MgSO_4$), filtered them, and then concentrated them under reduced pressure. A 1:1 combination of pentane and diethyl ether was used as the eluent in flash column chromatography on silica gel (60-120 mesh) to purify crude alkylated coumarin compounds. Periodically, the reaction's progress was monitored by thin-layer chromatography (TLC) on silica gel 60 F-254 plates at 254 nm under ultraviolet light Jaafar et al.(2025).

IV.2.3. Synthesis of Asymmetric Coumarin 1,3,4-thiadiazole Derivatives:

Pre-synthesized asymmetric alkylated coumarin derivatives were used as starting materials in the synthesis of twelve asymmetric coumarin–1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, T3a–T3b), employing a previously reported modified cyclic condensation protocol. In a 100 mL double-necked round-bottom flask equipped with a magnetic stirrer and a reflux condenser, the appropriate coumarin derivative (1.0 mmol, 1.0 equiv.) was dissolved together with thiosemicarbazide (1.0 mmol, 1.0 equiv.) in 15 mL of glacial acetic acid at a solvent-to-reactant ratio of approximately 15:1 (v/v). A

catalytic amount of piperidine was added to the mixture, and the resulting mixture was stirred at room temperature for 15 min to ensure complete homogeneity.

The mixture was then heated under reflux at 120 °C for 4–6 h, with the reaction progress monitored periodically by thin-layer chromatography (TLC) on silica gel plates (E. Merck Kieselgel 60 F-254) using a 7:3 (v/v) hexane/ethyl acetate solvent system.

Upon completion of the reaction and the disappearance of the coumarin spot, the mixture was cooled to room temperature, and 25 mL of cold distilled water was added. The resulting precipitate was extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with saturated sodium chloride solution (2 × 20 mL), dried over anhydrous magnesium sulfate (MgSO₄), filtered, and concentrated under reduced pressure using a rotary evaporator. The crude residue was then purified by flash column chromatography on silica gel (60–120 mesh) using a pentane/diethyl ether gradient (50:50 to 20:80, v/v).

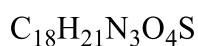
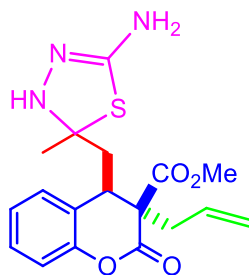
The purified compounds were recovered as crystalline solids with colours ranging from yellow to pale orange, yielding between 78% and 88% depending on the nature of the coumarin substituent.

IV.2.4. Structural and Spectroscopic Characterization of Synthesized Coumarin–1,3,4-Thiadiazole Derivatives:

A range of analytical techniques was employed to assess the quality of the recently produced coumarin 1,3,4-thiadiazole derivatives (T1a–T3b). These included thin-layer chromatography (TLC) for retention factor (R_f) evaluation, uncorrected melting point determination, Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy for proton and carbon-13 (¹H and ¹³C NMR). A Stuart SMP30 digital equipment was used for the purpose of measuring melting points. A hexane/ethyl acetate combination (7:3, v/v) was used as the mobile phase for the TLC analyses, which were carried out on silica gel 60 F-254 plates (Merck). Under ultraviolet light at 254 nm, the formed dots were observed. In order to identify distinctive absorption bands, FTIR spectra were acquired using KBr pellets in a PerkinElmer Spectrum Two spectrometer.

^1H and ^{13}C NMR spectra were obtained at $25 \pm 1^\circ\text{C}$ in either CDCl_3 or DMSO-d_6 , contingent upon the solubility of each compound, utilising a Bruker Avance 600 spectrometer (400 MHz for ^1H , 101 MHz for ^{13}C). Tetramethylsilane (TMS) functioned as the internal standard ($\delta = 0.00$ ppm). Pulse repetition intervals were established at 2 seconds for ^1H and 5 seconds for ^{13}C , with spectral widths of 12 ppm (^1H) and 240 ppm (^{13}C). To attain an adequate signal-to-noise ratio, 64 images were acquired for ^1H and 1024 scans for ^{13}C . Coupling constants (J) were expressed in Hz, and signal multiplicities were indicated as s, d, t, q, m, or br.

Through TLC and ^1H NMR tests, the purity of all substances (>95%) was validated. A C18 column and a MeOH/ H_2O (70:30) mobile phase operated at 1 mL/min were used in a high-performance liquid chromatography (HPLC) analysis of the chosen samples. The compounds were all recrystallised from ethanol unless otherwise indicated, and the spectrum data that was acquired agreed with the structures that had been proposed. In the section that follows, you will find comprehensive physical attributes (such as yield, colour, melting point, and R_f) and thorough spectrum information (from ^1H and ^{13}C NMR).

Compound T1a**Structure of Compound T1a**

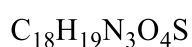
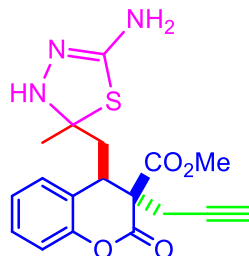
Methyl 3-allyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-2-oxo-3,4-dihydro-2H-chromene-3-carboxylate (T1a) was obtained as a brown solid with an 87% yield, melting point of 201–202 °C, and an R_f value of 0.6 (gradient: P–Et₂O, 1:1).

¹H NMR (400 MHz, CDCl₃) δ :

1.70 (s, 1H), 2.63 (dd, J = 17.2, 4.5 Hz, 1H), 2.92–2.81 (m, 1H), 3.41–3.21 (m, 3H), 4.10 (dd, J = 12.1, 7.1 Hz, 1H), 5.22–5.17 (m, 1H), 5.27 (d, J = 7.0 Hz, 1H), 5.99–5.79 (m, 3H), 7.11–7.00 (m, 1H), 7.19–7.11 (m, 1H), 8.76 (s, 1H).

¹³C NMR (101 MHz, CDCl₃) δ :

27.9 (CH), 31.2 (CH₃), 48.3 (CH₂), 52.01 (CH₃), 52.02 (C), 61.3 (C), 114.2 (CH₂), 117.5 (CH, aromatic), 124.5 (CH, aromatic), 127.4 (CH, aromatic), 128.0 (CH, aromatic), 128.7 (CH, aromatic), 135.9 (C), 136.9 (CH), 148.08 (C), 151.3 (C), 168.6 (C), 171.96 (C).

Compound T1b**Structure of Compound T1b**

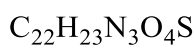
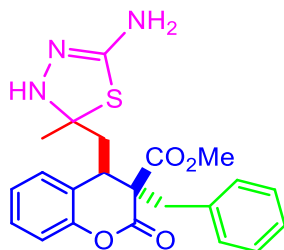
Methyl 2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(prop-2-yn-1-yl)-3,4-dihydro-2H-chromene-3-carboxylate (T1b) was obtained as a white solid with an 88% yield, melting point of 222 °C, and R_f value of 0.6 (gradient: P–Et₂O, 1:1).

¹H NMR (400 MHz, DMSO-d₆) δ:

1.64 (s, 1H), 2.27 (dd, J = 14.3, 4.2 Hz, 1H), 2.36 (dd, J = 17.3, 2.5 Hz, 1H), 2.80 (dd, J = 34.8, 15.8 Hz, 1H), 3.15 (t, J = 2.3 Hz, 1H), 3.33 (s, 1H), 3.76 (s, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.94 (t, J = 7.2 Hz, 1H), 7.13 (s, 0H), 7.42–7.19 (m, 1H), 8.13 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ:

30.7 (CH₃), 33.4 (CH), 43.7 (CH₂), 46.7 (CH₂), 52.9 (C), 53.1 (CH₃), 71.1 (CH), 75.5 (CH₂), 79.3 (C), 118.2 (C, aromatic), 120.6 (C, aromatic), 124.3 (C, aromatic), 129.1 (C, aromatic), 130.2 (C, aromatic), 150.7 (C), 150.9 (C, aromatic), 167.8 (C), 169.03 (C).

Compound T1c**Structure of Compound T1c**

Methyl-3-benzyl-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T1c) was obtained as a yellow solid with an 85% yield, melting point of 232–234 °C, and an R_f value of 0.6 (gradient: P–Et₂O, 1:1).

¹H NMR (400 MHz, CDCl₃) δ :

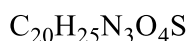
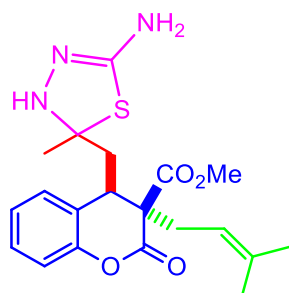
1.27 (s, 1H), 1.73 (d, J = 17.9 Hz, 1H), 2.27 (d, J = 10.4 Hz, 4H), 3.55 (s, 3H), 3.69 (dd, J = 8.7 Hz, 2H), 3.81 (d, J = 12.7 Hz, 2H), 7.14 (s, 1H), 7.24 (m, 5H), 7.34 (m, 4H), 8.76 (s, 1H).

¹³C NMR (101 MHz, CDCl₃) δ :

31.27 (CH₃), 32.6 (CH), 33.3 (CH₂), 42.4 (CH₂), 47.5 (CH₂), 52.60 (CH₃), 52.96 (CH₃), 117.4 (C, aromatic), 121.4 (C, aromatic), 126.9 (C, aromatic), 127.3 (C, aromatic), 127.9 (C, aromatic), 128.5 (C, aromatic), 128.9 (C, aromatic), 130.9 (C), 136.16 (C), 137.17 (C), 150.8 (C), 151.1 (C), 155.6 (C), 183.74 (C).

Compound T1d

Structure of Compound T1d



Methyl 3-(3-methylbut-2-en-1-yl)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T1d) was acquired as a white solid with an 87% yield, melting point of 208 °C, and R_f value of 0.6 (gradient: P–Et₂O, 1:1).

¹H NMR (400 MHz, DMSO-d₆) δ:

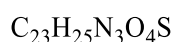
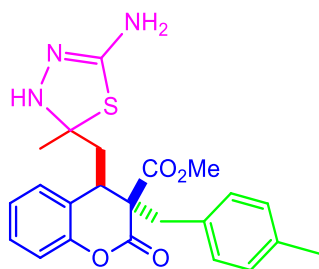
1.57 (s, 3H), 1.63 (s, 3H), 1.68 (s, 3H), 2.50 (dd, J = 59.0, 10.5 Hz, 3H), 2.82–2.70 (m, 1H), 3.10 (dd, J = 18.8, 3.6 Hz, 1H), 3.68 (s, 3H), 3.78 (d, J = 7.4 Hz, 2H), 5.33 (t, J = 7.1 Hz, 1H), 6.96–6.75 (m, 3H), 7.06 (s, 1H), 7.23 (t, J = 7.8 Hz, 2H), 8.18 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ:

17.23 (CH₃), 25.44 (CH₃), 31.07 (CH₃), 32.47 (CH₂), 34.98 (CH), 46.26 (CH₂), 52.04 (CH₃), 52.45 (CH₃), 59.12 (C), 117.6 (CH, aromatic), 119.97 (CH, aromatic), 128.59 (CH, aromatic), 130.58 (CH, aromatic), 133.71 (CH, aromatic), 134.2 (C, aromatic), 148.65 (C), 150.60 (C, aromatic), 169.10 (C), 170.69 (C), 182.28 (C).

Compound T1e

Structure of Compound T1e



With an 82% yield, a melting point of 248°C, an R_f value of 0.6, and a gradient of P-Et₂O, 1:1, **Methyl 3-(4-methylbenzyl)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T1e)** was produced as a white solid.

¹H NMR (400 MHz, DMSO-d₆) δ :

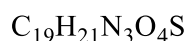
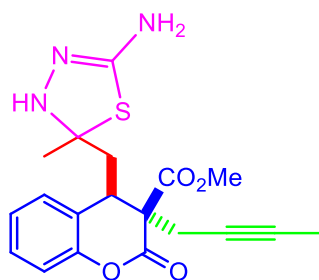
1.62 (s, 3H), 2.31 (s, 4H), 2.33 (d, J = 1.7 Hz, 1H), 2.70–2.63 (m, 2H), 3.01–2.76 (m, 3H), 3.07 (d, J = 10.7 Hz, 1H), 3.26 (d, J = 4.0 Hz, 1H), 3.31 (s, 23H), 3.75 (m, 0H), 5.49 (dd, J = 11.9, 4.5 Hz, 0H), 6.71 (d, J = 1.2 Hz, 1H), 7.10–6.75 (m, 2H), 7.24 (dd, J = 13.0, 8.0 Hz, 4H), 7.36 (d, J = 7.9 Hz, 0H), 8.42 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ :

20.3 (CH₃), 26.5 (CH), 31.6 (CH₃), 35.2 (CH₂), 46.7 (CH₂), 50.8 (CH₃), 51.8 (C), 72.3 (C), 116.0 (C, aromatic), 125.6 (C, aromatic), 127.8 (C, aromatic), 128.28 (C, aromatic), 128.4 (C, aromatic), 128.9 (C, aromatic), 135.1 (C, aromatic), 136.0 (C, aromatic), 150.4 (C), 151.3 (C, aromatic), 168.1 (C), 172.26 (C)

Compound T1f

Structure of Compound T1f



Methyl 3-(but-2-yn-1-yl)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T1f) was acquired as a brown solid with an 85% yield, melting point of 228–229 °C, and R_f value of 0.6 (gradient: P–Et₂O, 1:1).

Its ¹H NMR spectrum (400 MHz, DMSO-*d*₆) showed signals at δ

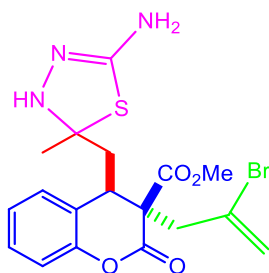
1.64 (s, 1H), 1.82 (s, 1H), 2.89–2.19 (m, 4H), 3.83 (s, 11H), 5.07 (t, $J = 13.4$ Hz, 1H), 7.02 (s, 1H), 7.51–7.29 (m, 5H), 7.73 (t, $J = 7.8$ Hz, 4H), 7.90 (d, $J = 7.7$ Hz, 5H), 8.76 (s, 1H).

The ¹³C NMR spectrum (101 MHz, DMSO-*d*₆) displayed resonances at δ

10.4 (CH₃), 17.3 (CH₂), 29.05 (CH), 31.5 (CH₃), 43.7 (CH₂), 52.4 (CH₃), 52.9 (C), 60.09 (C), 73.2 (C), 77.9 (C), 117.3 (CH, aromatic), 124.8 (CH, aromatic), 129.5 (CH, aromatic), 130.3 (CH, aromatic), 134.5 (C, aromatic), 149.06 (C), 151.0 (C), 163.1 (C), 170.9 (C).

Compound T1g

Structure of Compound T1g



With an R_f value of 0.6 (gradient: P-Et₂O, 1:1), A white solid was obtained in 84% yield, with a melting point of 216–218 °C, known as **Methyl 3-(2-bromoallyl)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T1g)**.

¹H NMR (400 MHz, DMSO-d₆) δ :

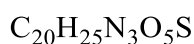
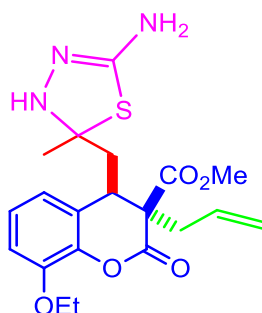
1.64 (s, 3H), 2.33–2.07 (m, 2H), 2.64 (d, $J = 12.8$ Hz, 1H), 3.77 (s, 3H), 3.97–3.84 (m, 3H), 5.61 (s, 1H), 5.73 (s, 1H), 6.98–6.80 (m, 3H), 7.00 (s, 1H), 7.43–7.08 (m, 2H), 8.22 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ :

29.3 (CH), 30.58 (CH₃), 35.2 (CH₂), 41.3 (C), 41.8 (CH₂), 52.7 (C), 52.92 (CH₃), 88.5 (CH₂), 115.4 (CH, aromatic), 118.3 (CH₂), 120.9 (CH, aromatic), 126.8 (CH, aromatic), 127.3 (C, aromatic), 128.8 (CH, aromatic), 130.7 (C), 149.4 (C), 150.6 (C), 182.2 (C).

Compound T2a

Structure of Compound T2a



Methyl 3-allyl-8-ethoxy-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T2a) was acquired as a yellow viscous substance with an 87% yield, melting point of 220–222 °C, and R_f value of 0.5 (gradient: P–Et₂O, 1:1).

¹H NMR (400 MHz, DMSO-d₆) δ:

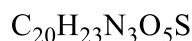
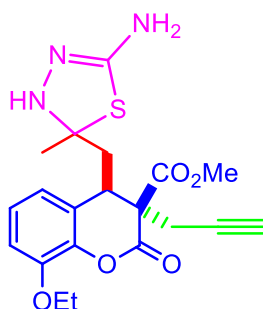
1.40 (t, J = 7.0 Hz, 3H), 1.66 (s, 1H), 2.08 (s, 1H), 2.67 (dd, J = 3.6, 1.8 Hz, 1H), 3.01–2.96 (m, 2H), 3.04 (dd, J = 9.5, 3.3 Hz, 1H), 3.21 (dd, J = 18.9, 3.3 Hz, 1H), 3.83 (s, 3H), 4.02–3.94 (m, 1H), 4.16 (q, J = 7.0 Hz, 2H), 4.34 (d, J = 7.0 Hz, 1H), 4.52 (d, J = 5.8 Hz, 2H), 4.78 (dt, J = 5.3, 1.5 Hz, 6H), 6.81 (d, J = 6.7 Hz, 0H), 6.90 (d, J = 3.1 Hz, 2H), 7.46–7.21 (m, 2H), 8.72 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ:

14.5 (CH₃), 31.41 (CH₃), 35.9 (CH), 41.6 (CH₂), 46.6 (CH₂), 52.45 (CH₃), 52.91 (C), 59.9 (C), 64.5 (CH₂), 112.5 (C, aromatic), 118.3 (C, aromatic), 121.1 (C, aromatic), 124.7 (C, aromatic), 134.4 (CH, aromatic), 140.9 (CH), 143.9 (C, aromatic), 145.4 (C, aromatic), 149.3 (C), 163.1 (C), 169.2 (C).

Compound T2b

Structure of Compound T2b



With an 88% yield, m.p. of 230-231°C, $R_f = 0.5$ (gradient: P-Et₂O, 1:1), and a brown solid, **Methyl 8-ethoxy-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(prop-2-yn-1-yl)-3,4-dihydro-2H-chromene-3-carboxylate (T2b)** was produced.

¹H NMR (400 MHz, CDCl₃) δ :

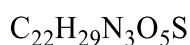
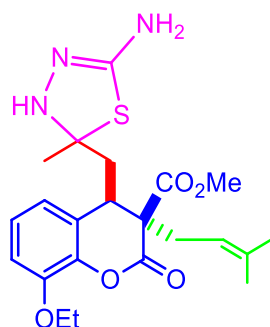
1.47 (t, $J = 7.0$ Hz, 3H), 1.75 (s, 2H), 2.12 (t, $J = 2.6$ Hz, 1H), 2.52 (s, 1H), 3.19 (dd, $J = 19.0, 2.9$ Hz, 2H), 3.75 (dd, $J = 28.8, 18.6$ Hz, 2H), 3.92 (s, 3H), 4.06–3.96 (m, 9H), 4.16 (q, $J = 7.0$ Hz, 4H), 6.40 (d, $J = 9.5$ Hz, 3H), 7.01 (s, 1H), 7.16 (t, $J = 7.9$ Hz, 9H), 7.66 (d, $J = 9.5$ Hz, 2H), 8.50 (s, 1H).

¹³C NMR (101 MHz, CDCl₃) δ :

14.81 (CH₃), 27.89 (CH₂), 29.75 (CH₂), 31.98 (CH₃), 48.98 (CH₂), 52.74 (C), 52.95 (CH₃), 64.68 (C), 65.07 (CH₂), 82.40 (CH), 83.89 (C), 115.15 (C, aromatic), 119.32 (C, aromatic), 124.37 (C, aromatic), 124.83 (C, aromatic), 143.81 (C, aromatic), 146.66 (C, aromatic), 149.53 (C), 160.57 (C), 173.95 (C).

Compound T2c

Structure of Compound T2c



(3S,4R)-Methyl 8-ethoxy-3-(3-methylbut-2-en-1-yl)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T2c) was acquired as a yellowish solid with an 82% yield, melting point of 206 °C, and *R_f* value of 0.5 (gradient: P–Et₂O, 1:1).

¹H NMR (400 MHz, DMSO-*d*₆) δ:

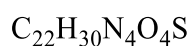
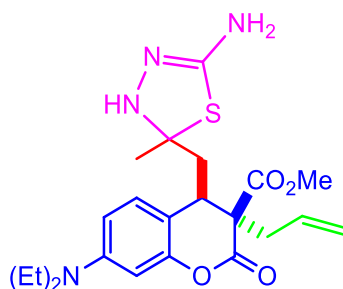
1.06 (t, *J* = 7.0 Hz, 2H), 1.62 (s, 1H), 1.76 (s, 1H), 1.89 (s, 2H), 2.31 (d, *J* = 2.6 Hz, 1H), 2.85 (s, 2H), 2.95 (d, *J* = 4.5 Hz, 1H), 4.35 (t, *J* = 5.0 Hz, 1H), 5.09 (s, 2H), 5.49 (dd, *J* = 11.7, 4.7 Hz, 1H), 7.29–7.14 (m, 3H), 8.43 (s, 1H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ:

15.49 (CH₃), 18.55 (CH₃), 25.55 (CH₃), 27.35 (CH), 31.17 (CH₃), 31.92 (CH₂), 46.93 (CH₂), 51.07 (CH₃), 54.82 (C), 61.80 (C), 69.22 (CH₂), 112.37 (CH), 120.91 (CH), 121.70 (CH), 125.87 (CH), 134.04 (C), 135.34 (C), 136.31 (C), 149.40 (C), 154.80 (C), 166.62 (C), 172.32 (C).

Compound T3a

Structure of Compound T3a



A greenish solid with a melting point of 238°C, an R_f value of 0.7, and a gradient of P-Et₂O in a 1:1 ratio was produced as **Methyl 3-allyl-7-(diethylamino)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T3a)** 86% yield).

¹H NMR (400 MHz, DMSO-d₆) δ :

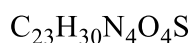
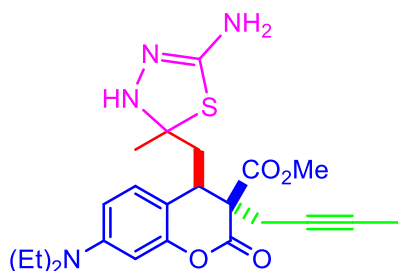
1.06 (t, J = 6.8 Hz, 3H), 1.60 (s, 1H), 1.96 (s, 1H), 2.19 (dd, J = 14.1, 3.9 Hz, 1H), 2.96 (dd, J = 12.0, 7.9 Hz, 1H), 3.27 (q, J = 7.1 Hz, 2H), 3.36 (dd, J = 9.9, 5.6 Hz, 1H), 3.45 (dd, J = 13.9, 7.0 Hz, 1H), 3.69 (s, 1H), 3.99 (dd, J = 15.2, 5.7 Hz, 1H), 4.60 (d, J = 5.2 Hz, 1H), 5.06 (t, J = 13.7 Hz, 1H), 6.63–5.68 (m, 1H), 6.99 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 9.0 Hz, 1H), 8.56 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ :

12.33 (CH₃), 12.54 (CH₃), 23.83 (CH), 32.36 (CH₃), 35.42 (CH₂), 43.69 (CH₂), 44.39 (CH₂), 46.68 (CH₂), 51.75 (CH₃), 52.83 (C), 60.59 (C), 104.88 (CH, aromatic), 109.82 (CH, aromatic), 118.23 (CH₂), 129.97 (C), 131.36 (CH, aromatic), 134.67 (CH), 148.17 (C, aromatic), 149.48 (C), 151.83 (C), 169.66 (C), 171.35 (C).

Compound T3b

Structure of Compound T3b



Using a 1:1 gradient of P-Et₂O, a brown solid with a molecular weight of 241-242°C and an R_f of 0.7 was produced as **Methyl 3-(but-2-yn-1-yl)-7-(diethylamino)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T3b)**, with an 87% yield.

¹H NMR (400 MHz, DMSO-d₆) δ:

1.13 (t, J = 7.0 Hz, 6H), 1.77 (s, 3H), 2.05–2.00 (m, 3H), 2.68–2.64 (m, 6H), 3.55–3.44 (m, 3H), 3.75 (s, 3H), 4.16–4.11 (m, 2H), 6.52 (d, J = 2.0 Hz, 2H), 6.79–6.74 (m, 3H), 7.28 (d, J = 8.0 Hz, 2H), 8.56 (s, 1H).

¹³C NMR (101 MHz, DMSO-d₆) δ:

3.98 (C), 12.35 (CH₃), 13.92 (C), 23.79 (C), 31.29 (C), 44.40 (CH₂), 51.77 (C), 52.48 (C), 61.39 (C), 74.27 (C), 78.20 (C), 107.04 (C), 109.87 (C), 124.92 (C), 128.69 (C), 145.72 (C), 149.52 (C), 150.06 (C), 168.87 (C), 176.76 (C).

Table 10. General physical properties of synthesized coumarin–1,3,4-thiadiazole derivatives (T1a–T3b).

Compound	Yield (%)	m.p. (°C)	R _f (solvent system)	Appearance
T1a	87	201-202	0.6 (pentane/Et ₂ O 1:1)	Brown solid
T1b	88	222	0.6 (pentane/Et ₂ O 1:1)	White solid
T1c	85	232-234	0.6 (pentane/Et ₂ O 1:1)	Yellow solid
T1d	87	208	0.6 (pentane/Et ₂ O 1:1)	White solid
T1e	82	248	0.6 (pentane/Et ₂ O 1:1)	White solid
T1f	85	228-229	0.6 (pentane/Et ₂ O 1:1)	Brown solid
T1g	84	216-218	0.6 (pentane/Et ₂ O 1:1)	White solid
T2a	87	220-222	0.5 (pentane/Et ₂ O 1:1)	Yellow viscous
T2b	88	230-231	0.5 (pentane/Et ₂ O 1:1)	Brown solid
T2c	82	206	0.5 (pentane/Et ₂ O 1:1)	Yellowish Solid
T3a	86	238	0.7 (pentane/Et ₂ O 1:1)	Greenish solid
T3b	87	241-242	0.7 (pentane/Et ₂ O 1:1)	Brown solid

IV.3. Biological Methods:

The synthesized coumarin derivatives underwent a series of in vitro biological assays to evaluate their potential as antioxidant and neuroprotective agents. Their antioxidant activity was assessed using three complementary methods: the ABTS radical scavenging assay, the o-phenanthroline reducing power assay, and the FRAP assay. Additionally, the inhibitory activity against acetylcholinesterase (AChE) was evaluated.

These assays provide a comprehensive assessment of the biological potential of the synthesized compounds.

IV.3.1. Determination of Antioxidant Activity by the ABTS Radical Cation

Method:

We used the ABTS radical cation decolourisation assay to comprehensively assess the ability of the novel coumarin 1,3,4-thiadiazole derivatives (T1a–T3b) to scavenge free radicals. This widely acknowledged spectrophotometric approach was carried out following the protocol established by Re et al. (1999) [145], with slight modifications to accommodate the current study. ABTS was oxidised with potassium persulfate to make the ABTS radical cation (ABTS^{•+}). In particular, a 7 mM solution of ABTS in water was mixed with 2.45 mM of potassium persulfate (K₂S₂O₈). The mixture was left in the dark at room temperature for 12 to 16 hours so that the ABTS^{•+} chromophore could fully develop and stabilise.

Before the test, the radical solution was mixed with 100% ethanol to make a working solution with an absorbance of 0.703 ± 0.025 at 734 nm. We used a UV–Vis microplate reader (EnSpire, PerkinElmer, USA) to verify that the radical concentration was consistent and correct before it interacted with the test compounds.

Each chemical solution, produced in methanol at concentrations ranging from 0.0625 to 4.0 mM, was divided into 40 μ L portions and placed into the wells of a 96-well microplate for the scavenging experiment. To start the reaction, 160 μ L of the newly made ABTS^{•+} working solution was added to every well. A decrease in absorbance at 734 nm was measured after the reaction mixtures were mixed gently and left to incubate in the dark at room temperature (25 ± 2 °C) for 10 minutes. This allowed the antioxidant molecules and radical species to interact sufficiently. For this comparative evaluation, we utilised butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as positive reference antioxidants, and we corrected the background absorbance with methanol.

Radical scavenging activity was quantified as the percentage inhibition of ABTS^{•+} according to the following equation:

$$\text{ABTS scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}} / A_{\text{control}})] \times 100$$

where A_{control} and A_{sample} represent the absorbance values of the control and test samples, respectively.

The data are shown as the mean $\pm$ standard deviation (SD), and all experimental determinations were carried out in triplicate (three separate trials, $n = 3$). Assuming differences to be statistically significant at $p < 0.05$, one-way analysis of variance (ANOVA) was employed to conduct statistical comparisons. Using XLSTAT software for nonlinear regression analysis, the IC_{50} values were determined. These values are defined as the concentration needed to block 50% of $ABTS^{\bullet+}$ radicals.

IV.3.2. Determination of Antioxidant Activity Using the 1,10-Phenanthroline Reducing Power Assay:

The reducing properties of the synthesised compounds were evaluated using the o-phenanthroline method, as described by Szydłowska–Czerniak et al. (2008) [146], with minor adjustments to the experimental procedure.

A 96-well microplate with 50 μL of 0.2% FeCl_3 , 30 μL of 0.5% o-phenanthroline, and 110 μL of methanol was supplemented with 10 μL of each chemical solution, which ranged from 0.0625 to 4.0 mM in methanol. The EnSpire Multimode Plate Reader (PerkinElmer, USA) was used to measure absorbance at 510 nm after incubating the reaction mixture at 30 $^{\circ}\text{C}$ for 20 minutes. Under the same testing circumstances, BHA was used as the reference antioxidant. The concentration needed to achieve an absorbance value of 0.50 was specified as $A_{0.5}$ (μM), which is the unit of reducing activity. A stronger antioxidant reduction potential is indicated by lower $A_{0.5}$ values.

The data are shown as the mean $\pm$ standard deviation (SD), and each experiment was carried out three times. The values of $A_{0.5}$ were found by linear regression analysis with the help of the XLSTAT program.

IV.3.3. Antioxidant Activity by the Ferric Reducing Antioxidant Power (FRAP) Assay:

The antioxidant reducing capacity of the synthesised derivatives was determined using the Ferric Reducing Antioxidant Power (FRAP) method, according to Oyaizu (1986) , with minor modifications.

In a 96-well plate, the test components included 10 μL of each drug solution at different concentrations, 40 μL of phosphate buffer solution (pH 6.6), and 50 μL of 1% potassium ferricyanide solution (w/v). After that, the mixture was heated to 50 $^{\circ}\text{C}$ for 20 minutes to reduce the Fe^{3+} ions. Then, 50 μL of 10% (w/v) trichloroacetic acid was added to stop the reaction. After that, 40 μL of distilled water was added to the solution that had been produced, and then 10 μL of a ferric chloride solution with a concentration of 0.1% (w/v) was added. An EnSpire Multimode Plate Reader (PerkinElmer, USA) was used to record the absorbance at 700 nm after full mixing. The studied substances exhibited improved reducing activity as demonstrated by an increase in absorbance.

The findings are shown as the average plus or minus the standard deviation (SD), and every measurement was taken three times.

IV.3.4. Anti-Enzymatic (Anti-AChE) Activity:

The inhibitory effect of the synthesised 1,3,4-thiadiazole coumarin derivatives (T1a–T3b) against acetylcholinesterase (AChE) was evaluated using the spectrophotometric method originally described by Ellman et al. (1961) [147] with slight adaptations to the experimental procedure. This assay is based on quantifying thiocholine produced by the enzymatic hydrolysis of a thioester substrate, which reacts with DTNB to generate a yellow product that can be measured at 412 nm.

The experiment was conducted in a microplate with 96 wells. First, a 100 mM sodium phosphate buffer solution (pH 8.0) was mixed with 150 μL of each drug that was evaluated, with 10 μL of each molecule being produced in ethanol at concentrations ranging from 0.0625 to 2.0 mM. Afterwards, 20 μL of a solution containing acetylcholinesterase, which was derived from an electric eel (Type VI-S, Sigma-Aldrich) and had 5.32×10^{-3} U per well, was added to every well. The enzyme and any potential inhibitors were given enough time to interact by gently shaking the mixture and incubating it at 25 ± 1 $^{\circ}\text{C}$ for 15 minutes. Then, the substrate was added. Proceeding with the addition of 10 μL each of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 0.5 mM) and acetylthiocholine iodide (ATCI, 0.71 mM), the enzymatic reaction was thereafter started.

The absorbance was measured at 412 nm using an EnSpire Multimode Plate Reader (PerkinElmer, USA) twice: once after 0 minutes of substrate addition and again after 15 minutes. The solvent control, ethanol, was utilised to control for background activity, and the standard reference inhibitor, galantamine hydrobromide (10 μ M), was employed for comparison. The data were presented as a percentage of inhibition (I%), and all determinations were done in triplicate (n = 3). The following equation was used to calculate inhibitory activity:

$$I\% = A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}$$

Where A_{control} and A_{sample} represent the absorbance readings of the reaction mixture in the absence and presence of the test compounds, respectively. Galantamine hydrobromide was used as the standard reference inhibitor.

IV.4. Statistical analysis:

All experimental measurements were performed in triplicate (n = 3), and the results are presented as the mean $\pm$ standard deviation (SD). The half-maximal inhibitory concentration (IC_{50}) values for acetylcholinesterase (AChE) inhibition and radical scavenging assays (ABTS), as well as the half-maximal effective concentration ($A_{0.5}$) values for the reducing power assays (FRAP and phenanthroline), were determined by linear regression analysis using XLSTAT 2023 (Addinsoft, France). Graphical representations of the experimental data were generated using OriginPro 2022b (OriginLab Corporation, Northampton, MA, USA). The reported IC_{50} and $A_{0.5}$ values were obtained from the corresponding dose–response curves and are expressed as mean $\pm$ SD of three independent experiments.

IV.5. In silico Computational studies:

Molecular docking was performed using AutoDock Vina to investigate the binding affinity of the selected compounds toward the target proteins. The docking poses were ranked according to their predicted binding free energy (ΔG), and the ligand–protein interactions were analyzed using Discovery Studio Visualizer.

A selection of compounds (T1g, T3a, T1f, and T1c) were subjected to geometry optimisation and electronic property analysis utilising Density Functional Theory (DFT) at the B3LYP/def2-SVP level, implemented in ORCA 5.0.4 [148] [149] [150] [151].

Optimisations were conducted in the gas phase, followed by single-point energy calculations employing an implicit water solvent model (CPCM) to approximate physiological conditions.

The molecular structures were then subjected to a rigorous examination using vibrational frequency analysis to ascertain the absence of imaginary frequencies. This process confirmed that the structures indeed represent true minima on their respective potential energy surfaces. Subsequently, a range of significant quantum chemical parameters were computed, including HOMO and LUMO energies, energy gap (ΔE), chemical hardness, softness, chemical potential, and electronegativity. Electron density distributions were examined, and potential reactive regions were visualised using electrostatic potential (ESP) maps generated by Multiwfn 3.8. The compounds were constructed in ACD/ChemSketch 2022 and subsequently converted to PDBQT format using AutoDockTools 1.5.6 [152], with protonation adjusted to align with physiological pH (7.4).

The human acetylcholinesterase crystal structure (PDB ID: 4EY7; resolution 2.35 Å) was obtained from the Protein Data Bank. Protein preparation involved the removal of crystallographic water molecules and the co-crystallized ligand, followed by the addition of polar hydrogen atoms and Kollman charges. Molecular docking simulations were performed using AutoDock Vina 1.2.3 [153] with a grid box ($40 \times 40 \times 40$ Å) centered on the catalytic active site containing Ser203, His447, and Glu334. An exhaustiveness value of 32 was employed to improve the conformational search. The resulting docking poses were ranked according to their predicted binding free energies (ΔG), and the most favorable binding conformations were further analyzed using BIOVIA Discovery Studio Visualizer to identify hydrogen bonds, π - π stacking, π -cation, and hydrophobic interactions. ADMET profiles were predicted using SwissADME, ADMETlab 2.0, and ProTox-II, with consideration given to Lipinski's rule of five, bioavailability, TPSA, logP, gastrointestinal absorption, blood-brain barrier penetration, CYP450 inhibition, and LD50.

In summary, all synthesized coumarin derivatives were successfully prepared and fully characterized using standard spectroscopic and chromatographic techniques. We established and optimized biological evaluation protocols, which included antioxidant

and acetylcholinesterase inhibition assays. Statistical analysis procedures were applied to ensure the reliability of the experimental data. Additionally, computational studies provided preliminary insights into molecular interactions and pharmacokinetic properties. This comprehensive methodology lays a solid foundation for the subsequent presentation and discussion of the results.

General conclusion

Conclusion

This study shows that rational modifications of the coumarin–1,3,4-thiadiazole scaffold represent an effective and versatile strategy for developing multifunctional small molecules with promising pharmacological profiles. By combining synthetic chemistry, biological evaluation, and computational modeling, this research establishes clear structure-activity relationships and provides a coherent framework for understanding the molecular factors that influence both chemical reactivity and biological activity. The integration of these complementary approaches offers deeper insights into how structural variations impact chemical properties and biological responses, thereby aiding in the rational design of more efficient and potent derivatives.

From a synthetic perspective, the alkylation methodology used was robust, reproducible, and operationally straightforward. The targeted derivatives were consistently obtained in high yields (82%–88%), demonstrating the reliability of this method. Variations in reaction efficiency across the series reflected the combined influence of the electronic and steric effects of the substituents. These insights are particularly valuable for the rational fine-tuning of the coumarin–thiadiazole scaffold in future synthetic optimization efforts. Furthermore, the successful synthesis of structurally diverse analogues highlights the suitability of this methodology for medicinal chemistry programs aimed at developing multifunctional drug candidates.

Biological investigations into coumarin 1,3,4-thiadiazole derivatives that have been synthesised, revealed that several compounds display pronounced antioxidant capacity as well as acetylcholinesterase (AChE) inhibitory activity, underscoring the multifunctional potential of this hybrid molecular framework. Notably, compounds T1c and T1f exhibited significant radical scavenging activity against ABTS, with T1c achieving an IC_{50} value of $24.32 \pm 0.36 \mu\text{M}$, indicating strong electron-donating and redox properties. In parallel, compounds T1g and T1f were identified as the most effective AChE inhibitors, displaying IC_{50} values of $20.35 \pm 0.06 \mu\text{M}$ and $38.90 \pm 0.46 \mu\text{M}$, respectively, reflecting favorable enzyme–ligand interactions. These observations suggest that the electronic characteristics and the spatial orientation of substituents on the scaffold play a pivotal role in modulating both oxidative behavior and enzymatic

inhibition. Furthermore, the findings highlight that even subtle structural modifications can induce considerable variations in biological activity, thereby offering valuable insights for the rational design of multifunctional therapeutic agents.

Computational analyses strongly support the experimental findings. The calculated HOMO-LUMO energy gaps, which range from 1.65 to 2.19 eV, indicate favorable electronic properties that are consistent with enhanced molecular reactivity and stability. Molecular docking simulations demonstrated strong binding affinities toward acetylcholinesterase, with binding free energies reaching up to -12.1 kcal/mol. These *in silico* results offer a mechanistic explanation for the observed inhibitory activities and clarify the proposed binding modes at the molecular level. The alignment of computational predictions with experimental data reinforces the reliability and robustness of the integrated research approach, showcasing how combining synthetic, biological, and computational methods can accelerate drug discovery processes.

The coumarin–thiadiazole scaffold has emerged as a promising and versatile platform for lead discovery and optimization. While this study represents an early stage in developing this chemical series, the identification of T1f and T1g as lead candidates, along with the clarified structure-activity relationships, provides a solid foundation for further refinement. This work also emphasizes the importance of understanding both electronic and steric effects on biological activity, as well as the potential for rationally designing derivatives with enhanced multifunctionality.

Future studies should consider exploring the following areas:

- Conduct a systematic expansion of the scaffolds to investigate additional substituent patterns.
- Perform comprehensive pharmacokinetic and pharmacodynamic profiling to gain a deeper understanding of the absorption, distribution, metabolism, and excretion properties.
- Conduct advanced *in vivo* evaluations, focusing particularly on oxidative stress and cholinergic dysfunction associated with Alzheimer's disease.

- Explore combination strategies that involve other bioactive scaffolds to enhance therapeutic efficacy and selectivity.

In conclusion, this study makes a significant contribution to the growing body of knowledge in multifunctional drug design. It provides a rational and experimentally validated framework for developing novel therapeutic agents targeting complex neurodegenerative disorders. The insights gained here lay the groundwork for future research aimed at optimizing scaffold performance, enhancing pharmacological profiles, and ultimately facilitating the discovery of more effective and safer therapeutic candidates.

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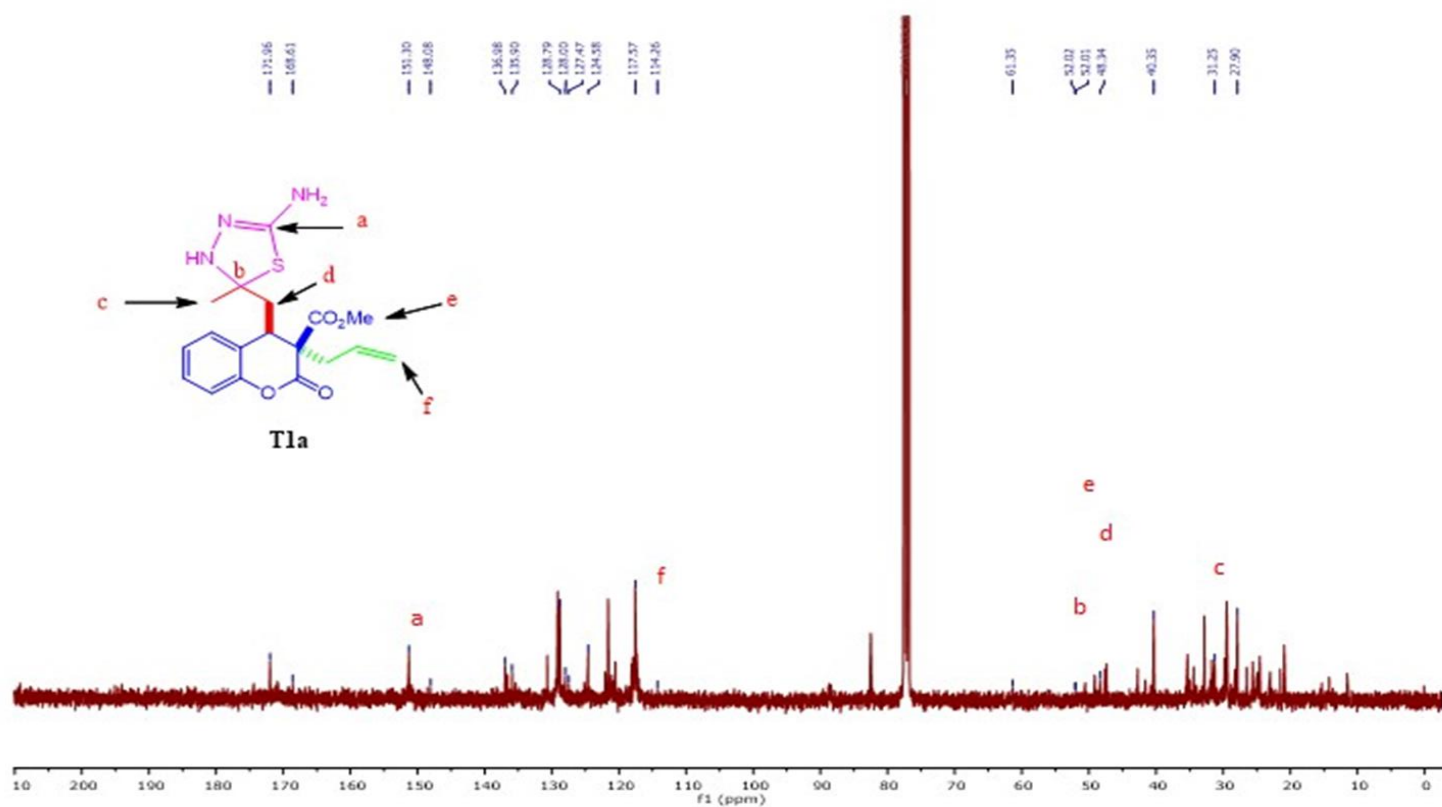
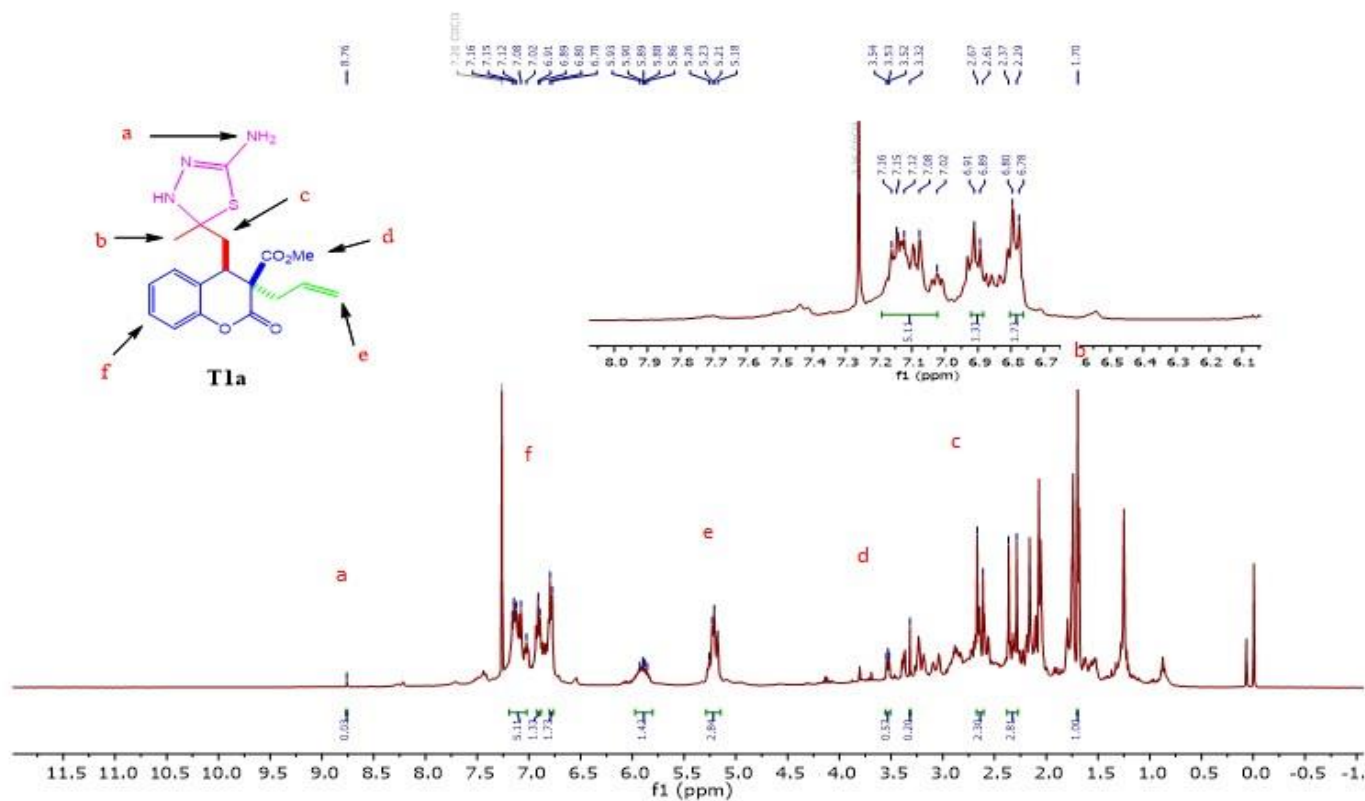
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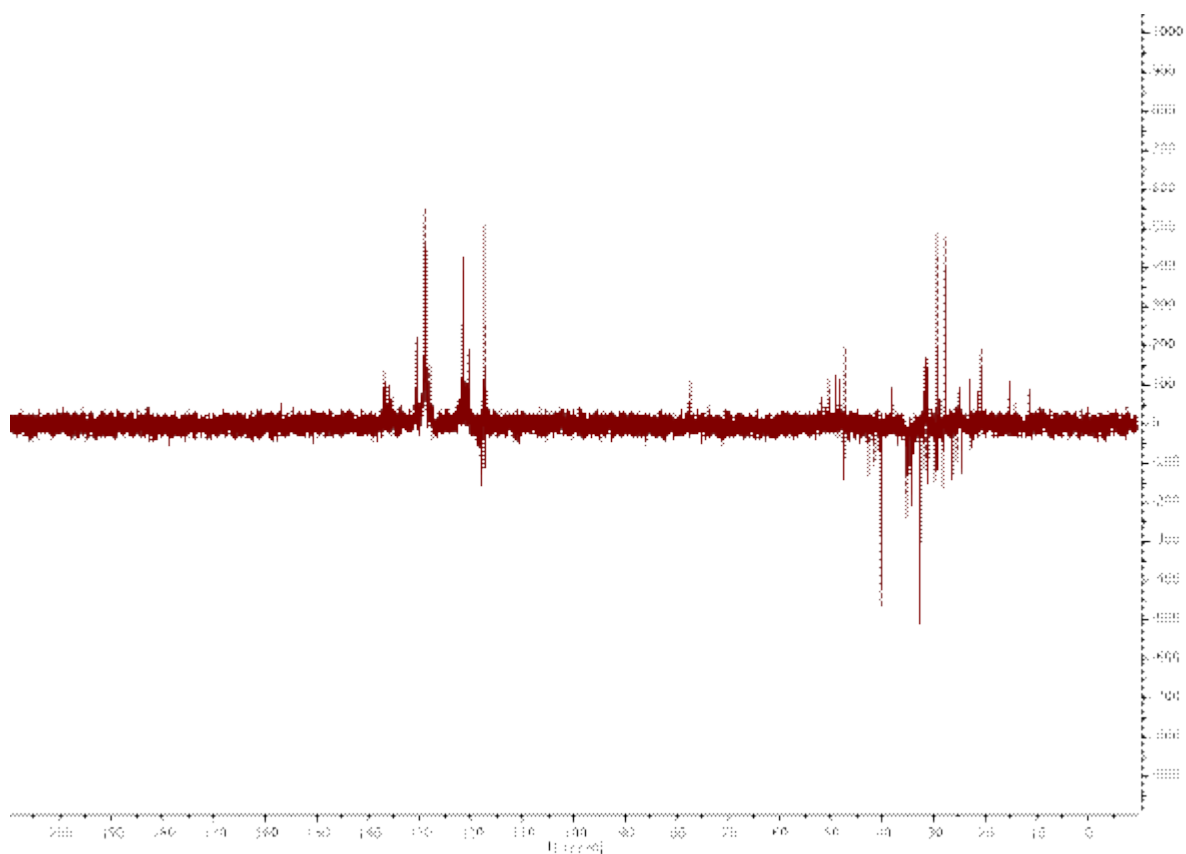
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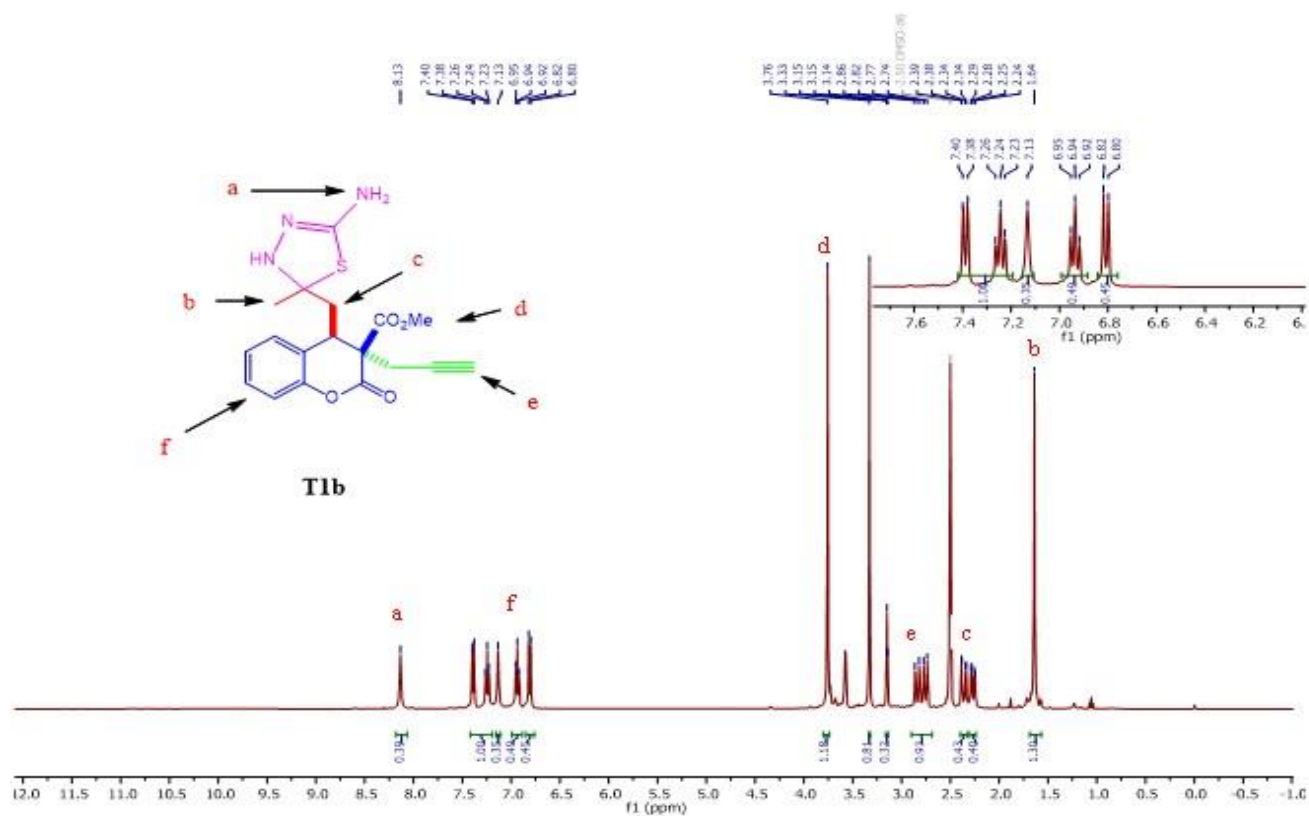
Appendices

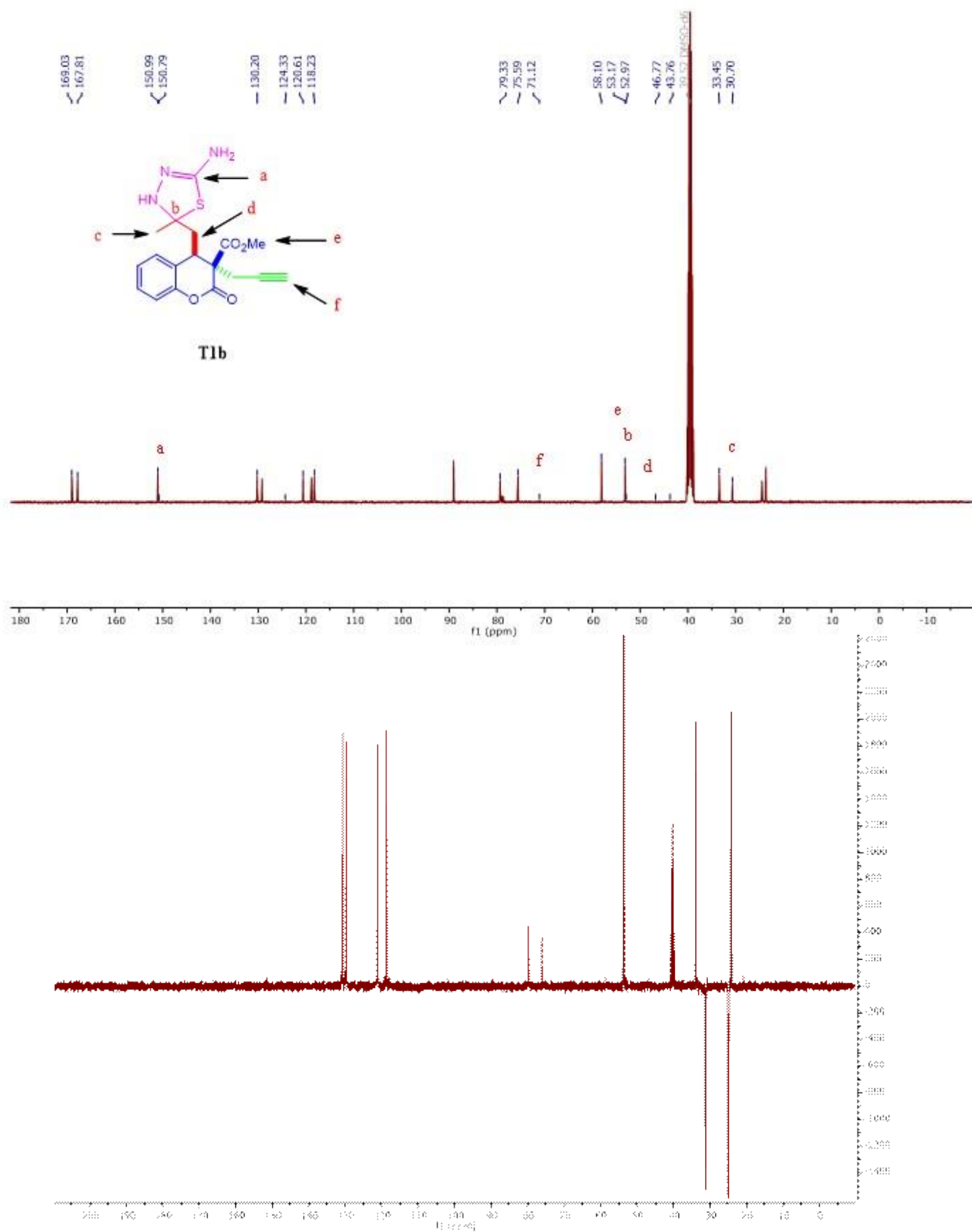
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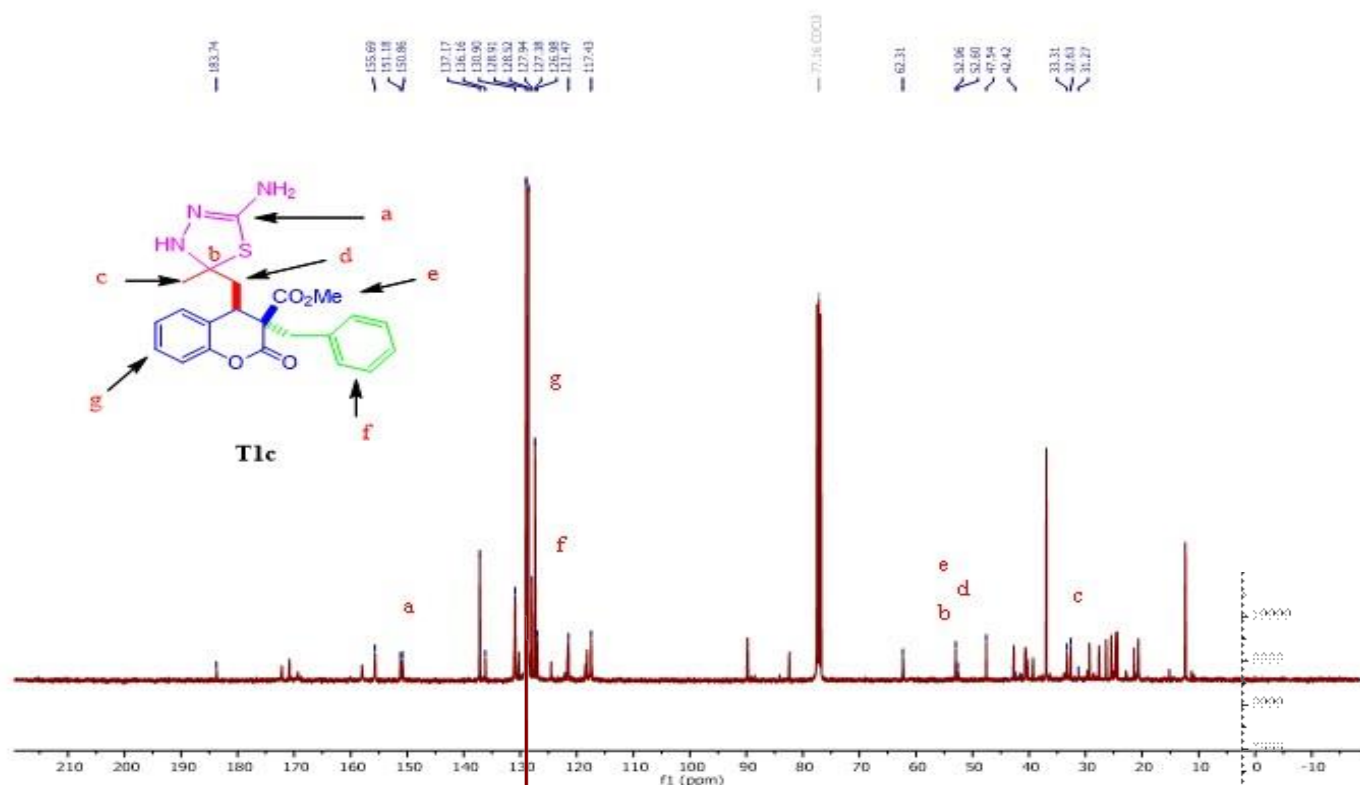
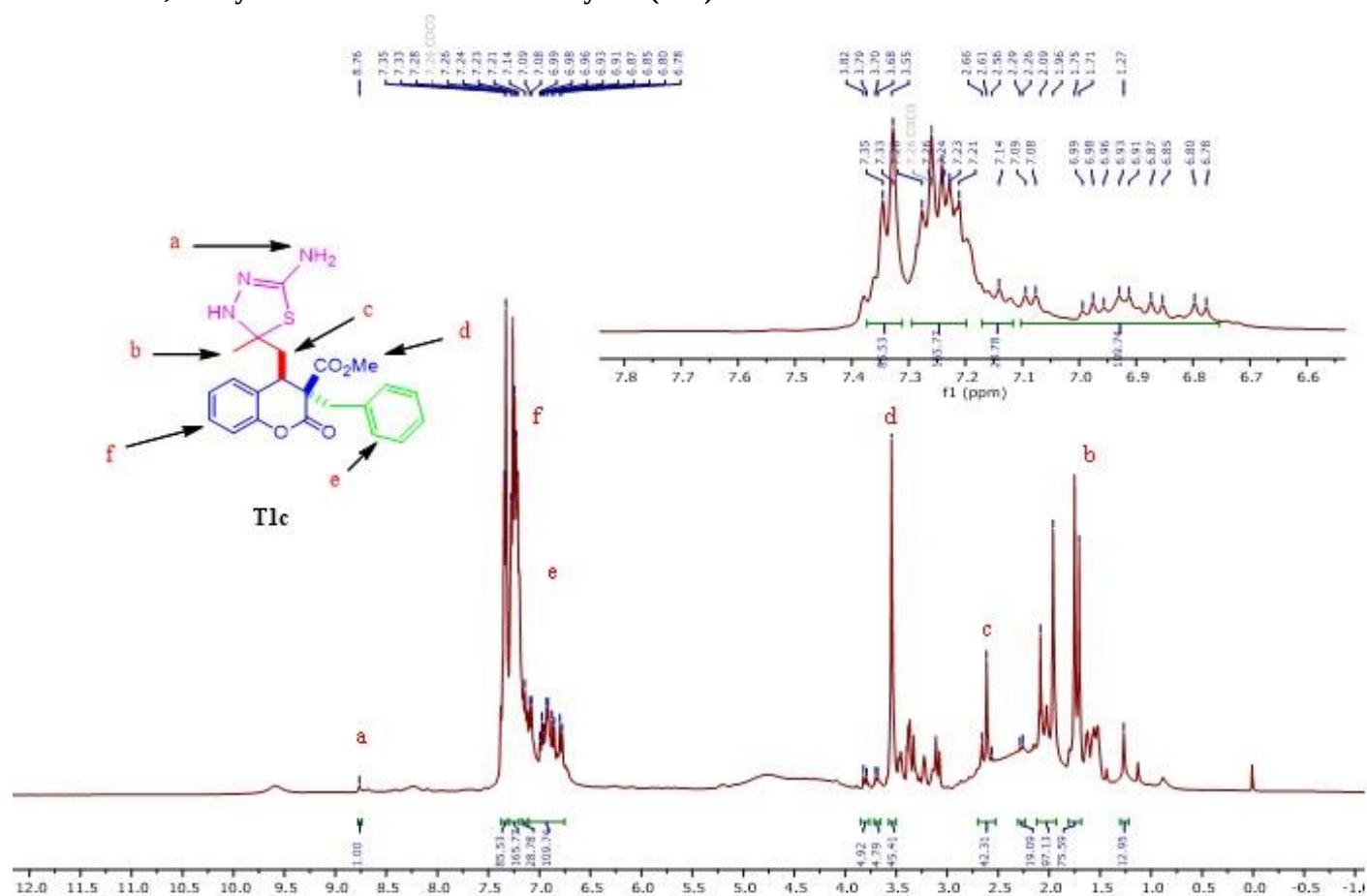


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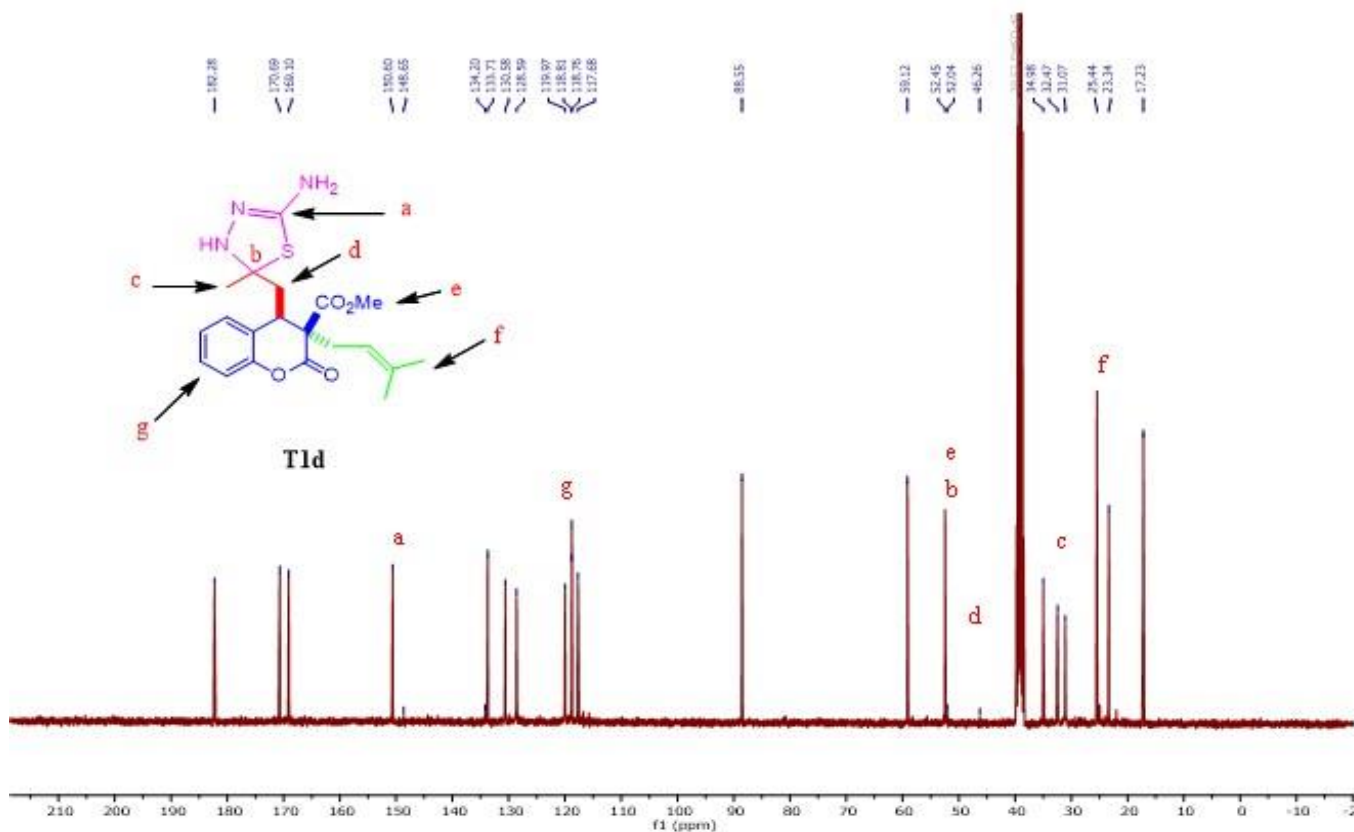
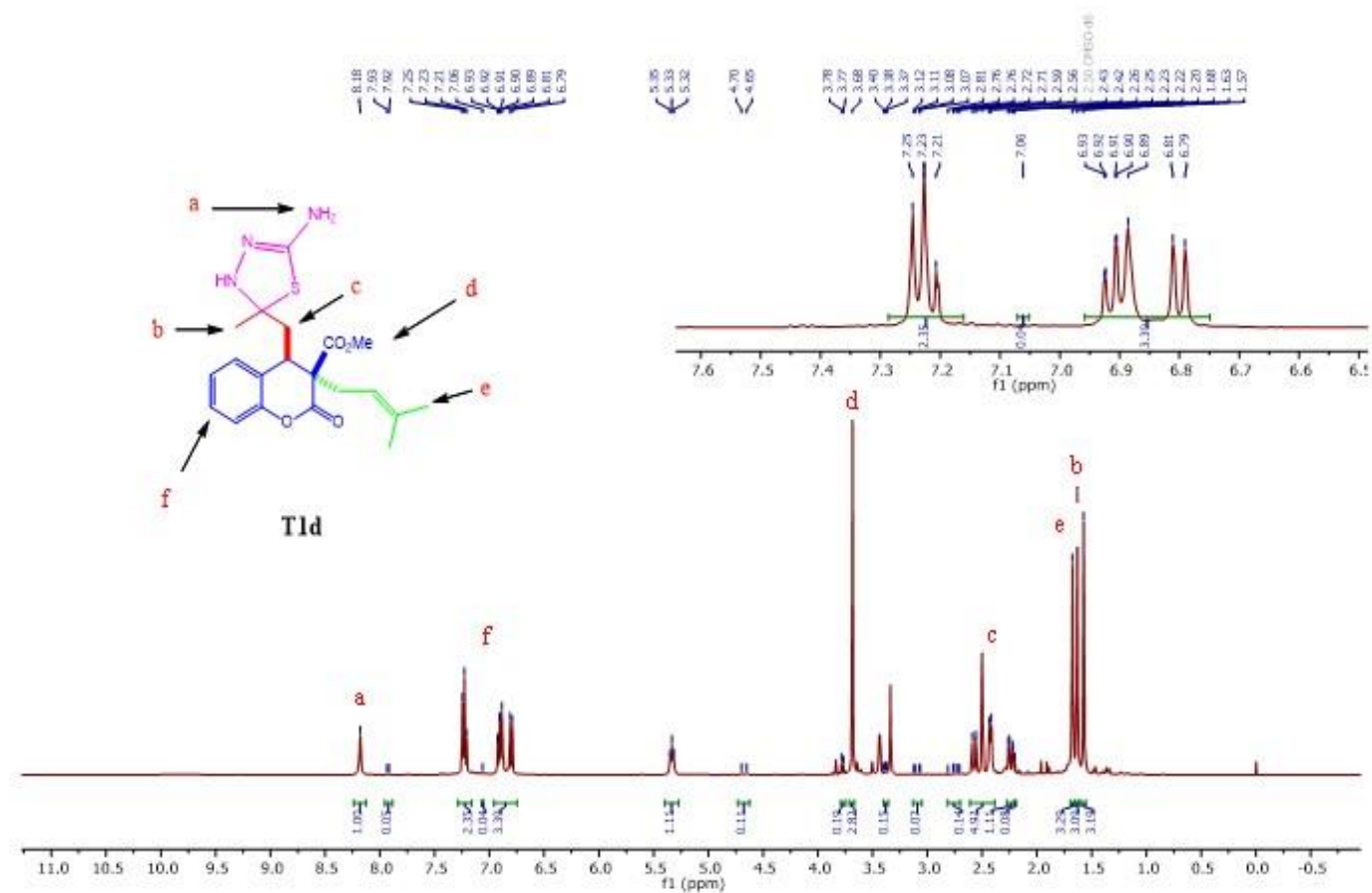


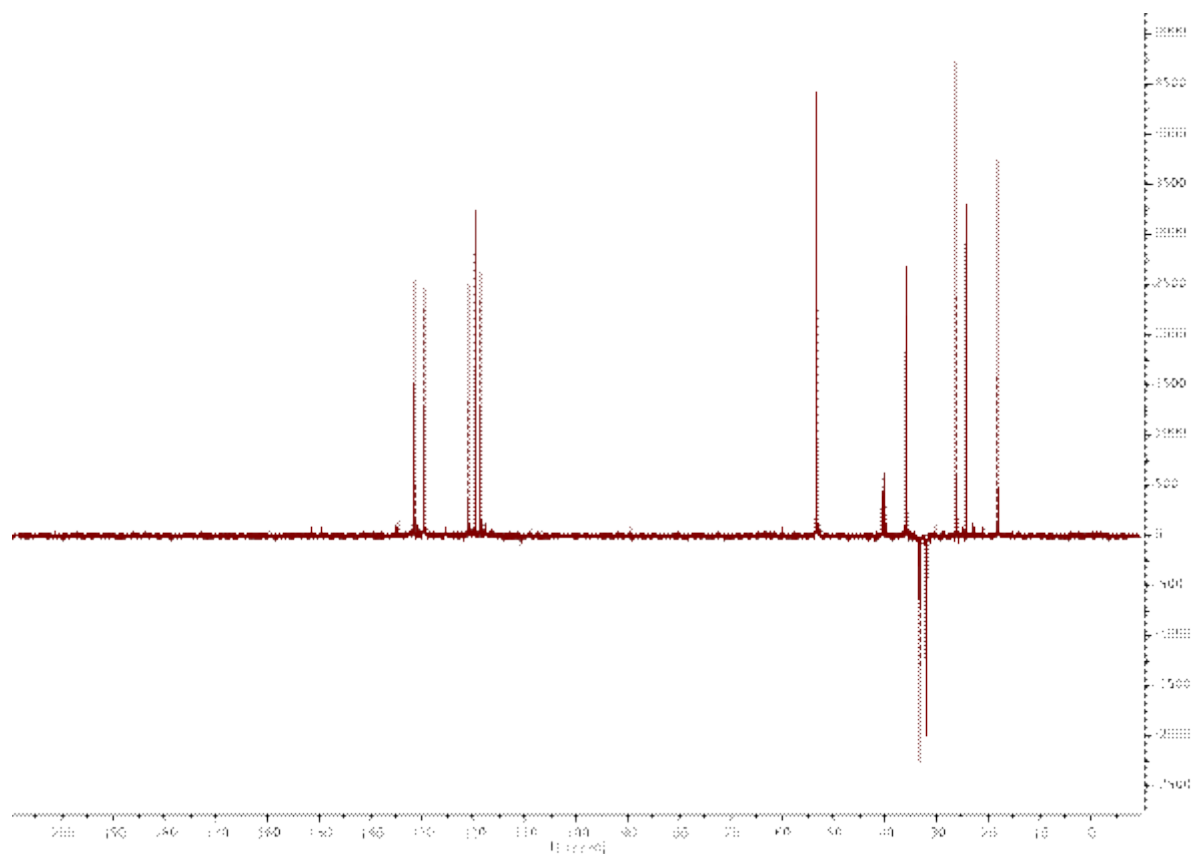


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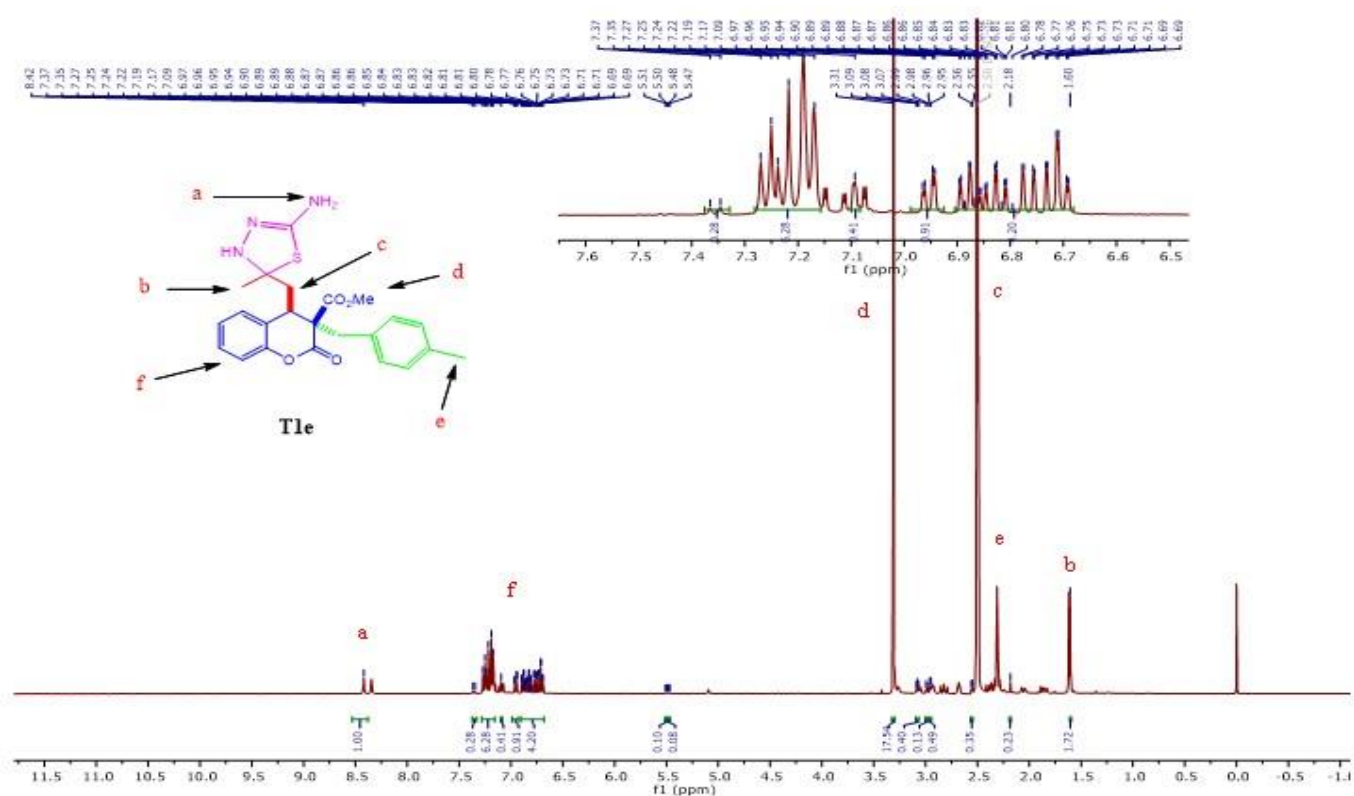


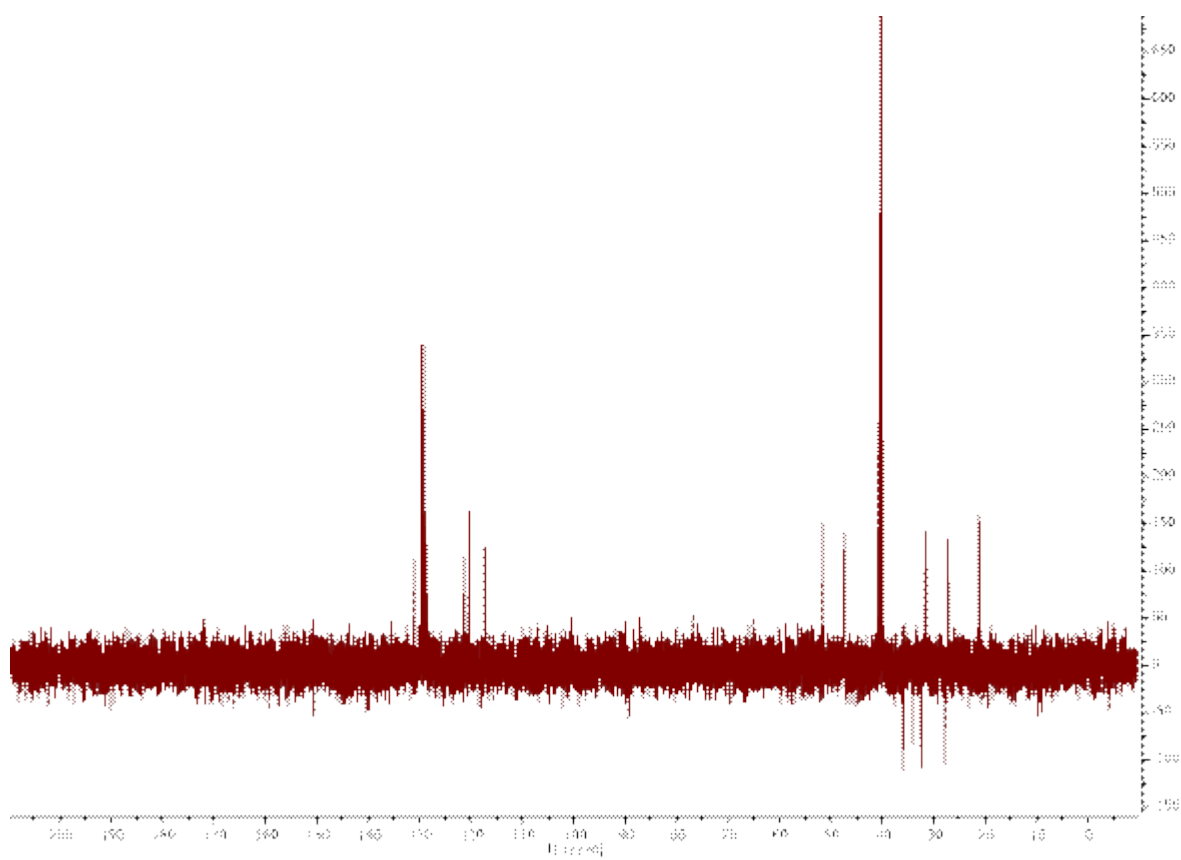
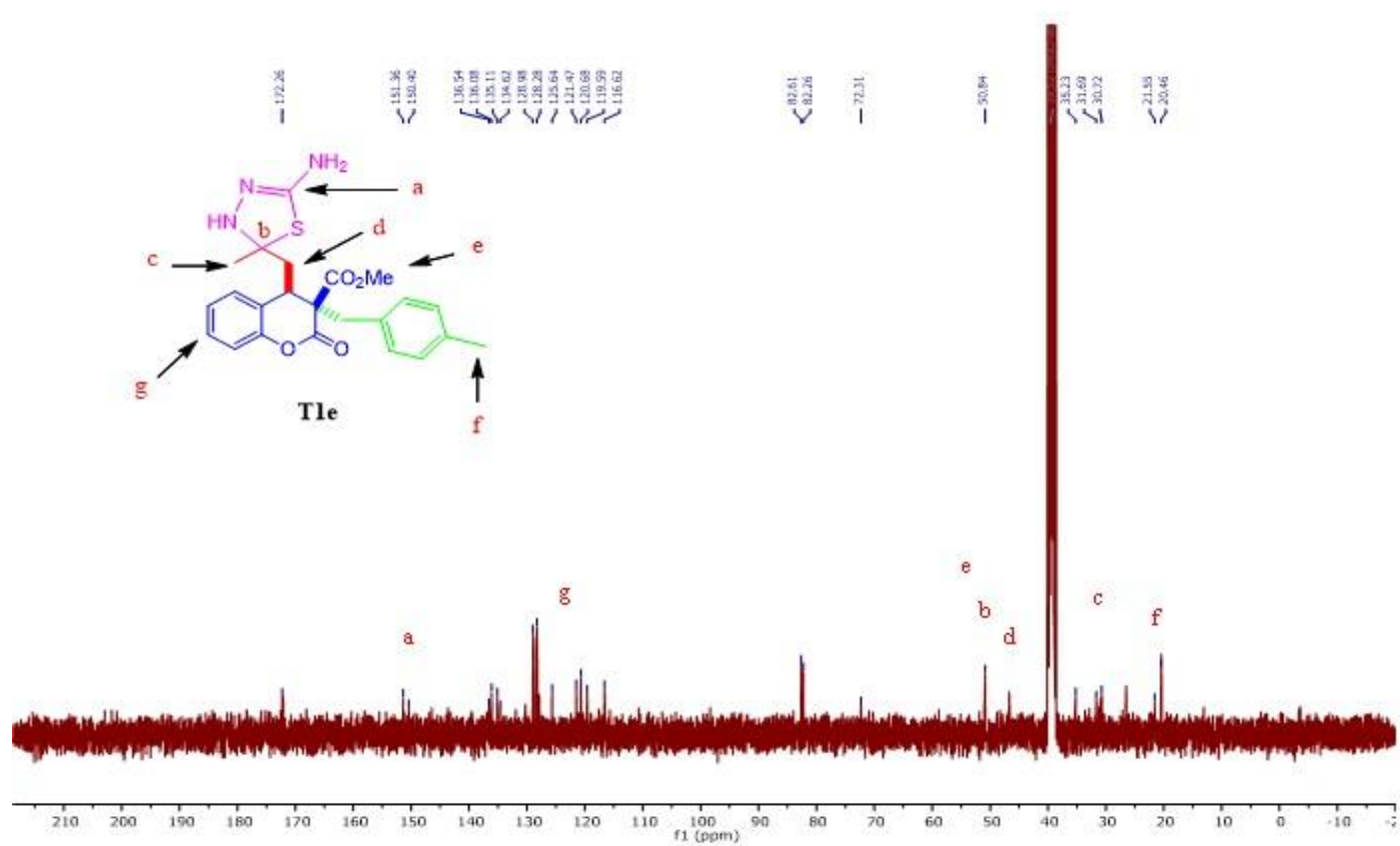
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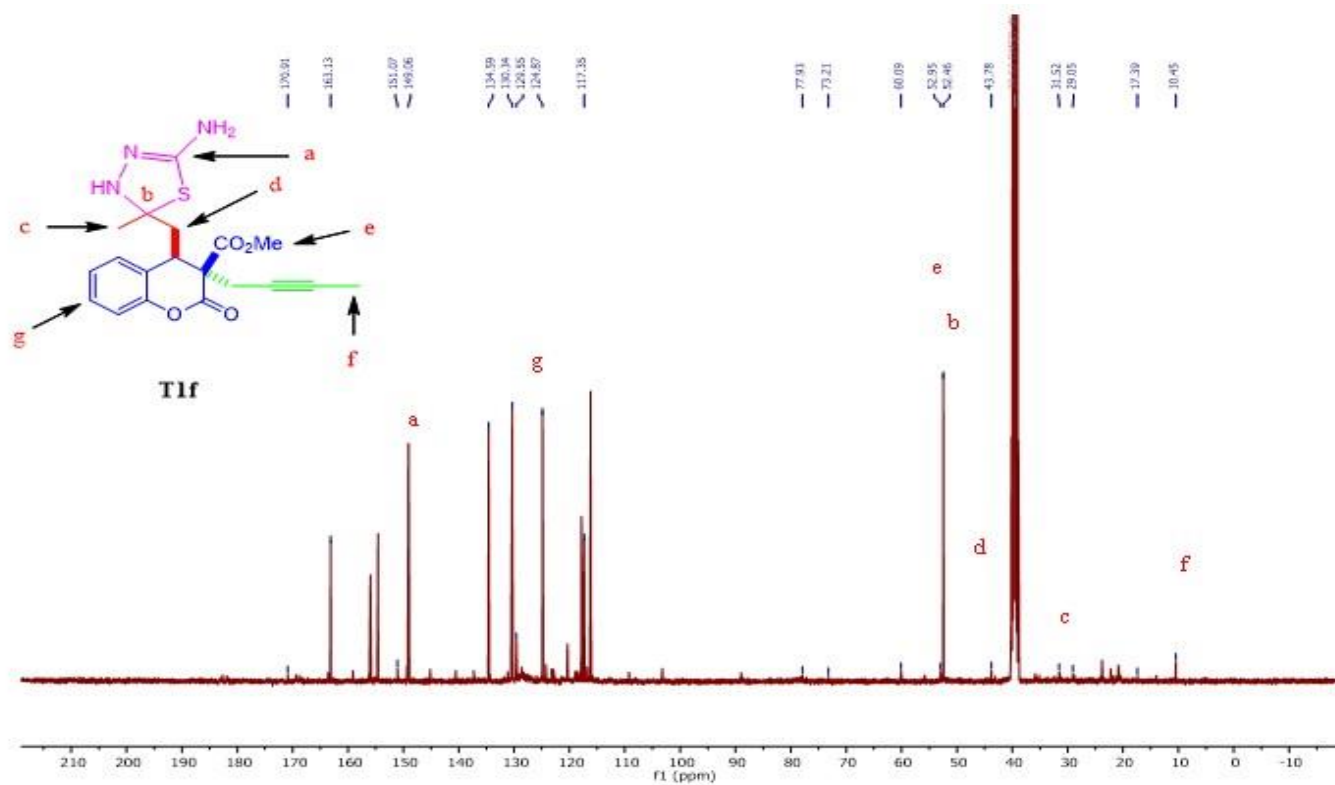
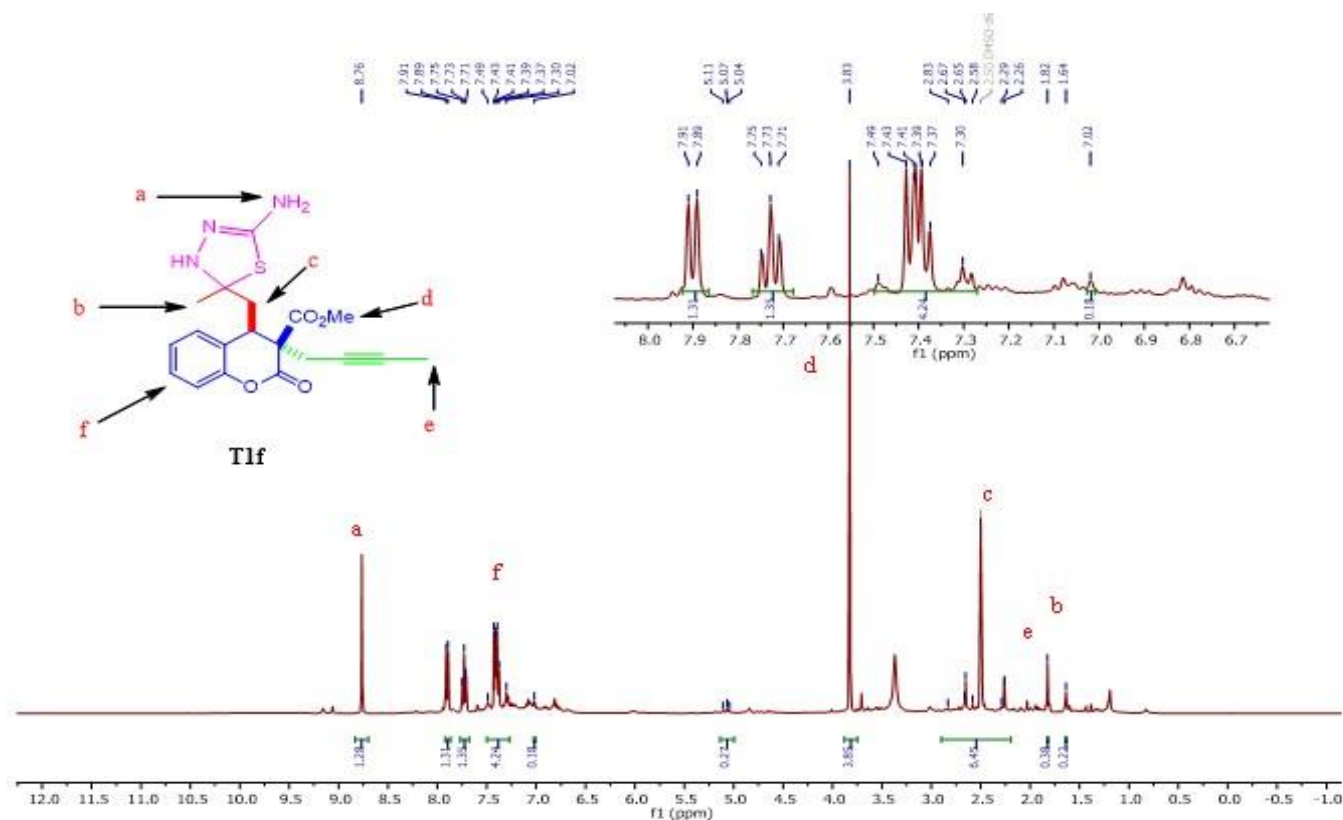


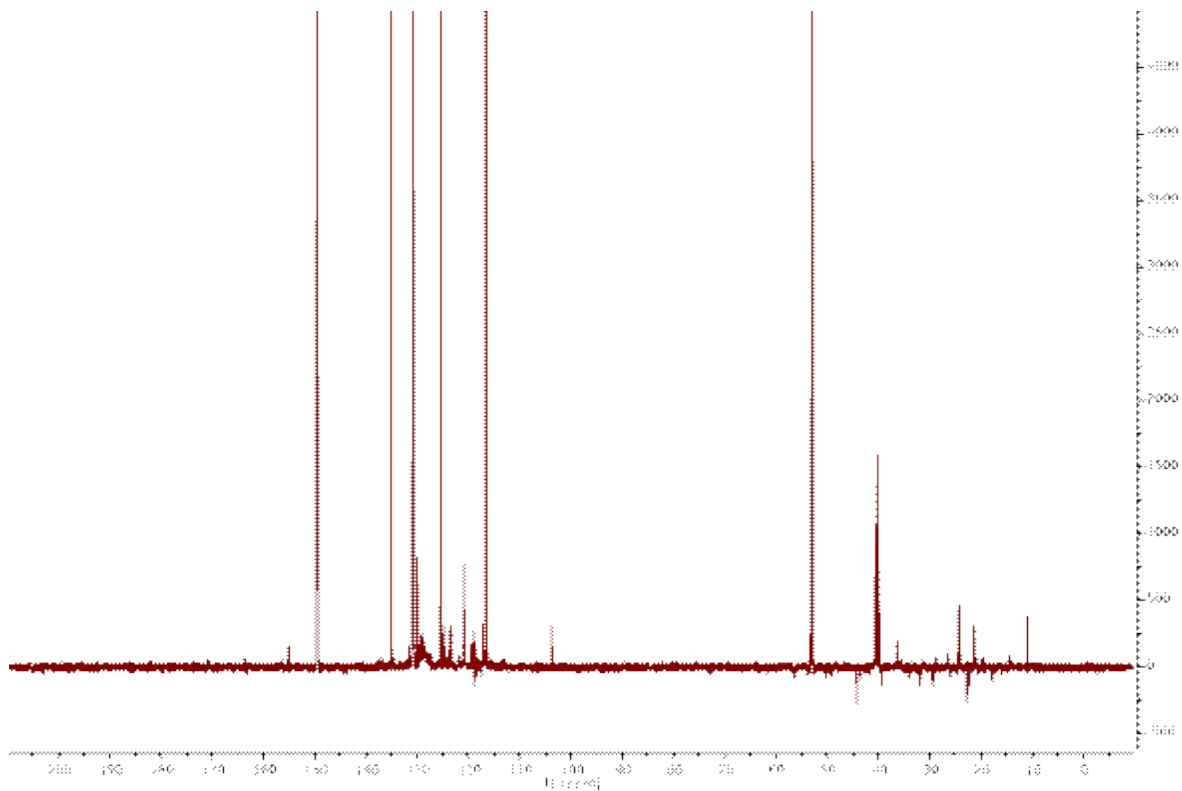
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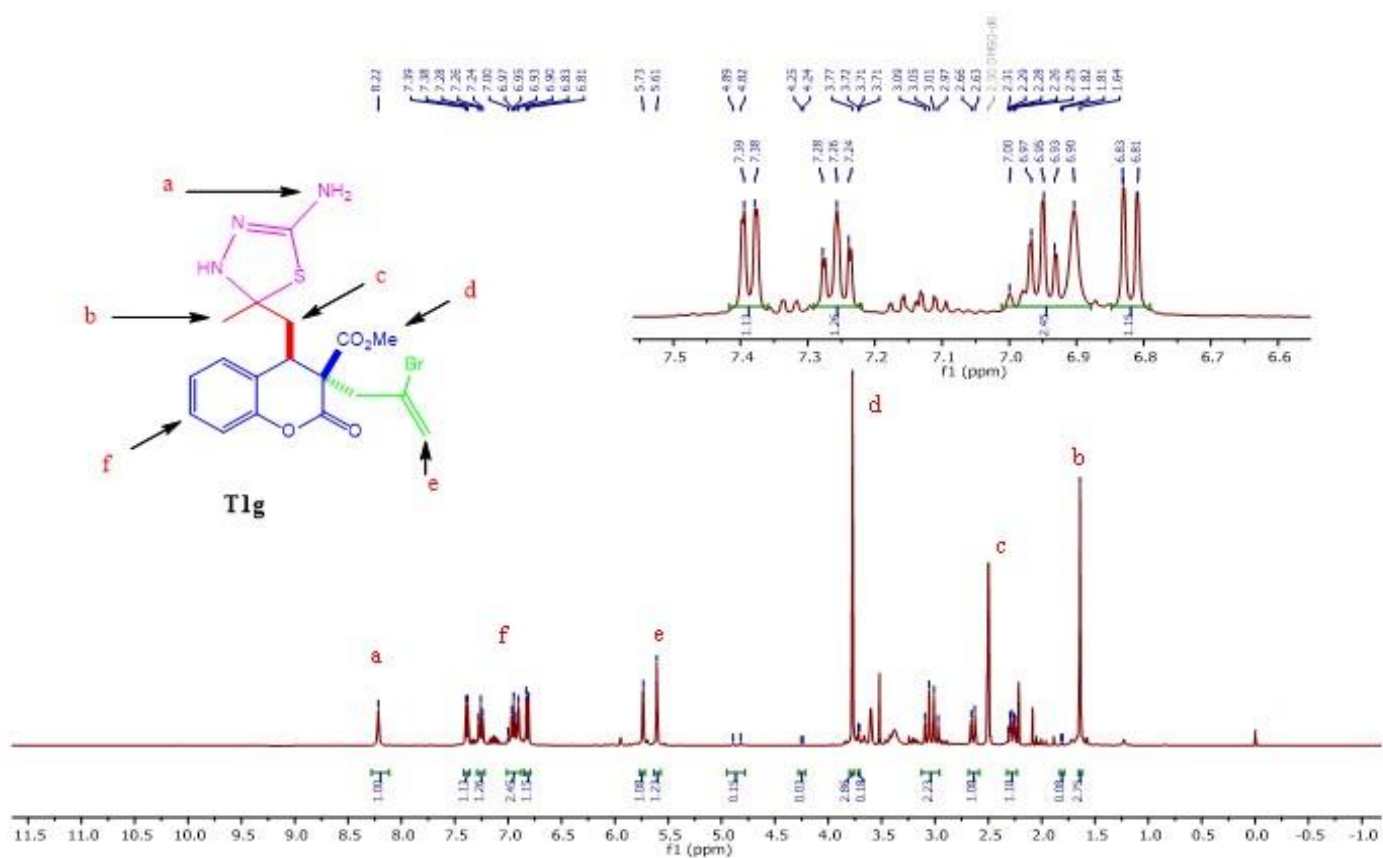


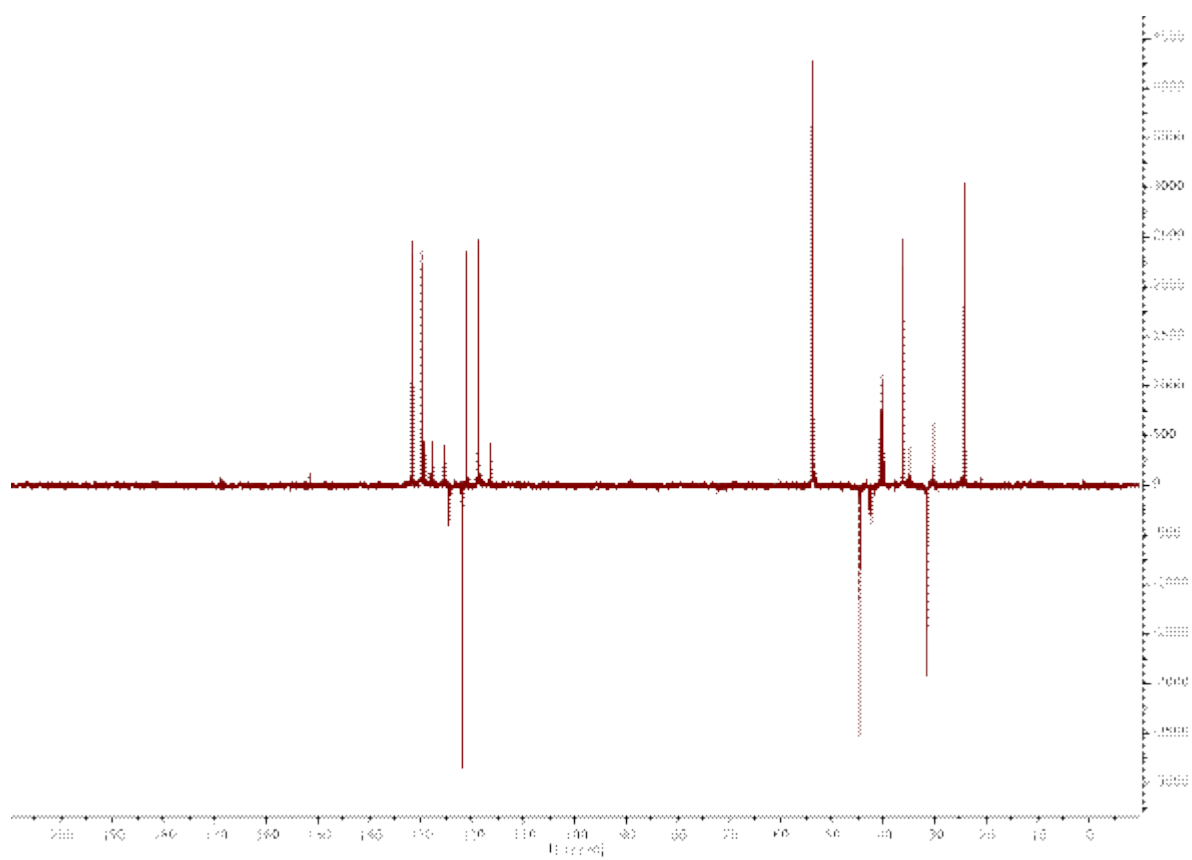
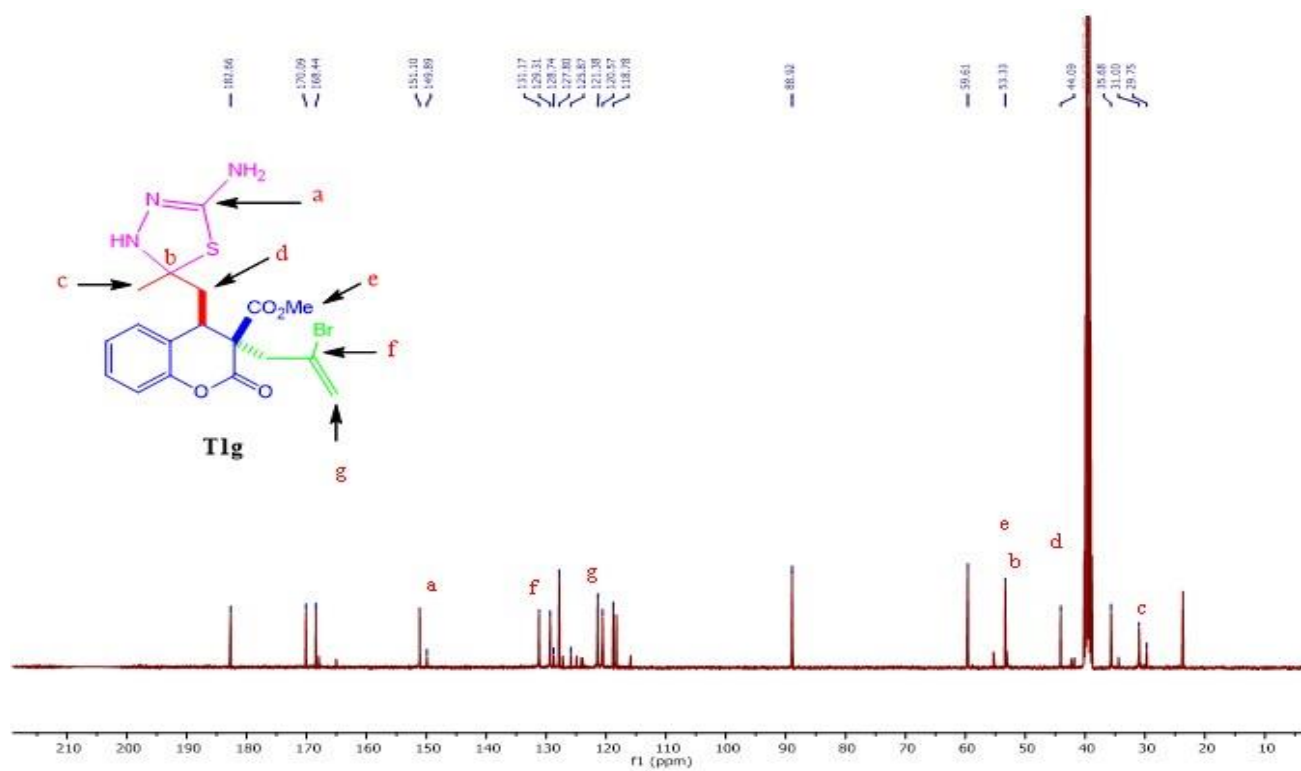
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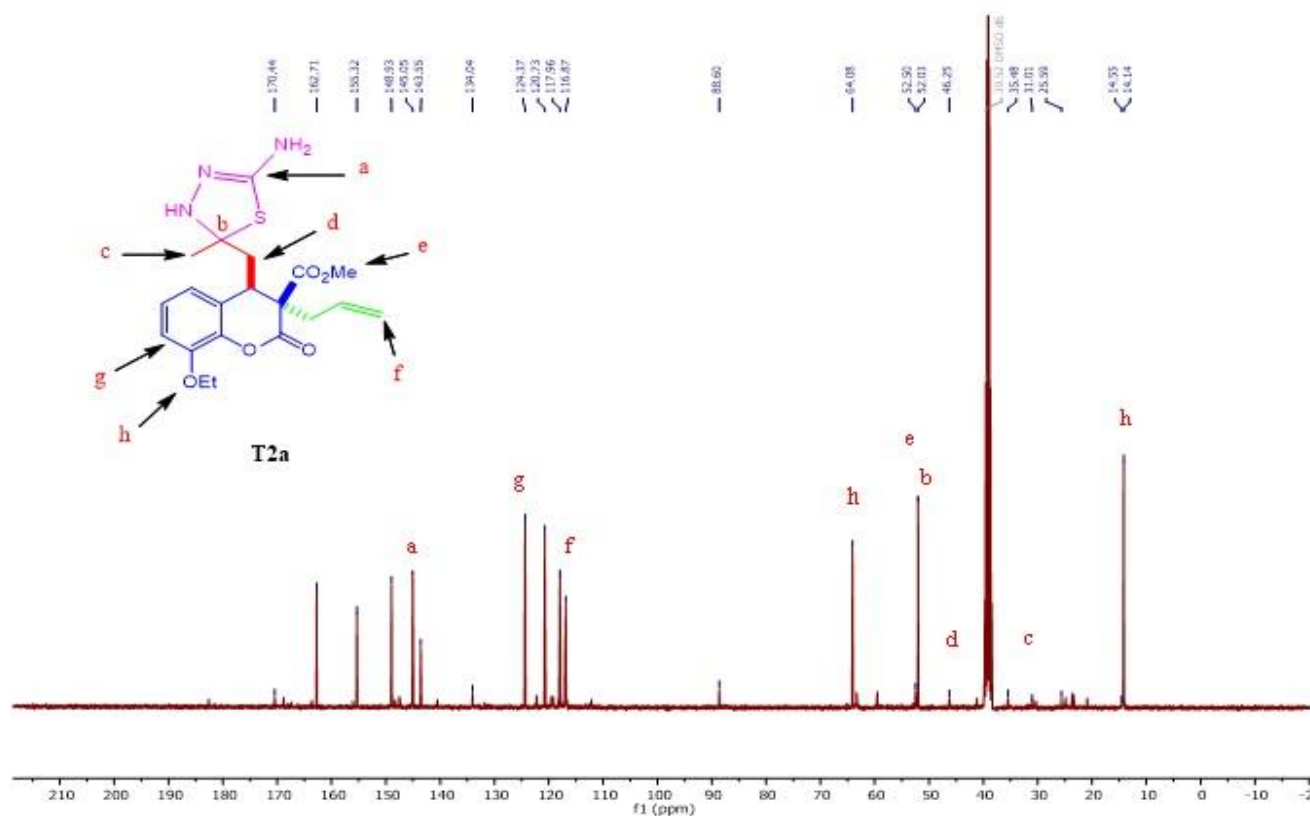
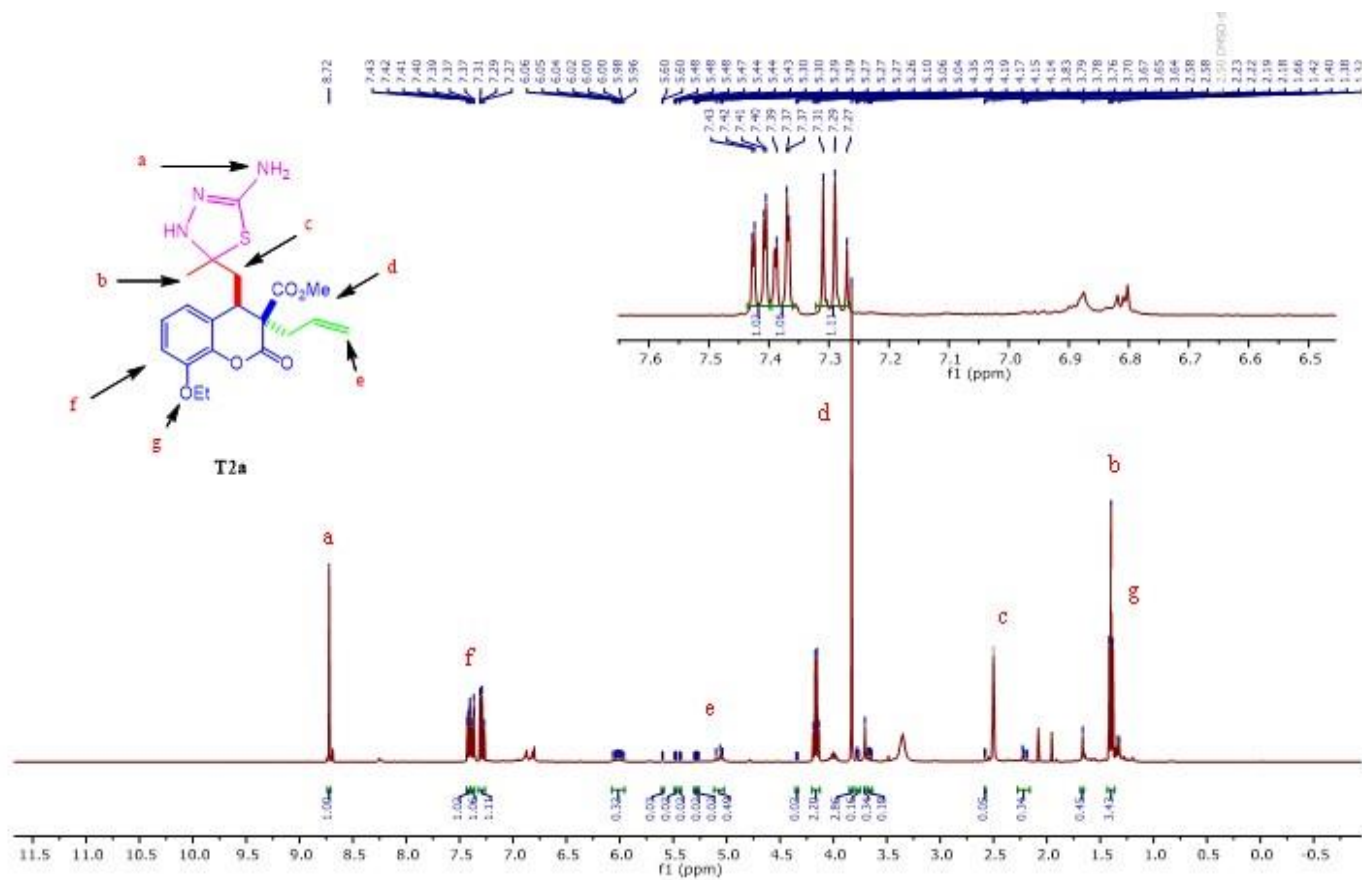


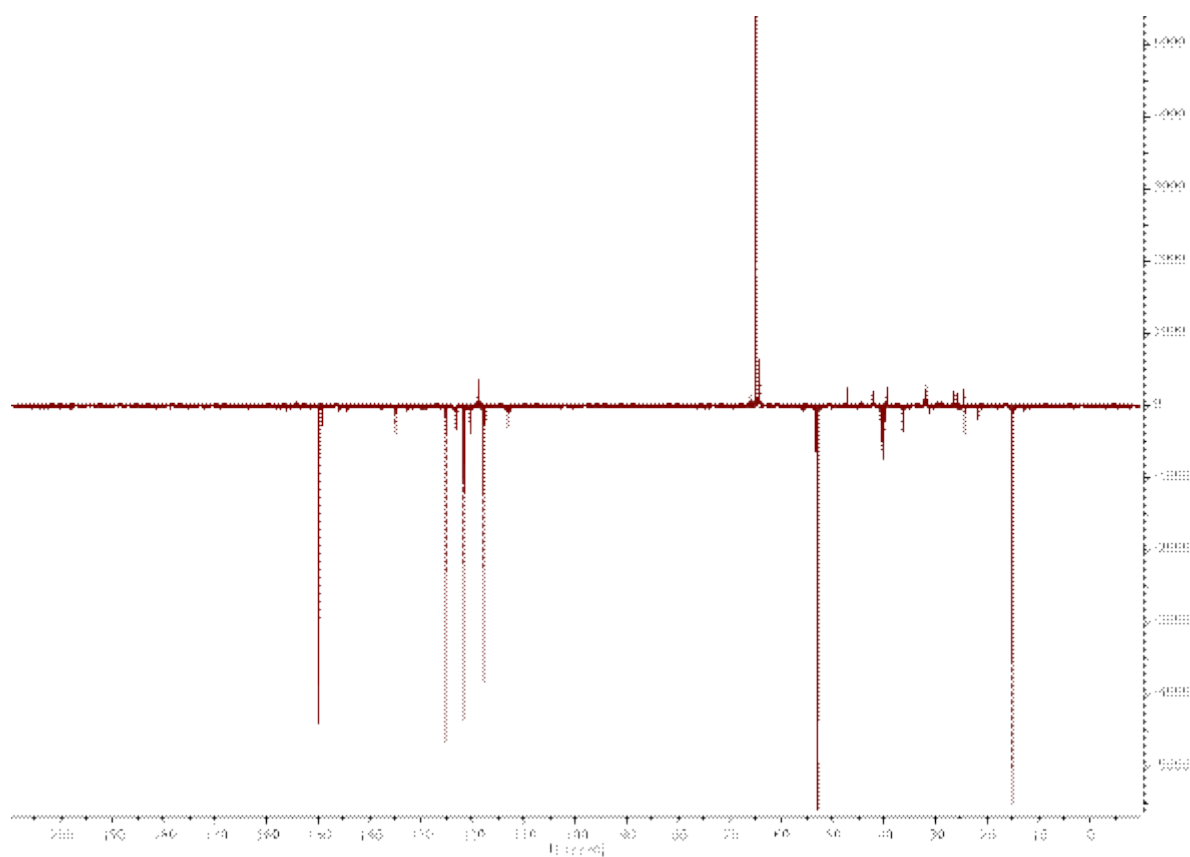
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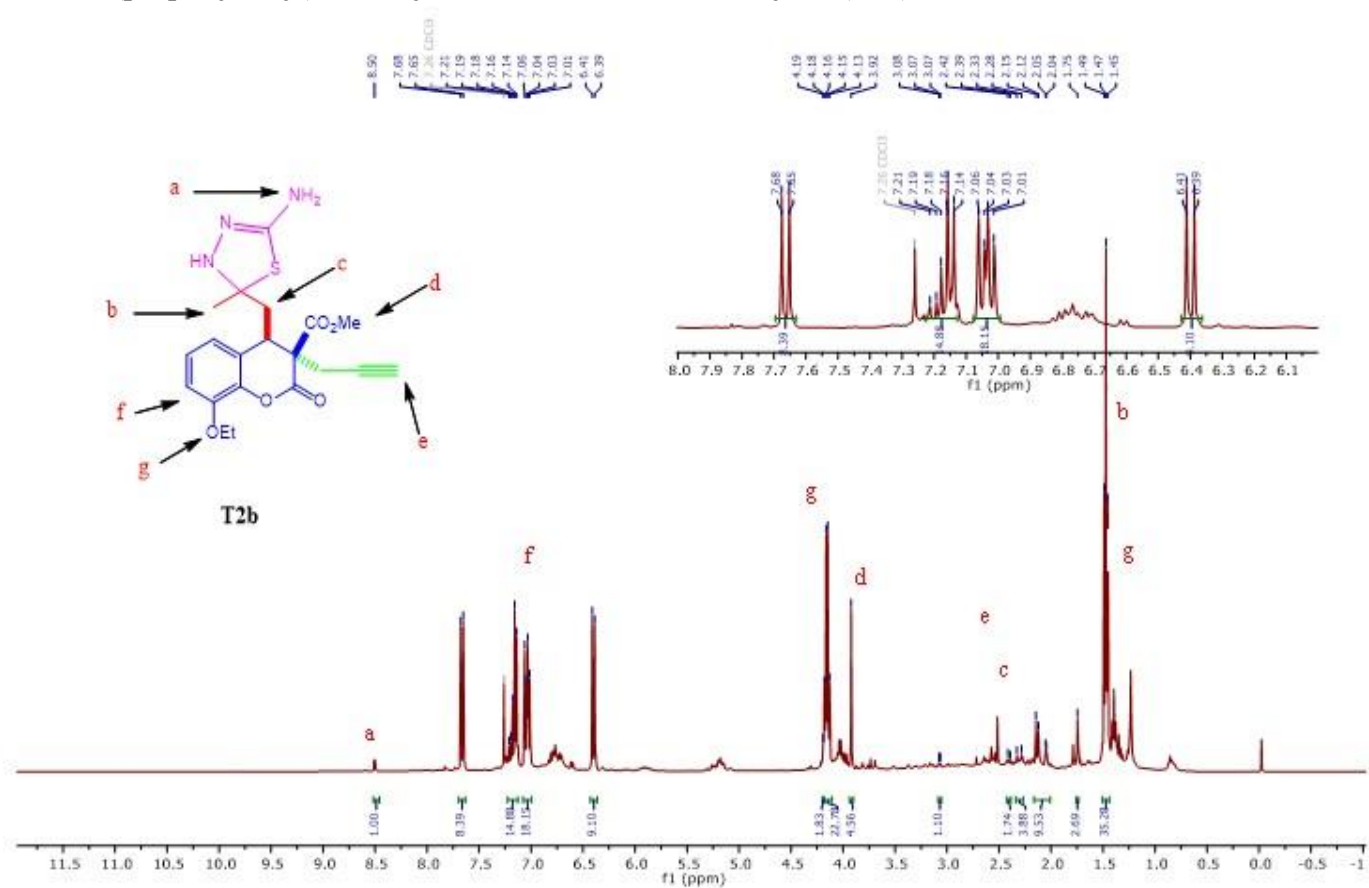


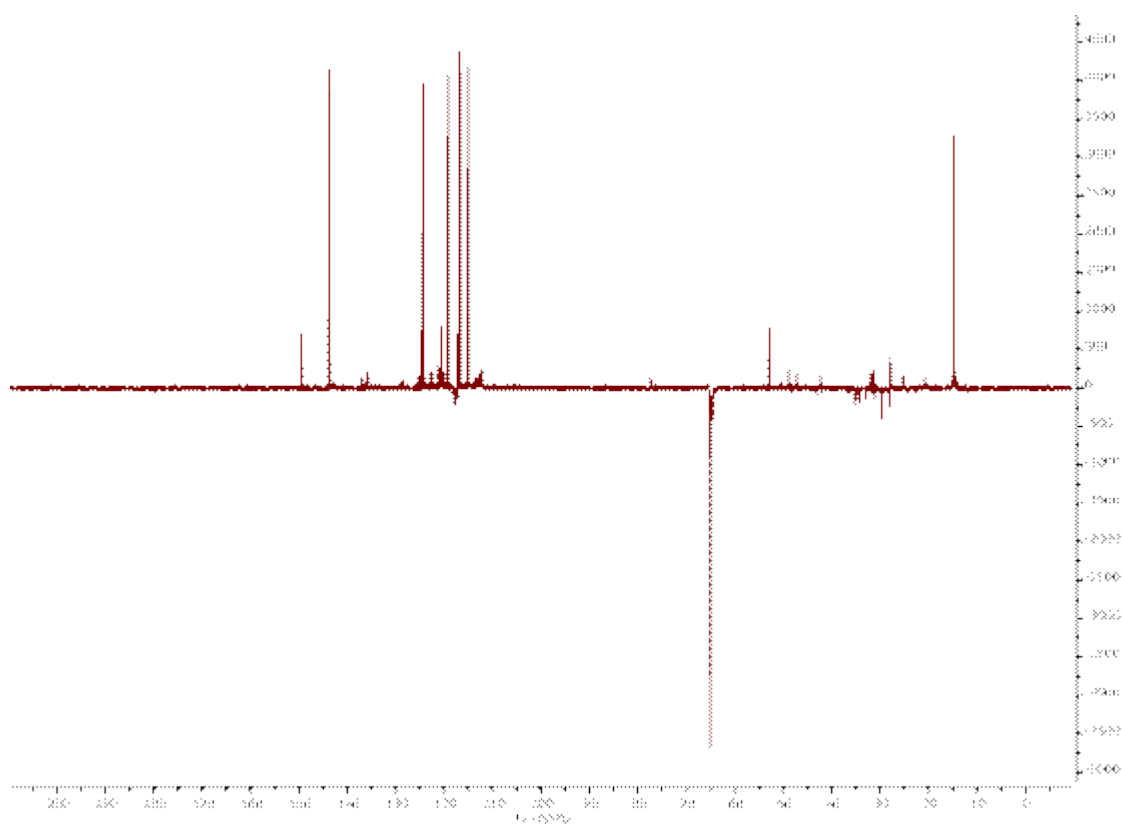
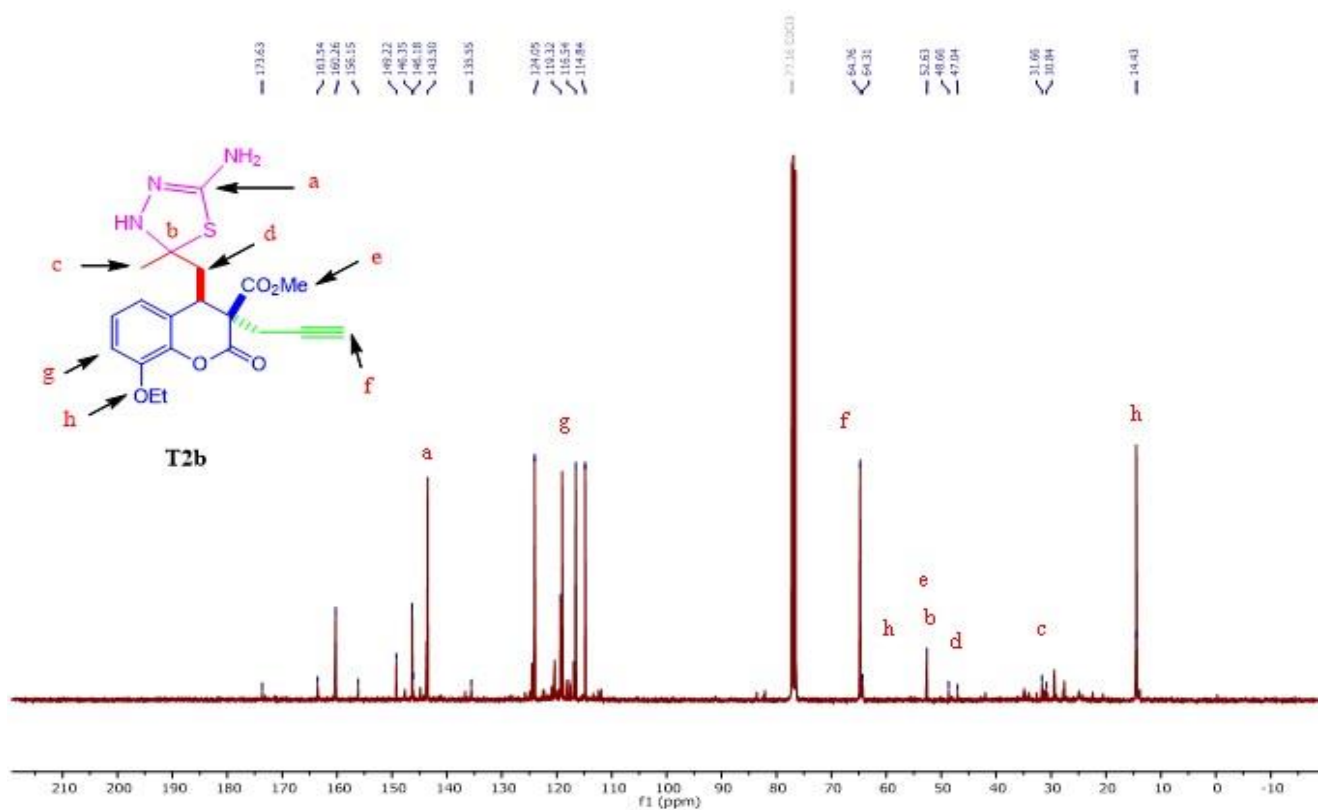
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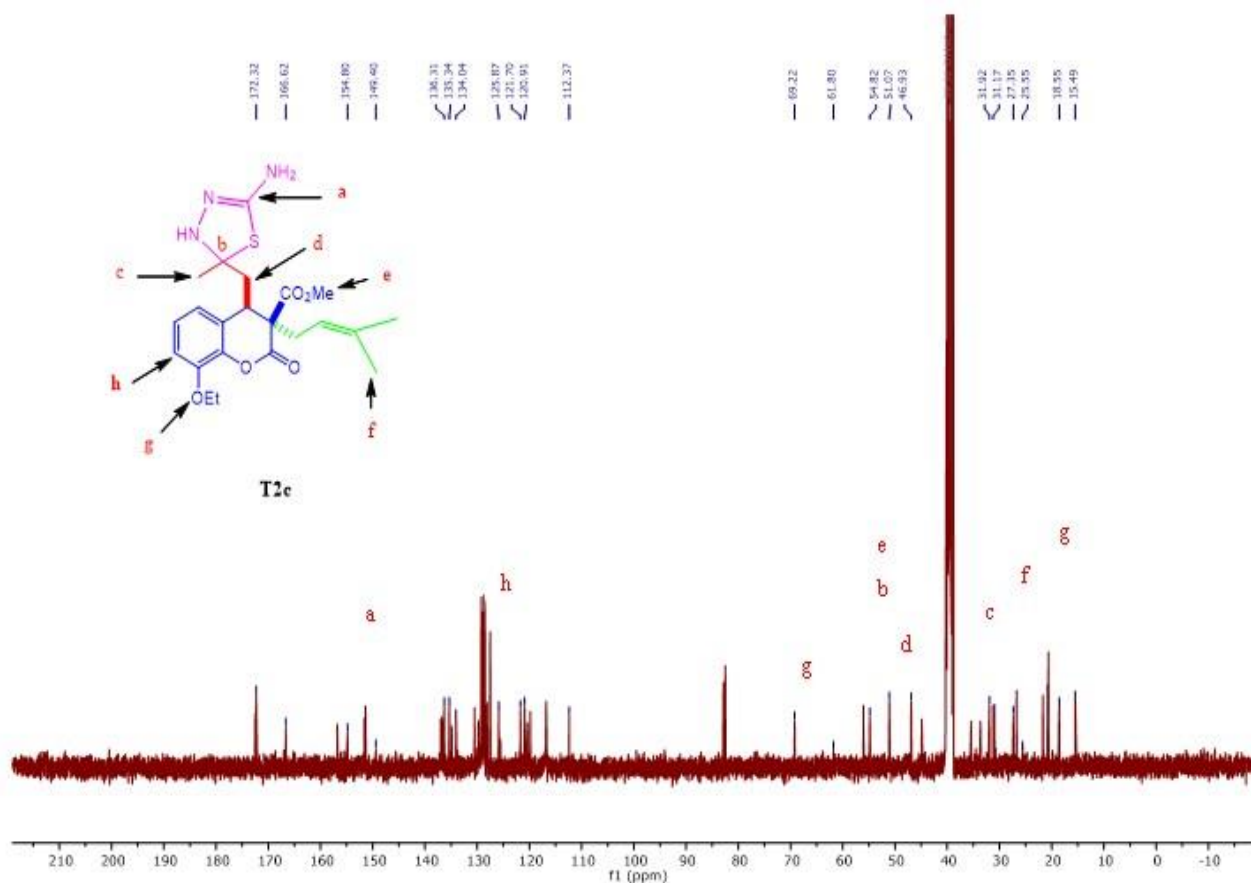
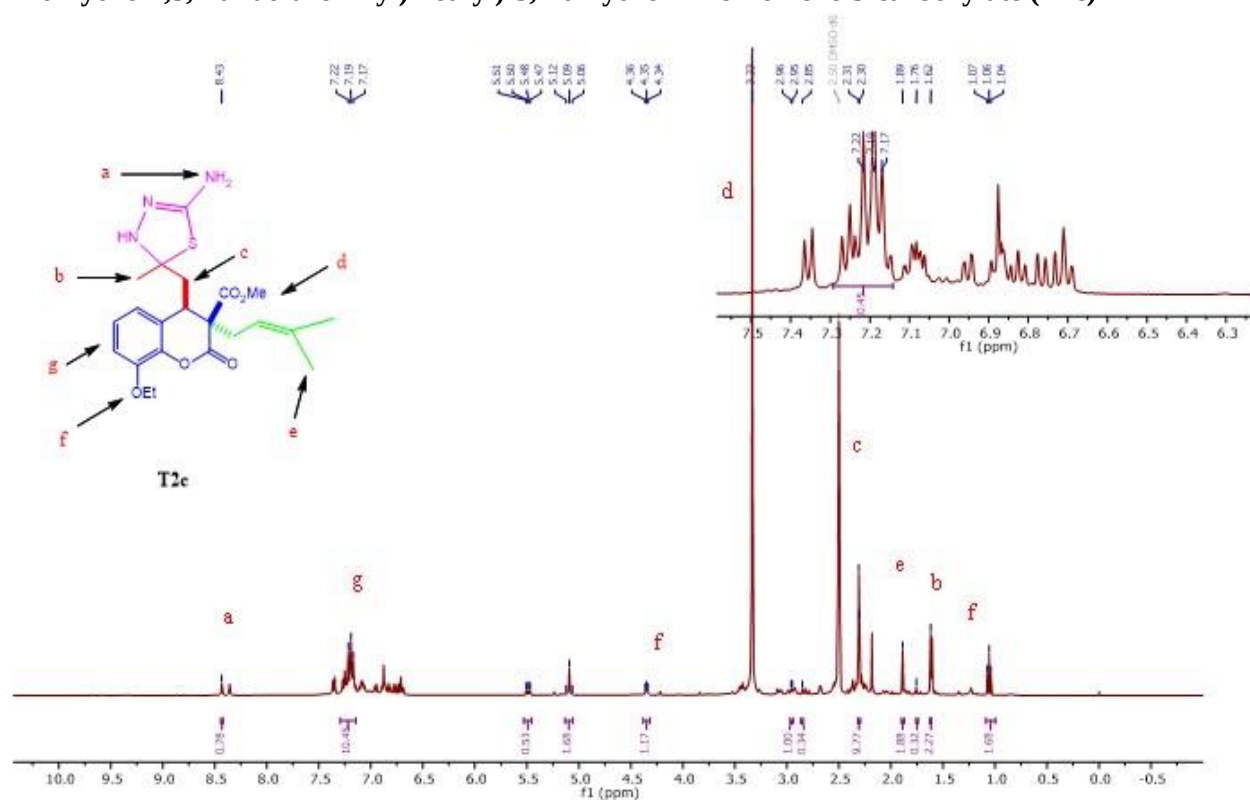


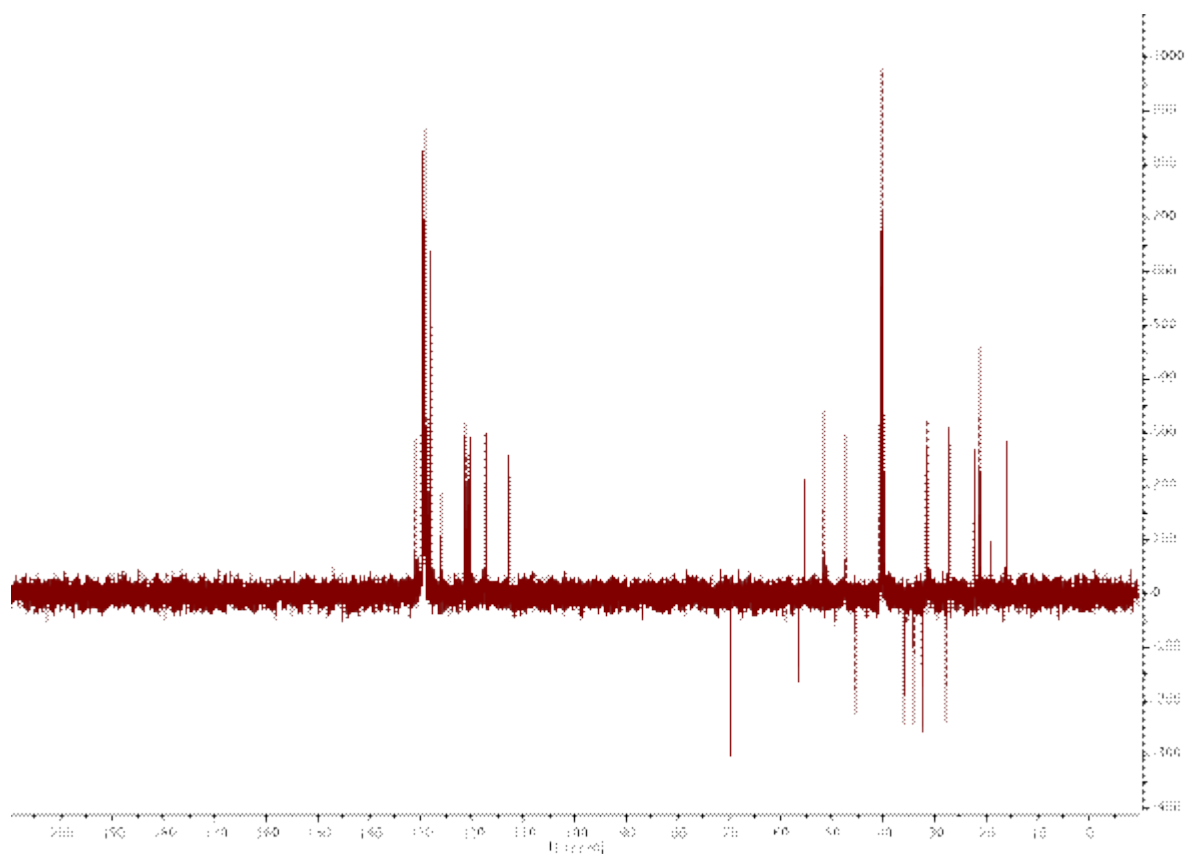
Methyl 8-ethoxy-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(prop-2-yn-1-yl)-3,4-dihydro-2H-chromene-3-carboxylate (T2b)



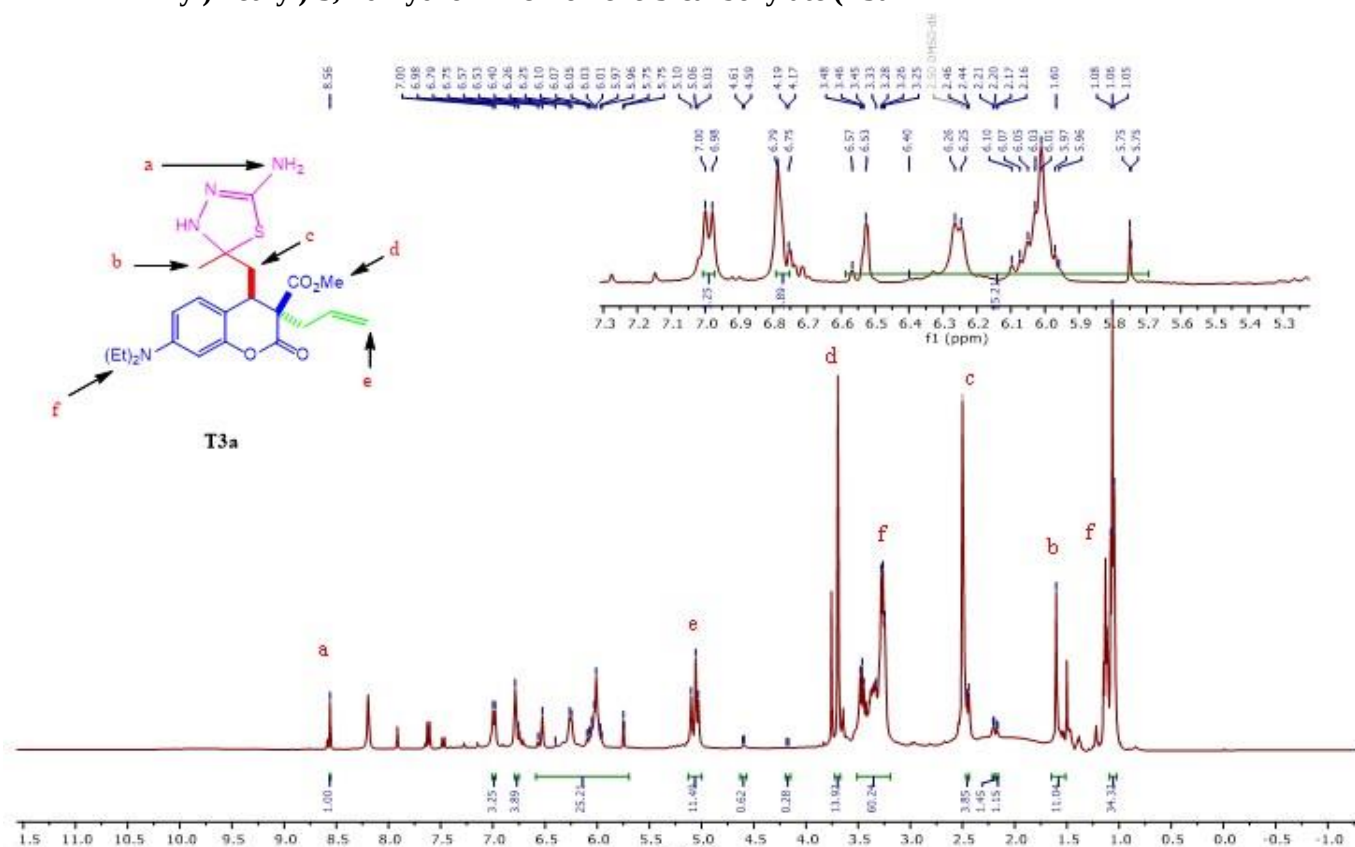


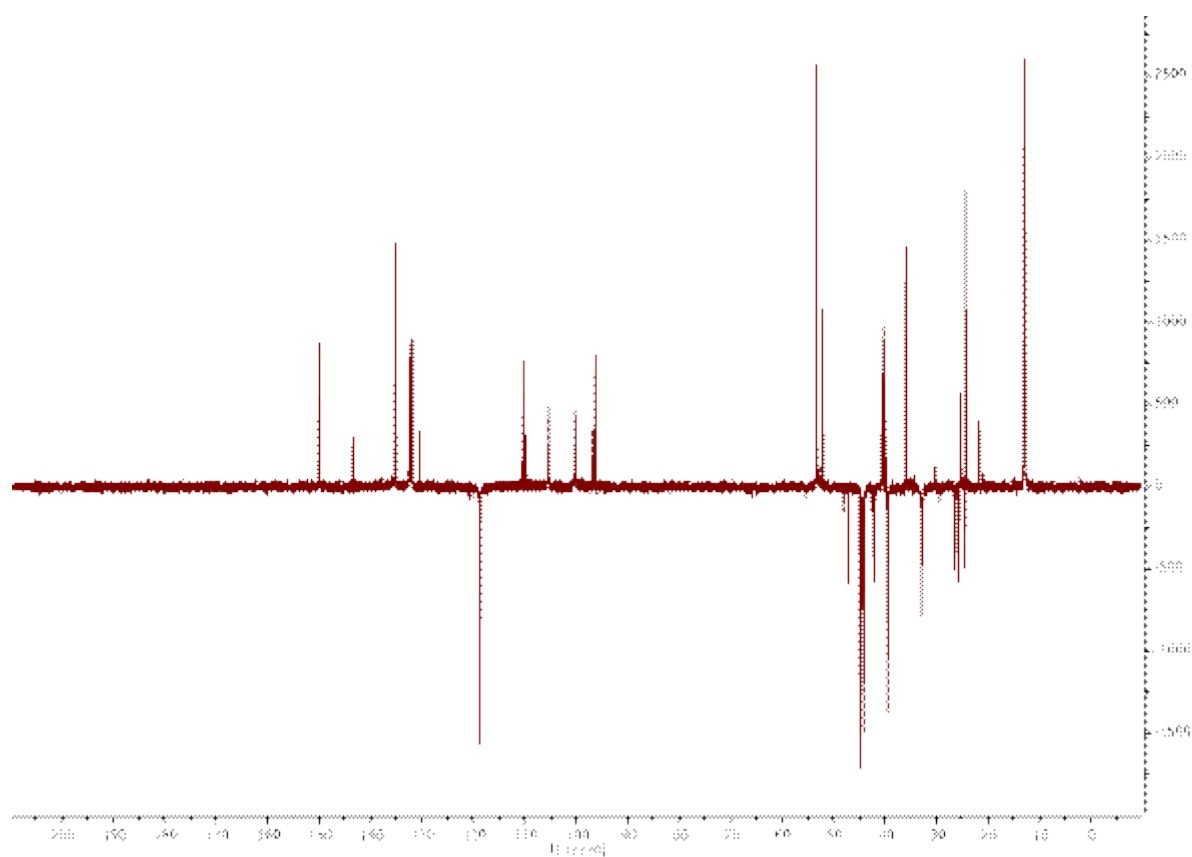
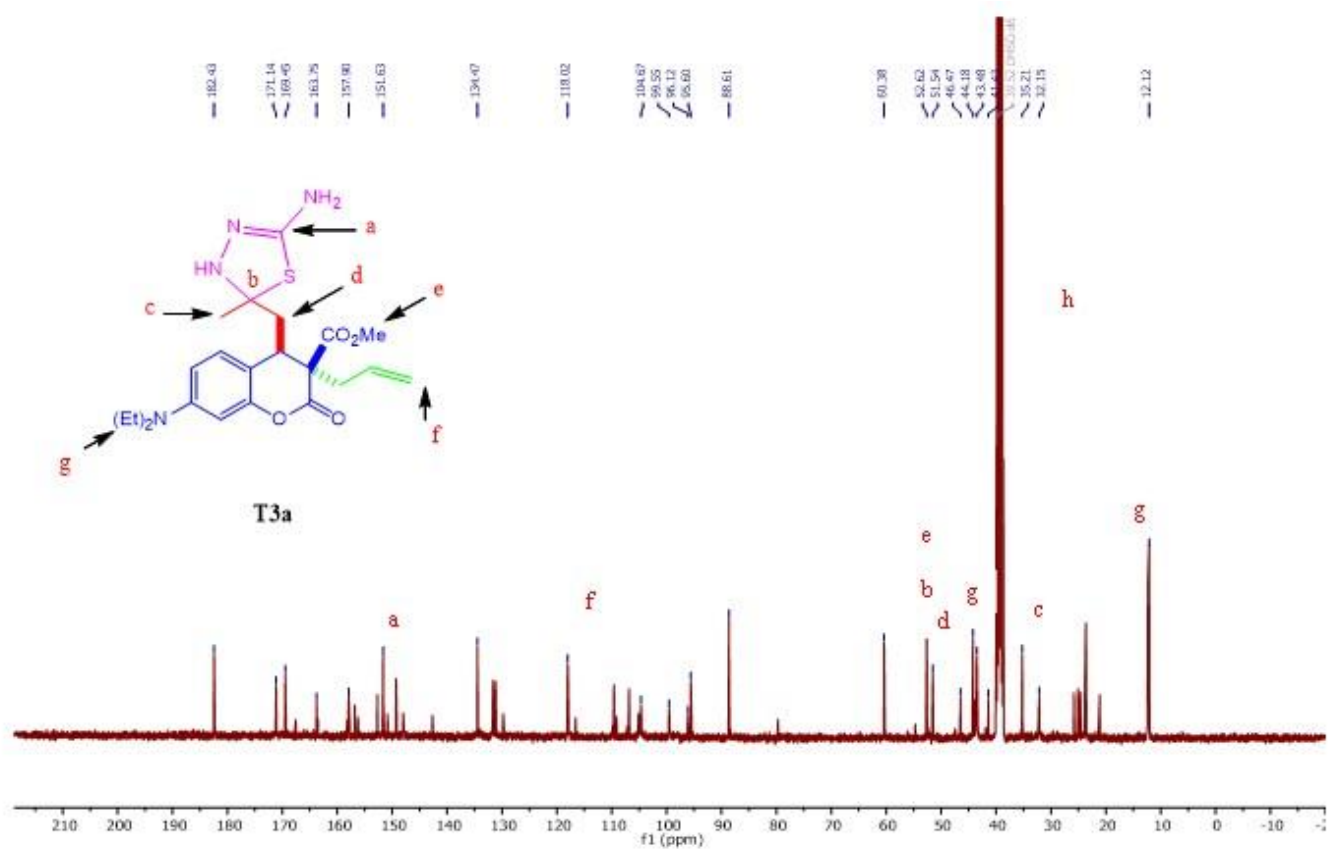
(3S,4R)-Methyl 8-ethoxy-3-(3-methylbut-2-en-1-yl)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T2c)



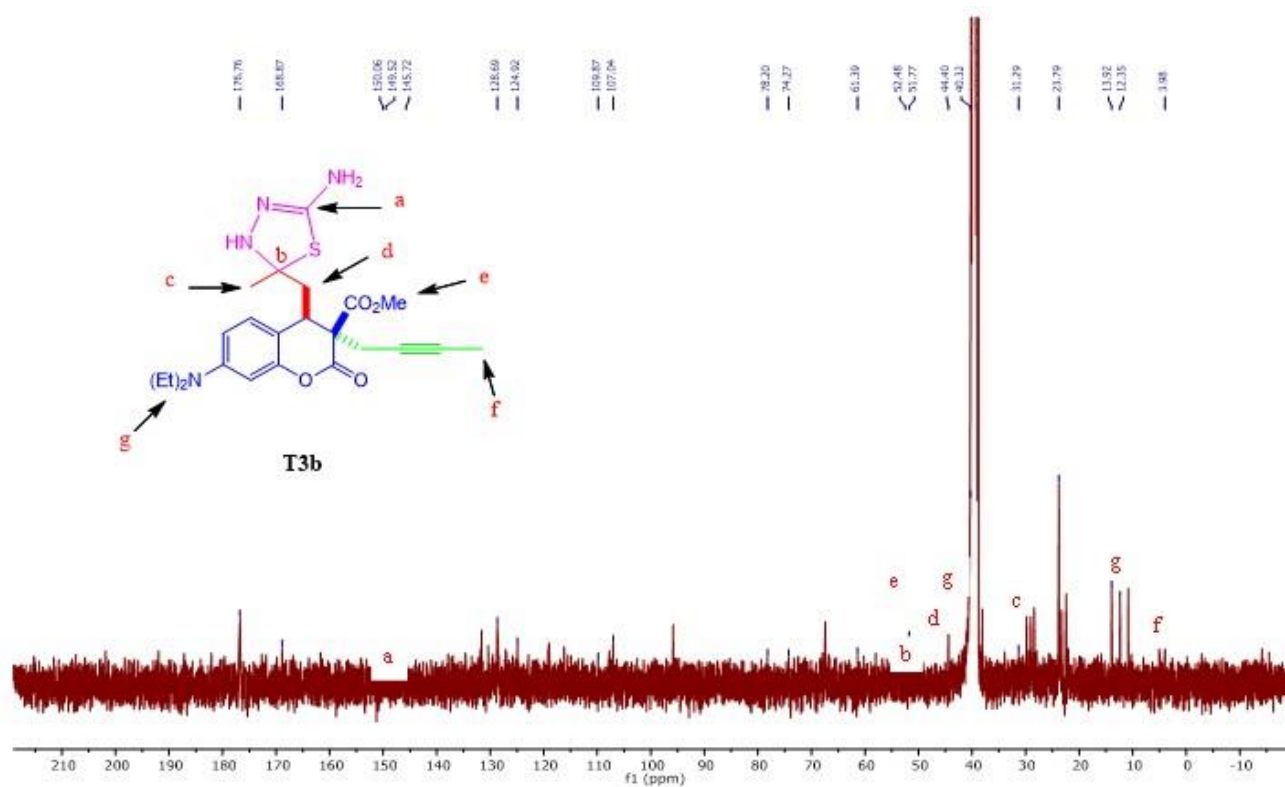
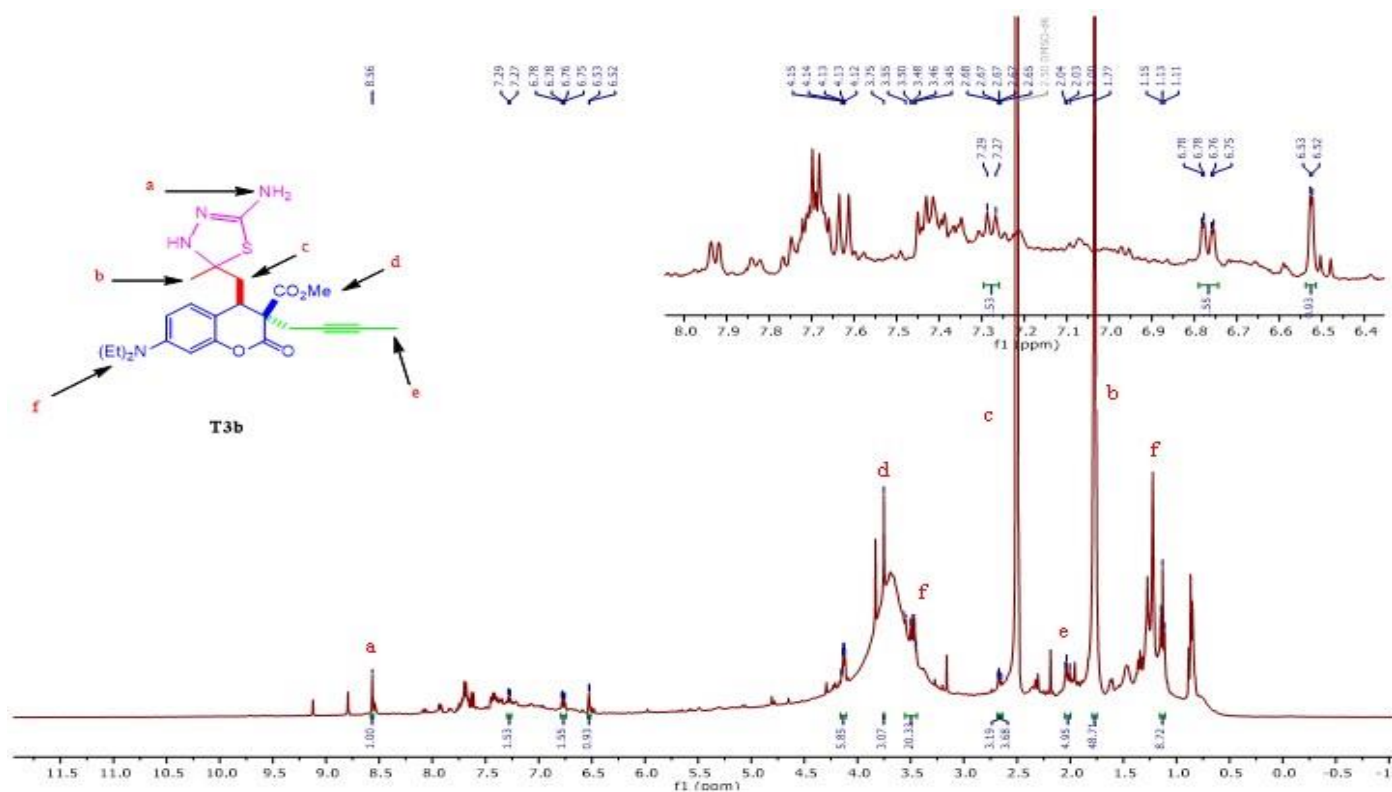


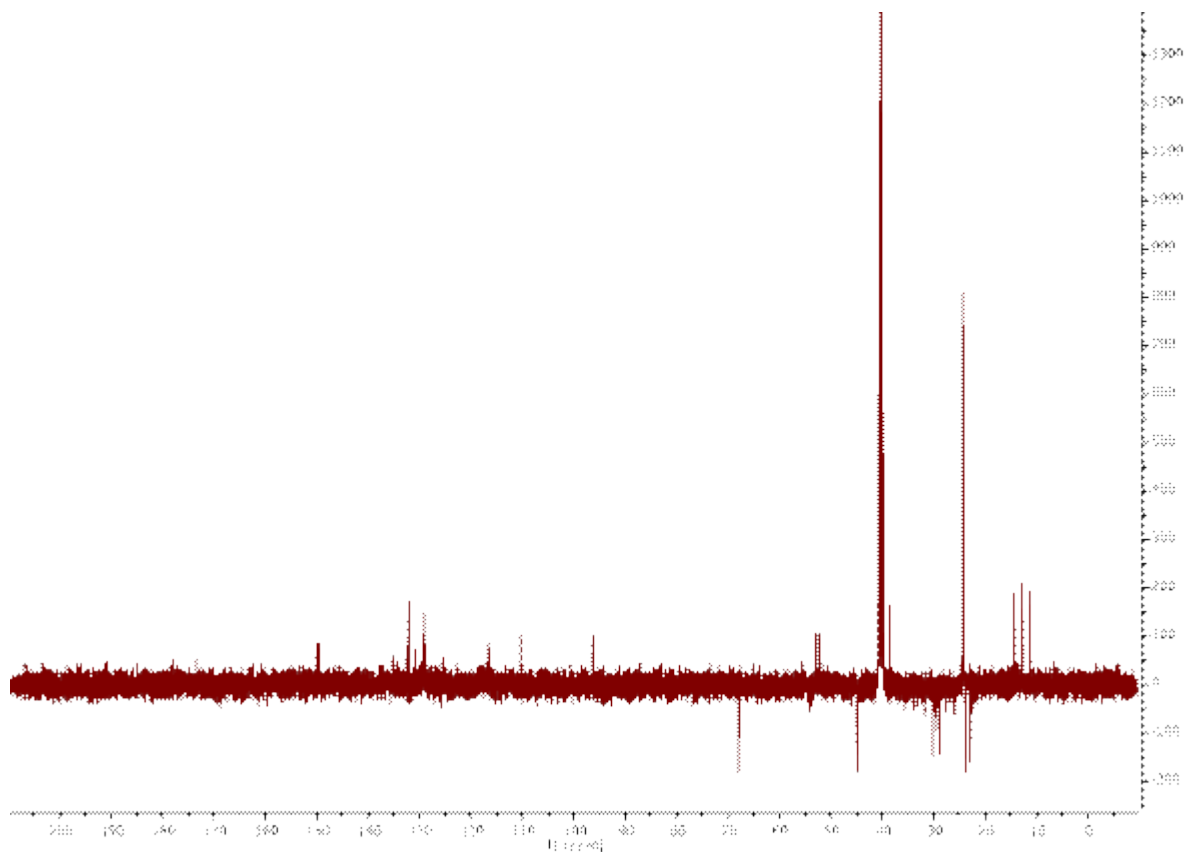
Methyl 3-allyl-7-(diethylamino)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T3a)





Methyl 3-(but-2-yn-1-yl)-7-(diethylamino)-2-oxo-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3,4-dihydro-2H-chromene-3-carboxylate (T3b)





The ABTS Assay:

	12.5	25	50	100	200	400	800	IC ₅₀ (μM)
T2c	11,10±1,72	17,32±1,24	24,81±0,92	0,76±0,50	49,99±0,76	55,38±3,97	52,41±7,44	194,31±0,72
T1d	25,92±1,05	35,55±2,30	53,07±0,86	59,79±3,19	74,50±0,38	78,85±0,99	82,59±1,99	45,56±0,89
T1b	27,07±2,42	41,39±1,63	52,85±0,10	60,62±2,57	72,13±3,55	79,95±3,61	82,92±0,91	43,73±0,70
T2a	8,12±0,57	13,58±3,03	24,15±2,01	34,40±2,40	44,37±3,00	73,01±1,81	79,90±1,67	222,95±2,59
T3a	22,63±3,42	34,53±2,36	44,57±2,79	60,01±3,78	76,49±0,70	85,37±2,39	76,78±4,73	69,40±3,89
T1g	24,38±0,53	33,72±0,44	41,15±2,71	60,19±0,10	64,83±3,08	75,39±2,09	80,15±3,06	72,28±2,11
T1e	10,85±2,96	14,80±3,97	21,13±3,94	23,04±1,49	24,49±2,64	22,40±0,70	Sat	>800
T3b	19,56±2,39	33,89±3,10	42,25±4,93	67,44±1,51	86,94±0,30	85,43±0,20	69,88±2,72	63,02±0,36
T1f	33,46±2,14	45,16±0,40	59,04±1,86	72,92±0,36	78,95±0,36	83,36±0,10	78,55±2,72	35,41±1,35
T1a	25,48±1,24	33,40±4,03	54,40±3,28	66,21±0,98	73,56±3,17	87,15±0,85	87,55±0,55	43,02±1,16
T2b	22,21±0,60	32,37±3,03	47,86±3,87	65,98±1,99	84,22±3,32	-0,56±0,65	90,13±0,55	50,14±0,17
T1c	28,75±2,65	51,30±0,89	63,11±1,29	82,22±0,10	90,59±0,70	92,71±0,10	91,51±0,10	24,32±0,36
	1.56	3.12	6.25	12. 5	25	50	100	IC ₅₀ (μM)
BHA	NT	29.17 ± 4.42	66.20 ± 1.83	88.43 ± 3.04	92.45 ± 0.11	92.45 ± 0.28	92.14± 0.32	42.85±0.82
BHT	NT	36.11 ± 5.67	56.27± 4.42	82.64 ± 1.59	88.67 ± 1.32	91.17 ± 0.11	90.01± 0.64	47.84±0.22

The Phenanthroline Assay:

	3.12	6.25	12.5	25	50	100	200	A _{0.5} (μM)
T2c	0,33±0,02	0,27±0,09	0,35±0,02	0,38±0,01	0,44±0,03	0,50±0,00	0,44±0,05	94,48±3,58
T1d	0,48±0,06	0,43±0,03	0,44±0,03	0,44±0,01	0,58±0,02	0,56±0,00	0,53±0,10	31,90±3,61
T1b	0,23±0,09	0,31±0,02	0,30±0,01	0,32±0,00	0,37±0,07	0,52±0,01	0,54±0,03	92,00±6,39
T2a	0,27±0,01	0,29±0,01	0,30±0,02	0,33±0,04	0,40±0,03	0,64±0,17	0,69±0,06	85,90±0,52
T3a	0,30±0,02	0,39±0,03	0,53±0,04	0,61±0,06	0,72±0,07	0,72±0,10	1,14±0,24	11,38±1,38
T1g	0,28±0,28	0,29±0,02	0,27±0,03	0,30±0,00	0,25±0,01	0,28±0,02	0,32±0,02	>200
T1e	0,25±0,01	0,26±0,01	0,27±0,01	0,26±0,01	0,27±0,01	0,30±0,01	0,32±0,01	>200
T3b	0,29±0,01	0,30±0,00	0,32±0,01	0,33±0,01	0,35±0,06	0,36±0,04	0,45±0,04	>200
T1f	0,30±0,02	0,39±0,03	0,53±0,04	0,61±0,06	0,72±0,07	0,72±0,10	1,14±0,24	11,38±1,38
T1a	0,28±0,03	0,29±0,02	0,27±0,03	0,30±0,00	0,25±0,01	0,28±0,02	0,32±0,02	>200
T2b	0,25±0,01	0,26±0,01	0,27±0,01	0,26±0,01	0,27±0,01	0,30±0,01	0,32±0,01	>200
T1c	0,29±0,01	0,30±0,00	0,32±0,01	0,33±0,01	0,35±0,06	0,36±0,04	0,45±0,04	>200
BHA	0,34±0,04	0,39±0,05	0,57±0,14	0,89±0,09	0,92±0,34	1,79±0,46	2,32±0,83	7,32±0,83
BHT	0,16±0,01	0,16±0,01	0,18±0,02	0,20±0,05	0,23±0,02	0,27±0,03	0,51±0,01	190,32±0,83

FRAP Assay:

	3.125	6.25	12.5	25	50	100	200	A ₀₅ (μM)
T2c	0,08±0,01	0,08±0,01	0,09±0,00	0,09±0,01	0,11±0,00	0,16±0,02	0,16±0,09	>200
T1d	0,09±0,01	0,08±0,01	0,08±0,01	0,10±0,01	0,09±0,00	0,09±0,00	0,15±0,03	>200
T1b	0,06±0,01	0,07±0,01	0,07±0,00	0,09±0,03	0,09±0,01	0,12±0,04	0,11±0,02	>200
T2a	0,08±0,01	0,07±0,01	0,08±0,01	0,10±0,01	0,09±0,00	0,14±0,03	0,20±0,02	>200
T3a	0,12±0,01	0,15±0,02	0,16±0,01	0,15±0,01	0,16±0,02	0,23±0,04	0,19±0,09	>200
T1g	0,06±0,01	0,06±0,00	0,07±0,01	0,08±0,01	0,13±0,00	0,14±0,01	0,12±0,03	>200
T1e	0,06±0,02	0,06±0,02	0,07±0,01	0,09±0,01	0,11±0,03	0,12±0,01	0,14±0,04	>200
T3b	0,07±0,01	0,09±0,00	0,10±0,01	0,11±0,01	0,13±0,01	0,19±0,02	0,15±0,07	>200
T1f	0,09±0,01	0,11±0,01	0,12±0,01	0,22±0,03	0,20±0,01	0,43±0,04	0,27±0,13	>200
T1a	0,07±0,00	0,09±0,00	0,14±0,05	0,14±0,03	0,16±0,03	0,24±0,02	0,17±0,04	>200
T2b	0,09±0,01	0,09±0,02	0,10±0,01	0,09±0,01	0,13±0,01	0,26±0,07	0,21±0,03	>200
T1c	0,07±0,01	0,10±0,01	0,11±0,01	0,11±0,01	0,23±0,03	0,20±0,02	0,34±0,13	>200
	0.39	0.78	1.56	3.12	6.25	12.5	25	A ₀₅ (μM)

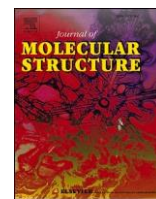
AChE assay:

	6.25	12.5	25	50	100	200	IC ₅₀ (μM)
T2c	NA	NA	NA	NA	47,20± 3,35	67,55± 2,96	121,72± 3,85
T1d	NA	NA	NA	NA	NA	9,17± 0,30	>200
T1b	NA	NA	NA	NA	20,32± 0,26	19,28± 7,90	>200
T2a	NA	NA	NA	23,69± 1,94	32,74± 1,62	42,72± 1,19	>200
T3a	NA	7,74± 2,35	25,37± 4,16	39,68± 2,32	54,98± 2,07	86,35± 3,69	86,33±3,71
T1g	8,75± 1,74	9,67± 1,77	73,90± 0,55	76,12± 3,35	87,77± 0,31	88,59± 0,92	20,35±0,06
T1e	NA	NA	NA	16,32± 1,09	78,76± 2,30	88,90± 1,54	76,99± 1,17
T3b	NA	NA	11,83± 0,41	28,37± 6,47	43,47± 0,04	60,95± 1,90	143,71± 2,53
T1f	NA	13,12± 4,71	48,73± 2,66	57,92± 0,00	73,02± 6,96	74,22± 3,01	38,90± 0,46
T1a	NA	NA	18,31± 1,49	24,59± 5,09	30,16± 4,15	46,31± 0,03	>200
T2b	NA	NA	NA	37,56± 2,09	43,68± 4,22	44,04± 1,70	>200
T1c	NA	NA	NA	NA	NA	17,75± 1,20	>200
	0.78	1.56	3.12	6.25	12.5	25	IC ₅₀ (μM)
Galantamine	29.30 ± 1.85	38.34 ± 0.72	69.71 ± 1.32	78.22 ± 0.45	86.58 ± 2.20	87.65 ± 1.50	4.14±0.07



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Coumarin 1,3,4-thiadiazole derivatives: Synthesis, bioactivity, and *In Silico* evaluation as Alzheimer's Disease inhibitors

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ABSTRACT

This study reports the synthesis of a new series of asymmetric coumarin-1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, T3a–T3b) targeting biochemical pathways relevant to Alzheimer's disease. Three coumarin precursors (C1–C3) were employed, using a one-pot sequential addition followed by alkylation to afford alkylated coumarin intermediates. Subsequent condensation with thiosemicarbazide under mild basic conditions and intramolecular cyclization yielded previously unreported asymmetric coumarin-thiadiazole derivatives. Twelve compounds were obtained in high yields (>82%) and fully characterized by ¹H and ¹³C NMR spectroscopy. Biological evaluation revealed significant antioxidant and anti-acetylcholinesterase (AChE) activities, supported by *In Silico* analyses. In the ABTS assay, compounds T1a, T1b, T1c, T1d, T1f, and T2b exhibited strong radical scavenging activity ($IC_{50} = 24.32 \pm 0.32$ – $50.14 \pm 0.17 \mu M$), comparable to BHT ($47.84 \pm 0.22 \mu M$). Phenanthroline reduction identified T1f, T3a, and T1d as the most potent reducing agents ($A_{0.5} = 11.38$ – $31.90 \mu M$), approaching BHA ($7.32 \pm 0.83 \mu M$). AChE inhibition was most pronounced for T1f and T1g ($IC_{50} = 38.90 \pm 0.46$ and $20.35 \pm 0.06 \mu M$, respectively), relative to galantamine ($IC_{50} = 4.14 \pm 0.07 \mu M$). Molecular docking confirmed strong binding within the AChE active site, while quantum chemical and ADMET analyses indicated favorable electronic properties, electron-donating substituent effects, and drug-like pharmacokinetic profiles. T1f, T1g, T1c, and T3a emerged as multifunctional lead candidates combining antioxidant and AChE inhibitory activities, highlighting the coumarin-thiadiazole scaffold as a promising platform for the development of therapeutics targeting oxidative stress-associated neurodegenerative disorders.

1. Introduction compounds widely distributed in nature [1]. These substances have been found to be present in the integument of various plant parts.

Coumarin and its derivatives are an important group of natural However, the highest concentrations of these subs

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found in fruits and flowers [2]. These compounds are biosynthesized from phenylalanine via the shikimic acid [3]. Generally, there are different classifications for the coumarin derivatives, they can be classified as: simple coumarins, furocoumarins, dihydrofurocoumarins, pyranocoumarins (linear and angular), phenylcoumarins, and biscoumarins [4]. Their bioactivity is important due to their distinctive structural properties, providing applications in areas of fundamental importance such as cancer, neuronal injury or degeneration, microbial and parasitic infections, and others. As a result, they are of great importance to the pharmaceutical industry [5].

Chemical rings, commonly found in various chemical compounds, are of pharmacological interest and represent the foundation for drug classes that target receptors [6]. Thiadiazoles are nitrogen- and sulfur-containing heterocycles that are commonly utilized as building blocks for biologically active molecules and are valuable to medicinal chemists. There are five isomers of the thiadiazole ring that are rarely found in nature, including: 1,2,3-thiadiazole (1); 1,2,4-thiadiazole (2); 1,2,5-thiadiazole (3); and 1,3,4-thiadiazole (4) [7]. Currently, the derivatives of 1,3,4-thiadiazole have gained significant attention and the research on this class of compounds has increased due to their range of pharmacological activities. 1,3,4-Thiadiazole derivatives are believed to exert their distinct biological activities owing to the presence of the =N-C-S- moiety [7]. Many compounds containing such a scaffold exhibit a wide spectrum of biological reactions and have been shown to have antifungal [8], antimicrobial [9], and anticancer [10] activities. In addition to other industrial applications.

In recent times, chemistry has increasingly relied on computing in its various aspects, with computers being used as essential tools in research, analysis, modelling and simulation. Molecular docking, which is an *In Silico* approach to simulate and predict the behavior of a molecule, such as a drug, to bind and interact with a target protein. This technique is fundamental to the design of new drugs. It helps determine the best way for a molecule to bind to a protein to form a stable compound [11]. We used this technique to provide valuable information on binding affinity and mechanism of action and to understand the relationships between

asymmetric coumarin thiadiazole compounds and specific proteins such as 1N8Q, 1OG5, 2CDU and 4JK4. and the enzyme 4MOE. In addition, SwissADME and ProTox-II were used to evaluate the properties of compounds ADMET (absorption, distribution, metabolism, excretion and toxicity), including bioavailability and toxicokinetic properties. The analysis was performed using laws that define drug properties, such as Lipinski, Egan, Egan, and Weber.

We report the design and synthesis of twelve asymmetric coumarin-1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, T3a–T3b) via an acid-catalyzed condensation of alkylated coumarin precursors with methyl-1,3,4-thiadiazole-2-amine, providing a straightforward route to structurally diverse hybrids with unique 3-position substituents. The structures were confirmed by ^1H and ^{13}C NMR spectroscopy, and their biological activities were evaluated using ABTS radical scavenging, phenanthroline reducing power, and acetylcholinesterase (AChE) inhibition assays. Importantly, AChE inhibition, a key therapeutic target in Alzheimer's disease, was specifically investigated to assess the potential of these compounds as modulators of cholinergic signaling. Complementary *In Silico* molecular docking studies provided mechanistic insights into interactions with Alzheimer's-relevant proteins. This integrated synthetic, experimental, and computational approach highlights the multifunctional potential of these asymmetric coumarin-thiadiazole derivatives as promising candidates for combating oxidative stress and neurodegeneration.

2. Experimental section

2.1. Materials and reagents

All commercial reagents and solvents were used as received unless otherwise stated. The alkylated coumarin derivatives used as starting materials were synthesized in-house following the procedure described by Jaafar et al. [12], using commercially available coumarin carboxylates (Sigma-Aldrich) as precursors. Analytical-grade reagents were obtained from Sigma-Aldrich and included acetic acid ($\geq 99.7\%$), thiosemicarbazide (98 %), piperidine (99 %), dichloromethane ($\geq 99.5\%$), sodium chloride (NaCl, 99 %) for washing the organic phase, magnesium sulfate (MgSO_4 , 99 %) and sodium sulfate (Na_2SO_4 , 99 %) for

drying organic extracts, and ethanol (96 %) for recrystallization. All moisture-sensitive reagents, particularly thiosemicarbazide, were stored in tightly sealed amber vials under dry nitrogen at room temperature. Solvents were freshly distilled when required; otherwise, high-purity commercial grades were used without further purification. **2.2. Synthesis of asymmetric coumarin–1,3,4-thiadiazole derivatives**

A series of twelve asymmetric coumarin–1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, T3a–T3b) were synthesized using pre-prepared asymmetric alkylated coumarin derivatives as starting materials, following a modified condensation–cyclization procedure described by Jaafar et al. [12]. In a 100 mL two-neck round-bottom flask equipped with a magnetic stirrer and reflux condenser, the corresponding coumarin derivative (1.0 mmol, 1 eq) and thiosemicarbazide (1.0 mmol, 1 eq) were dissolved in 15 mL of glacial acetic acid, corresponding to an approximate solvent-to-substrate ratio of 15:1 (v/v). To this mixture, one drop of piperidine (as catalyst) was added, and the solution was stirred at room temperature for 15 min to ensure homogeneity. The reaction mixture was then heated under reflux at 120 °C for 4–6 h, and the progress was monitored by thin-layer chromatography (TLC) using silica gel plates (E. Merck Kieselgel 60 F-254) and a hexane/ethyl acetate (7:3, v/v) solvent system.

Upon completion (disappearance of the coumarin spot), the reaction mixture was cooled to room temperature and quenched with 25 mL of cold distilled water. The resulting precipitate was extracted with dichloromethane (3 × 20 mL), and the combined organic layers were washed with saturated NaCl solution (2 × 20 mL), dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure using a rotary evaporator. The crude residue was purified by flash column chromatography on silica gel (60–120 mesh) using a gradient elution of pentane/ethyl ether (from 50:50 to 20:80, v/v) as the mobile phase.

The purified products were obtained as yellow to pale orange crystalline solids in yields ranging from 78 % to 88 %, depending on the substituents on the coumarin moiety. Each compound was characterized by melting point (Kofler heating bench, 50 to 260 °C, 230 V), TLC (Rf values), and spectroscopic techniques (1H 400 MHz,

Chloroform- d , DMSO- d_6 and 13C 101 MHz, Chloroform- d , DMSO- d_6 NMR). Representative spectral data for T1f are provided in Section 2.3. Mechanistically, the reaction proceeds via an initial condensation between the carbonyl group of the coumarin and the terminal amino group of thiosemicarbazide, forming a thiosemicarbazone intermediate, followed by intramolecular cyclization and dehydration under acidic conditions to yield the fused 1,3,4-thiadiazole ring. A schematic representation of the synthetic pathway is provided in Scheme 1 to illustrate the overall condensation–cyclization sequence.

2.3. Characterization of the synthesized coumarins 1.3.4-thiadiazols derivatives

The synthesized coumarin–1,3,4-thiadiazole derivatives (T1a–T3b) were characterized by their TLC retention factors (Rf), melting points, FTIR, and 1H and 13C NMR spectroscopy. Melting points were determined using a Stuart SMP30 digital melting point apparatus and are reported as uncorrected values. TLC analyses were carried out on silica gel 60 F-254 plates (E. Merck) using hexane/ethyl acetate (7:3, v/v) as the mobile phase, and spots were visualized under UV light at 254 nm. FTIR spectra were recorded on a PerkinElmer Spectrum Two spectrophotometer using KBr pellets, and the characteristic absorption bands confirmed the presence of key functional groups ($\text{C}=\text{O}$ at $\sim 1710\text{ cm}^{-1}$, $\text{C}=\text{N}$ at $\sim 1620\text{ cm}^{-1}$, and $\text{C}-\text{S}-\text{C}$ at $\sim 740\text{ cm}^{-1}$). NMR spectra were recorded at $25 \pm 1^\circ\text{C}$ in CDCl_3 or DMSO- d_6 solvents (depending on compound solubility) using a Bruker Avance 600 spectrometer operating at 400 MHz for 1H and 101 MHz for 13C. Tetramethylsilane (TMS) was used as an internal reference ($\delta = 0.00\text{ ppm}$). The pulse repetition times were 2 s for 1H and 5 s for 13C, with spectral widths of 12 ppm (1H) and 240 ppm (13C). A total of 64 scans were collected for 1H and 1024 scans for ^{13}C to ensure an adequate signal-to-noise ratio. Coupling constants (J) are reported in Hz, and multiplicities are denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Compound purity (> 95 %) was verified by the presence of a single sharp TLC spot and consistent 1H NMR integrations; for selected samples, purity was further confirmed by HPLC using an Agilent 1200 system (C18 column, 70:30 MeOH/ H_2O , 1 mL min^{-1}). All samples

were recrystallized from ethanol unless otherwise specified. Spectral data were consistent with the proposed structures. For example, in compound T1f, a diagnostic singlet was observed at δ 8.12 ppm (H-4, coumarin ring) and a signal at δ 161.4 ppm ($C=N$, thiadiazole ring), confirming the expected coumarin–thiadiazole framework. Detailed physical properties (yield, color, melting point, *R_f* value) and spectroscopic data

(^1H , ^{13}C NMR and FTIR) are summarized in Supplementary Tables S1–S2, while the corresponding NMR and FTIR spectra of representative compound T1f are provided in Supplementary

Figures S1–S25.

Methyl-3-allyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-2-oxochroman-3-carboxylate (T1a) was obtained as a brown solid (87 % yield), *m.p.* 201–202 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, CDCl_3) δ : 8.76 (s, 1H), 7.19–7.11 (m, 1H), 7.11–7.00 (m, 1H), 5.99–5.79 (m, 3H), 5.27 (d, *J* = 7.0 Hz, 1H),

5.22–5.17 (m, 1H), 4.10 (dd, *J* = 12.1, 7.1 Hz, 1H), 3.41–3.21 (m, 3H), 2.92–2.81 (m, 1H), 2.63 (dd, *J* = 17.2, 4.5 Hz, 1H), 1.70 (s, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ : 171.96 (C), 168.6 (C), 151.3 (C), 148.08 (C), 136.9 (CH), 135.9 (C), 128.7 (CH, aromatic), 128.0 (CH, aromatic), 127.4 (CH, aromatic), 124.5 (CH, aromatic), 117.5 (CH, aromatic), 114.2 (CH_2), 61.3 (C), 52.02 (C), 52.01 (CH_3), 48.3 (CH_2), 31.2 (CH_3), 27.9 (CH).

Methyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-2-oxo-3-(prop 2-yn-1-yl)chroman-3-carboxylate (T1b) was obtained as a white solid (88 % yield), *m.p.* 222 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 8.13 (s, 1H), 7.42–7.19 (m, 1H), 7.13 (s, 0H), 6.94 (t, *J* = 7.2 Hz, 1H), 6.81 (d, *J* = 8.0

Hz, 1H), 3.76 (s, 1H), 3.33 (s, 1H), 3.15 (t, *J* = 2.3 Hz, 1H), 2.80 (dd, *J* =

34.8, 15.8 Hz, 1H), 2.36 (dd, *J* = 17.3, 2.5 Hz, 1H), 2.27 (dd, *J* = 14.3, 4.2 Hz, 1H), 1.64 (s, 1H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 169.03 (C), 167.8 (C), 150.9 (C, aromatic), 150.7 (C), 130.2 (C, aromatic), 129.1 (C, aromatic), 124.3 (C, aromatic), 120.6 (C, aromatic),

118.2 (C, aromatic), 79.3 (C), 75.5 (CH_2), 71.1 (CH), 53.1 (CH_3), 52.9 (C), 46.7 (CH_2), 43.7 (CH_2), 33.4 (CH), 30.7 (CH_3).

Methyl 4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-benzyl-2-oxochroman-3-carboxylate (T1c) was obtained as a yellow solid (85 % yield), *m.p.* 232–234 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, CDCl_3) δ : 8.76 (s, 1H), 7.34 (m, 4H), 7.24 (m, 5H), 7.14 (s, 1H), 3.81 (d, *J* = 12.7 Hz, 2H), 3.69

(dd, *J* = 8.7 Hz, 2H), 3.55 (s, 3H), 2.27 (d, *J* = 10.4 Hz, 4H), 1.73 (d, *J* =

17.9 Hz, 1H), 1.27 (s, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ : 183.74 (C), 155.6 (C), 151.1 (C), 150.8 (C), 137.17 (C), 136.16 (C), 130.9 (C), 128.9 (C, aromatic), 128.5 (C, aromatic), 127.9 (C, aromatic), 127.3 (C, aromatic), 126.9 (C, aromatic), 121.4 (C, aromatic), 117.4 (C, aromatic),

52.96 (CH_3), 52.60 (CH_3), 47.5 (CH_2), 42.4 (CH_2), 33.3 (CH_2), 32.6 (CH),

31.27 (CH_3).

Methyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(3-methylbut-2-en-1-yl)-2-oxochroman-3-carboxylate (T1d) was obtained as a white solid (87 % yield), *m.p.* 208 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 8.18 (s, 1H), 7.23 (t, *J* = 7.8 Hz, 2H), 7.06 (s, 1H), 6.96–6.75 (m, 3H), 5.33 (t, *J* = 7.1 Hz, 1H), 3.78 (d, *J* = 7.4 Hz, 2H), 3.68 (s, 3H), 3.10 (dd, *J* = 18.8, 3.6 Hz, 1H), 2.82–2.70 (m, 1H), 2.50 (dd, *J* = 59.0, 10.5 Hz, 3H), 1.68 (s, 3H), 1.63 (s, 3H), 1.57 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 182.28 (C), 170.69 (C), 169.10 (C), 150.60 (C, aromatic), 148.65 (C), 134.2 (C, aromatic), 133.71 (CH, aromatic), 130.58 (CH, aromatic), 128.59 (CH, aromatic), 119.97 (CH, aromatic), 117.6 (CH, aromatic), 59.12 (C), 52.45 (CH_3), 52.04 (CH_3), 46.26 (CH_2), 34.98 (CH), 32.47 (CH_2), 31.07 (CH_3), 25.44 (CH_3), 17.23 (CH_3).

Methyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(4-methylbenzyl)-2-oxochroman-3-carboxylate (T1e) was obtained as a white solid (82 % yield), *m.p.* 248 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 8.42 (s,

1H), 7.36 (d, $J = 7.9$ Hz, OH), 7.24 (dd, $J = 13.0, 8.0$ Hz, 4H), 7.10–6.75 (m, 2H), 6.71 (d, $J = 1.2$ Hz, 1H), 5.49 (dd, $J = 11.9, 4.5$ Hz, OH), 3.75 (m, OH), 3.31 (s, 23H), 3.26 (d, $J = 4.0$ Hz, 1H), 3.07 (d, $J = 10.7$ Hz, 1H), 3.01–2.76 (m, 3H), 2.70–2.63 (m, 2H), 2.33 (d, $J = 1.7$ Hz, 1H), 2.31 (s, 4H), 1.62 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 172.26 (C), 168.1 (C), 151.3 (C, aromatic), 150.4 (C), 136.0 (C, aromatic), 135.1 (C, aromatic), 128.9 (C, aromatic), 128.4 (C, aromatic), 128.28 (C, aromatic), 127.8 (C, aromatic), 125.6 (C, aromatic), 116.0 (C, aromatic), 72.3 (C), 51.8 (C), 50.8 (CH₃), 46.7 (CH₂), 35.2 (CH₂), 31.6 (CH₃), 26.5 (CH), 20.3 (CH₃).

Methyl 4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(but-2-yn-1-yl)-2-oxochroman-3-carboxylate (T1f) was obtained as a brown solid (85 % yield), *m.p.* 228–229 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). Its ^1H NMR spectrum (400 MHz, DMSO- d_6) showed signals at δ 8.76 (s, 1H), 7.90 (d, $J = 7.7$ Hz, 5H), 7.73 (t, $J = 7.8$ Hz, 4H), 7.51–7.29 (m, 5H), 7.02 (s, 1H), 5.07 (t, $J = 13.4$ Hz, 1H), 3.83 (s, 11H), 2.89–2.19 (m, 4H), 1.82 (s, 1H), and 1.64 (s, 1H). The ^{13}C NMR spectrum (101 MHz, DMSO- d_6) displayed resonances at δ 170.9 (C), 163.1 (C), 151.0 (C), 149.06 (C), 134.5 (C, aromatic), 130.3 (CH, aromatic), 129.5 (CH, aromatic), 124.8 (CH, aromatic), 117.3 (CH, aromatic), 77.9 (C), 73.2 (C), 60.09 (C), 52.9 (C), 52.4 (CH₃), 43.7 (CH₂), 31.5 (CH₃), 29.05 (CH), 17.3 (CH₂), and 10.4 (CH₃).

Methyl 4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(2-bromoallyl)-2-oxochroman-3-carboxylate (T1g) was obtained as a white solid (84 % yield), *m.p.* 216–218 °C, *R_f* = 0.6 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, DMSO- d_6) δ : 8.22 (s, 1H), 7.43–7.08 (m, 2H), 7.00 (s, 1H), 6.98–6.80 (m, 3H), 5.73 (s, 1H), 5.61 (s, 1H), 3.97–3.84 (m, 3H), 3.77 (s, 3H), 2.64 (d, $J = 12.8$ Hz, 1H), 2.33–2.07 (m, 2H), 1.64 (s, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 182.2 (C), 150.6 (C), 149.4 (C), 130.7 (C), 128.8 (CH, aromatic), 127.3 (C, aromatic), 126.8 (CH, aromatic), 120.9 (CH, aromatic), 118.3 (CH₂), 115.4 (CH, aromatic), 88.5 (CH₂), 52.92 (CH₃), 52.7 (C), 41.8 (CH₂), 41.3 (C), 35.2 (CH₂), 30.58 (CH₃), 29.3 (CH).

Methyl 3-allyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-8-ethoxy-2-oxochroman-3-carboxylate (T2a) was obtained as a yellow viscous

compound (87 % yield), *m.p.* 220–222 °C, *R_f* = 0.5 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, DMSO- d_6) δ : 8.72 (s, 1H), 7.46–7.21 (m, 2H), 6.90 (d, $J = 3.1$ Hz, 2H), 6.81 (d, $J = 6.7$ Hz, OH), 4.78 (dt, $J = 5.3, 1.5$ Hz, 6H), 4.52 (d, $J = 5.8$ Hz, 2H), 4.34 (d, $J = 7.0$ Hz, 1H), 4.16 (q, $J = 7.0$ Hz, 2H), 4.02–3.94 (m, 1H), 3.83 (s, 3H), 3.21 (dd, $J = 18.9, 3.3$ Hz, 1H), 3.04 (dd, $J = 9.5, 3.3$ Hz, 1H), 3.01–2.96 (m, 2H), 2.67 (dd, $J = 3.6, 1.8$ Hz, 1H), 2.08 (s, 1H), 1.66 (s, 1H), 1.40 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 169.2 (C), 163.1 (C), 149.3 (C), 145.4 (C, aromatic), 143.9 (C, aromatic), 140.9 (CH), 134.4 (CH, aromatic), 124.7 (C, aromatic), 121.1 (C, aromatic), 118.3 (C, aromatic), 112.5 (C, aromatic), 64.5 (CH₂), 59.9 (C), 52.91 (C), 52.45 (CH₃), 46.6 (CH₂), 41.6 (CH₂), 35.9 (CH), 31.41 (CH₃), 14.5 (CH₃).

Methyl 4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-8-ethoxy-2-oxo-3-(prop-2-yn-1-yl)chroman-3-carboxylate (T2b) was obtained as a brown solid (88 % yield), *m.p.* 230–231 °C, *R_f* = 0.5 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, CDCl₃) δ : 8.50 (s, 1H), 7.66 (d, $J = 9.5$ Hz, 2H), 7.16 (t, $J = 7.9$ Hz, 9H), 7.01 (s, 1H), 6.40 (d, $J = 9.5$ Hz, 3H), 4.16 (q, $J = 7.0$ Hz, 4H), 4.06–3.96 (m, 9H), 3.92 (s, 3H), 3.75 (dd, $J = 28.8, 18.6$ Hz, 2H), 3.19 (dd, $J = 19.0, 2.9$ Hz, 2H), 2.52 (s, 1H), 2.12 (t, $J = 2.6$ Hz, 1H), 1.75 (s, 2H), 1.47 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl₃) δ : 173.95 (C), 160.57 (C), 149.53 (C), 146.66 (C, aromatic), 143.81 (C, aromatic), 124.83 (C, aromatic), 124.37 (C, aromatic), 119.32 (C, aromatic), 115.15 (C, aromatic), 83.89 (C), 82.40 (CH), 65.07 (CH₂), 64.68 (C), 52.95 (CH₃), 52.74 (C), 48.98 (CH₂), 31.98 (CH₃), 29.75 (CH₂), 27.89 (CH₂), 14.81 (CH₃).

(3S,4R)-methyl4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-8-ethoxy-3-(3-methylbut-2-en-1-yl)-2-oxochroman-3-carboxylate (T2c) was obtained as a yellowish solid (82 % yield), *m.p.* 206 °C, *R_f* = 0.5 (gradient: P–Et₂ O, 1:1). ^1H NMR (400 MHz, DMSO- d_6) δ : 8.43 (s, 1H), 7.29–7.14 (m, 3H), 5.49 (dd, $J = 11.7, 4.7$ Hz, 1H), 5.09 (s, 2H), 4.35 (t, $J = 5.0$ Hz, 1H), 2.95 (d, $J = 4.5$ Hz, 1H), 2.85 (s, 2H), 2.31 (d, $J = 2.6$ Hz, 1H), 1.89 (s, 2H), 1.76 (s, 1H), 1.62 (s, 1H), 1.06 (t, $J = 7.0$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 172.32 (C), 166.62 (C), 154.80 (C), 149.40 (C), 136.31 (C), 135.34

(C), 134.04 (C), 125.87 (CH), 121.70 (CH), 120.91 (CH), 112.37 (CH), 69.22 (CH₂), 61.80 (C), 54.82 (C), 51.07 (CH₃), 46.93 (CH₂), 31.92 (CH₂), 31.17 (CH₃), 27.35 (CH), 25.55 (CH₃), 18.55 (CH₃), 15.49 (CH₃).

Methyl 3-allyl-4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-7-(diethylamino)-2-oxochroman-3-carboxylate

(T3a) was obtained as a greenish solid (86 % yield),

m.p. 238 °C, *Rf* =

0.7 (gradient: P–Et₂ O, 1:1). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.56 (s, 1H), 7.62 (d, *J* = 9.0 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.63–5.68 (m,

1H), 5.06 (t, *J* = 13.7 Hz, 1H), 4.60 (d, *J* = 5.2 Hz, 1H), 3.99 (dd, *J* =

15.2, 5.7 Hz, 1H), 3.69 (s, 1H), 3.45 (dd, *J* = 13.9, 7.0 Hz, 1H), 3.36 (dd, *J* = 9.9, 5.6 Hz, 1H), 3.27 (q, *J* = 7.1 Hz, 2H), 2.96 (dd, *J* = 12.0, 7.9 Hz, 1H), 2.19 (dd, *J* = 14.1, 3.9 Hz, 1H), 1.96 (s, 1H), 1.60 (s, 1H), 1.06 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 171.35 (C), 169.66 (C), 151.83 (C), 149.48 (C), 148.17 (C, aromatic), 134.67 (CH), 131.36 (CH, aromatic), 129.97 (C), 118.23 (CH₂), 109.82 (CH, aromatic), 104.88 (CH, aromatic), 60.59 (C), 52.83 (C), 51.75 (CH₃), 46.68 (CH₂), 44.39 (CH₂), 43.69 (CH₂), 35.42 (CH₂), 32.36 (CH₃), 23.83 (CH), 12.54 (CH₃), 12.33 (CH₃).

Methyl 4-((5-amino-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)methyl)-3-(but-2-yn-1-yl)-7-(diethylamino)-2-oxochroman-3-carboxylate (T3b) was obtained as a brown solid (87 % yield), *m.p.* 241–242 °C, *Rf* = 0.7 (gradient: P–Et₂ O, 1:1). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.56 (s, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 6.79–6.74 (m, 3H), 6.52 (d, *J* = 2.0 Hz, 2H), 4.16–4.11 (m, 2H), 3.75 (s, 3H), 3.55–3.44 (m, 3H), 2.68–2.64 (m, 6H), 2.05–2.00 (m, 3H), 1.77 (s, 3H), 1.13 (t, *J* = 7.0 Hz, 6H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 176.76 (C), 168.87 (C), 150.06 (C), 149.52 (C), 145.72 (C), 128.69 (C), 124.92 (C), 109.87 (C), 107.04 (C), 78.20 (C), 74.27 (C), 61.39 (C), 52.48 (C), 51.77 (C), 44.40 (CH₂), 31.29 (C), 23.79 (C), 13.92 (C), 12.35 (CH₃), 3.98 (C).

2.4. Antioxidant ABTS cation radical scavenging assay

The antioxidant capacity of the synthesized coumarin–1,3,4-thiadiazole derivatives (T1a–T3b) was evaluated using the ABTS radical cation (ABTS•⁺) scavenging assay according to the method of Re et al. [13], with slight modifications. The ABTS•⁺ solution was generated by mixing 7 mM ABTS in distilled water with 2.45 mM potassium persulfate (K₂ S₂ O₈), followed by incubation in the dark at room temperature for 12–16 h to ensure complete radical formation. Before use, the solution was diluted with absolute ethanol to achieve an absorbance of 0.703 ± 0.025 at 734 nm using a UV–Vis microplate reader (EnSpire, PerkinElmer, USA). In a 96-well microplate, 40 μL of compound solution (prepared in methanol at concentrations ranging from 0.0625 to 4.0 mM) was mixed with 160 μL of the ABTS•⁺ working solution. The mixture was incubated in the dark at room temperature (25 ± 2 °C) for 10 min, and the absorbance was measured at 734 nm. Methanol was used as a blank, and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were included as standard antioxidant controls. The percentage inhibition of the ABTS radical was calculated using the equation:

$$\text{ABTScavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}} / A_{\text{control}})] \times 100$$

where *A*_{control} and *A*_{sample} represent the absorbance values of the control and test samples, respectively.

All measurements were performed in triplicate (three independent experiments, *n* = 3), and results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA (*p* < 0.05). The IC₅₀ values (concentrations required to scavenge 50 % of ABTS•⁺ radicals) were determined by nonlinear regression using XLSTAT software.

2.5. Antioxidant activity by the 1,10-Phenanthroline reducing power assay

The antioxidant reducing potential of the synthesized compounds was determined using the o-phenanthroline reducing power assay, as described by Szydłowska–Czerniak et al. (2008), with slight modifications. In a 96-well plate, 10 μL of compound solution (0.0625–4.0 mM in methanol) was added to a reaction mixture

containing 50 μL of 0.2 % FeCl_3 , 30 μL of 0.5 % o-phenanthroline, and 110 μL of methanol. The mixture was incubated at 30 °C for 20 min, and the absorbance was recorded at 510 nm using the EnSpire Multimode Plate Reader (PerkinElmer, USA). BHA was used as the positive antioxidant control under identical conditions. The antioxidant reducing capacity of each compound was expressed as $A_{0.5}$ (μM), defined as the concentration of test compound required to produce an absorbance of 0.50 under the assay conditions. Lower $A_{0.5}$ values indicate higher reducing potential. All experiments were conducted in triplicate, and data were expressed as mean $\pm$ SD. Linear regression analysis using XLSTAT was employed to calculate $A_{0.5}$ values.

2.6. Anti-Enzymatic (Anti-AChE) activity

The acetylcholinesterase (AChE) inhibitory activity of the synthesized coumarin-1,3,4-thiadiazole derivatives (T1a–T3b) was determined following the Ellman et al. [14] colorimetric method, with minor modifications. Briefly, 10 μL of each test compound (prepared in ethanol at concentrations ranging from 0.0625 to 2.0 mM) was mixed with 150 μL of 100 mM sodium phosphate buffer (pH 8.0) in a 96-well microplate. Then, 20 μL of AChE enzyme solution (5.32×10^{-3} U/well) from electric eel (Type VI-S, Sigma-Aldrich) was added. The reaction mixture was incubated at 25 ± 1 °C for 15 min to allow enzyme–inhibitor interaction. The enzymatic reaction was initiated by adding 10 μL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, 0.5 mM) and 10 μL of acetylthiocholine iodide (ATCI, 0.71 mM) as the substrate. In some cases, butyrylthiocholine chloride (BTC, 0.2 mM) was used to assess butyrylcholinesterase (BChE) inhibition under the same conditions. Absorbance was recorded at 412 nm at 0 min and 15 min using a Multimode Plate Reader (EnSpire, PerkinElmer, USA). Ethanol served as the solvent control, and galantamine hydrobromide (10 μM) was used as the reference AChE inhibitor. Each test was conducted in triplicate ($n = 3$). The percentage inhibition (%) was calculated using the equation:

$$\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} and A_{sample} correspond to the absorbance values of the control and test sample, respectively. Galantamine hydrobromide served as the reference inhibitor.

2.7. Statistical analysis

All experimental results are reported as mean $\pm$ standard deviation (SD) of three independent measurements ($n = 3$). The IC_{50} (for radical scavenging or enzyme inhibition assays) and $A_{0.5}$ (for reducing power assays) values were calculated using linear regression analysis in XLSTAT 2023 (Addinsoft, France). Where applicable, the significance of differences between test compounds and reference controls (e.g., BHA, BHT, galantamine) was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with a confidence level of 95 % ($p < 0.05$). Graphical representations of data were generated using OriginPro 2022b (OriginLab, USA). All calculations and analyses were performed under standard assumptions of normality and homogeneity of variance.

2.8. Computational studies

Geometry optimization and electronic property calculations of selected compounds (T1g, T3a, T1f, and T1c) were performed using Density Functional Theory (DFT) with the B3LYP hybrid functional and def2-SVP basis set, as implemented in ORCA 5.0.4 [15–20]. The geometry optimizations were conducted in the gas phase, followed by single-point energy corrections in implicit solvent (CPCM, water) to simulate physiological conditions. Vibrational frequency analysis confirmed the absence of imaginary frequencies, ensuring all optimized structures corresponded to true minima on the potential-energy surface.

Quantum-chemical descriptors (HOMO and LUMO energies (eV), energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$), chemical hardness ($\eta = \Delta E / 2$), softness ($S = 1 / \eta$), chemical potential ($\mu = (E_{\text{HOMO}} + E_{\text{LUMO}}) / 2$), and electronegativity ($\chi = -\mu$)) were computed to evaluate electronic reactivity and stability. Molecular electrostatic potential (ESP) maps were generated with Multiwfn 3.8 to visualize the electron-density distribution and identify potential electrophilic and nucleophilic regions relevant to ligand–receptor interactions. Key quantitative results are summarized in Table S3.

The optimized ligands were built in ACD/ [21], converted to PDBQT format using AutoDockTools 1.5.6

[22], and protonated according to physiological pH 7.4. The target enzyme acetylcholinesterase (AChE) from *Homo sapiens* (PDB ID: 4EY7, 2.35 Å) was retrieved from the Protein Data Bank. All crystallographic water molecules and co-crystallized ligands were removed, and polar hydrogens and Kollman charges were added in preparation for docking.

Molecular docking simulations were performed with AutoDock Vina 1.2.3 [23]. The grid box was centered at the active-site gorge ($x = 117.5$, $y = 117.2$, $z = 132.3$ Å) with dimensions $40 \times 40 \times 40$ Å and grid spacing 1.0 Å, fully encompassing the catalytic triad (Ser203, His447, Glu334). The exhaustiveness parameter = 32. Validation was carried out by re-docking the native ligand galantamine, yielding RMSD = 1.64 Å (< 2 Å), confirming the accuracy of the docking protocol. The

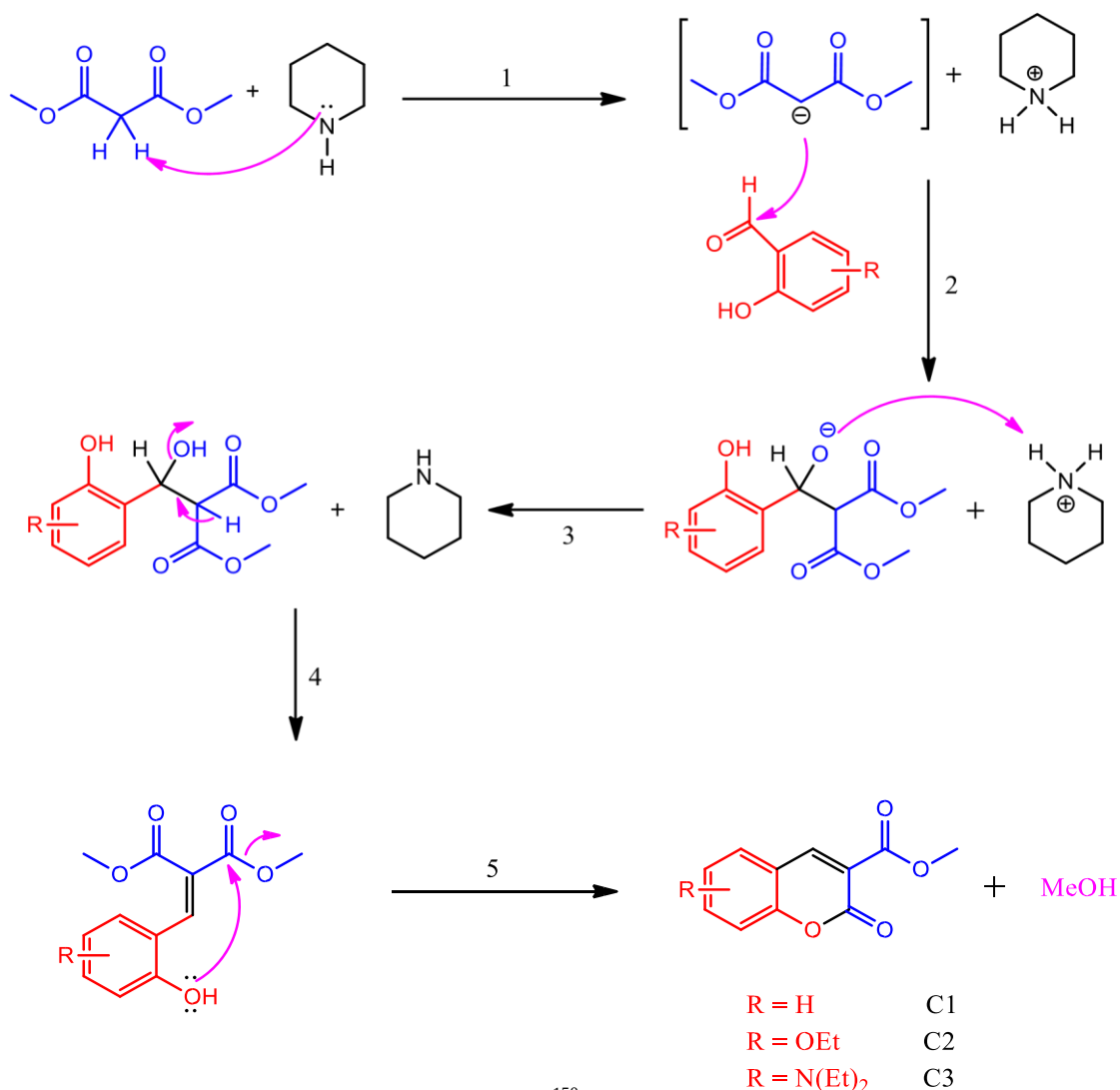


Fig. 1. Schematic representation of the synthesis of coumarin derivatives (C1–C3). The reaction involves the condensation of methyl malonate with salicylaldehyde (for Coumarin C1), o-vanillin (for Coumarin C2), or 4-(diethylamino)-2-hydroxybenzaldehyde (for Coumarin C3) in ethanol, catalyzed by piperidine under mild conditions, via a Knoevenagel condensation followed by intramolecular lactonization.

top-ranked poses were selected based on binding free energy (ΔG) and analyzed in BIOVIA Discovery Studio Visualizer 2021 to identify hydrogen-bond, π - π , π -cation, and hydrophobic interactions.

Pharmacokinetic and toxicity predictions (ADMET) were evaluated using SwissADME (v2023, <https://www.swissadme.ch>), ADMETlab 2.0 (v2.0, <https://admetmesh.scbdd.com/>), and ProTox-II v3.0 (https://tox-new.charite.de/protox_ii). Parameters included Lipinski's Rule of Five, bioavailability score, TPSA, logP, GI absorption, BBB permeability, CYP-450 inhibition, and LD₅₀ toxicity class. Compounds displaying strong in vitro AChE inhibition (T1f and T1g) also exhibited the lowest predicted binding energies (ΔG), in line with experimental findings. A schematic summary of the computational workflow is presented in Fig. 1, illustrating DFT optimization, docking, and ADMET

analysis stages.

3. Results and discussion

3.1. Structure and yield of coumarins 1.3.4-thiadiazols derivatives

The synthetic pathway to coumarin-1,3,4-thiadiazole derivatives involved three main steps: preparation of parent coumarins, selective alkylation, and subsequent cyclization with thiosemicarbazide.

The parent coumarins (C1–C3) were obtained via a Knoevenagel condensation of methyl malonate with salicylaldehyde, o-vanillin, or 4- (diethylamino)-2-hydroxybenzaldehyde in ethanol using piperidine as a mild base catalyst (Fig. 1). This transformation proceeds through enolate formation at the active methylene

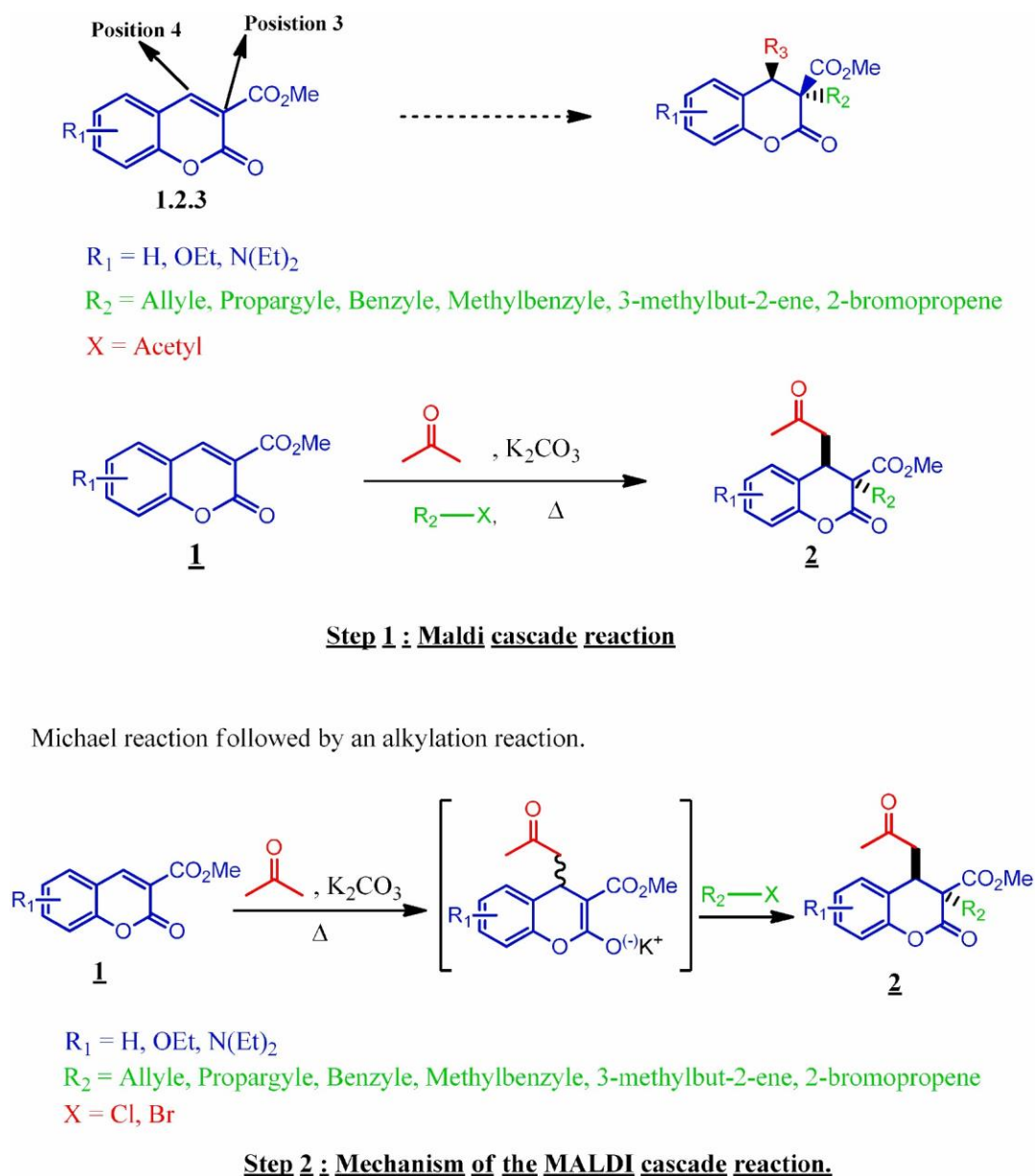


Fig. 2. The general preparation of asymmetric coumarins and their subsequent derivatization. Coumarin precursors (C1–C3) undergo a cascade addition followed by alkylation to afford diverse asymmetric derivatives.

[27].

Alkylation of the 3-position afforded a library of asymmetric coumarin derivatives (C1a–C3b) (Fig. 2). The nature of the substituent significantly affected the yields (80 %– 90 %, Table 1). Allyl and propargyl groups provided the highest yields (90 %), reflecting their low steric hindrance and favorable electronic conjugation with the aromatic nucleus [28–31]. In contrast, bulky benzyl or methylbenzyl substituents reduced efficiency (80 %), likely due to steric clashes during nucleophilic substitution. The observed variations align with known steric and electronic effects in electrophilic substitutions of activated aromatic systems.

As shown in Fig. 3, a clear structure–yield relationship is observed for substitutions at the C-3 position of the coumarin core. Linear and minimally sterically hindered substituents, such as allyl and propargyl groups, consistently provide higher synthetic yields, while bulkier or branched substituents result in moderate yield decreases. This trend indicates that steric effects and substituent linearity are the primary factors controlling reaction efficiency at the C-3 position, with electronic effects playing a secondary role.

The coumarin–1,3,4-thiadiazole derivatives (T1a–T1g, T2a–T2c, and T3a–T3b) were subsequently synthesized by condensation of the alkylated coumarins with thiosemicarbazide under basic catalysis (piperidine) (Fig. 4). The activated carbonyl group of coumarins facilitated nucleophilic attack by thiosemicarbazide, followed by intramolecular cyclization to form the heteroaromatic thiadiazole ring. The efficiency of this step was generally high, with most products obtained in yields above 82 % (Table 2) [32–34].

From a mechanistic perspective, the reactivity of the carbonyl function is explained by its polarized $C=O$ bond, which renders the carbonyl carbon a strong electrophilic center [32]. Protonation or electron withdrawal further enhances this effect, while the nucleophilic $-NH_2$ group of thiosemicarbazide ensures a facile condensation. The subsequent cyclization is driven by intramolecular proximity effects, which favor ring closure and stabilize the final thiadiazole system [33].

The synthesis of seven compounds (T1a, T1b, T1c, T1d, T1e, T1f and T1g) derived from

coumarin C1 was carried out by introducing different functional groups at the 3-position, and their yields were compared.

- (1) **Compound T1a**, derived from coumarin C1, bears an allyl substituent at the 3-position and affords an 87 % yield. The high yield is attributed to the favorable electronic nature and low steric demand of the allyl group, which facilitates nucleophilic substitution and promotes a smoother reaction course compared to bulkier substituents.
- (2) **Compound T1b**, obtained from coumarin C1 and featuring a propargyl group at the 3-position, exhibits a yield of 88 %. The highly reactive alkyne moiety facilitates efficient nucleophilic substitution, while its linear geometry minimizes steric hindrance. Although the triple bond may induce side reactions, the reaction proceeds efficiently, affording a yield comparable to T1a.
- (3) **Compound T1c**, synthesized from coumarin C1 and bearing a benzyl group at the 3-position, provides a yield of 85 %. The benzyl substituent stabilizes the intermediate via resonance effects; however, its bulkiness introduces steric hindrance that retards nucleophilic substitution, leading to a lower yield than T1a and T1b.
- (4) **Compound T1d**, obtained from coumarin C1 and incorporating a 3-methylbut-2-yn-1-yl group, delivers a yield of 87 %. The alkenyl functionality enhances electrophilicity at the reaction center, thereby facilitating faster substitution, though steric and electronic effects slightly influence the overall efficiency.
- (5) **Compound T1e**, derived from coumarin C1 and bearing a methylbenzyl group at position 3, affords a yield of 82 %. While the methylbenzyl moiety confers resonance stability, its large size induces steric congestion around the reaction site, diminishing reactivity and reducing yield.
- (6) **Compound T1f**, synthesized from coumarin C1 and characterized by the presence of a triple bond at position 3, demonstrates a yield of 85 %. The unsaturated alkenyl group enhances reactivity and promotes efficient substitution; despite possible side reactions, the process proceeds smoothly to give a high yield.
- (7) **Compound T1g**, obtained from coumarin C1 and featuring an (E)-2-bromobut-2-yne substituent,

yields 84 %. The bromine atom introduces steric and electronic effects that slightly retard nucleophilic substitution, resulting in a marginally reduced yield compared to the unsubstituted analogues.

The synthesis of three compounds (T2a, T2b, and T2c) derived from coumarin C2 was conducted by introducing various functional groups at distinct positions, and their respective yields were evaluated.

- (1) **Compound T2a** (derived from coumarin C2), bearing an ethoxy substituent at the 8-position, afforded a yield of 87 %. The ethoxy moiety enhances solubility and introduces only minimal steric hindrance, thereby facilitating efficient nucleophilic substitution. This notable yield serves as a benchmark for this series, reflecting the favorable electronic effects and moderate steric profile of the ethoxy group.
- (2) **Compound T2b** (derived from coumarin C2), bearing a propargyl substituent at the 3-position, afforded an 88 % yield. The propargyl moiety promotes efficient nucleophilic substitution; however, its increased reactivity and susceptibility to side reactions cause a slight reduction in yield compared to T2a. Nevertheless, the alkyne-based substitution proceeds effectively, producing a high overall yield.
- (3) **Compound T2c** (derived from coumarin C2), bearing a 3-methylbut-2-en-1-yl group, afforded an 82 % yield. The presence of the alkenyl group may enhance intermediate reactivity, accelerating nucleophilic substitution. However, the combined steric and electronic influences of the alkenyl moiety slightly decrease the yield relative to T2a.

The synthesis of two compounds (T3a and T3b) derived from coumarin C3 was carried out by introducing distinct functional groups at the 3-position, and their yields were comparatively evaluated.

- (1) **Compound T3a** (derived from coumarin C3), bearing an allyl group at the 3-position, afforded a high yield of 86 %. The allyl substituent effectively promotes nucleophilic substitution, and the elevated yield can be attributed to its low steric hindrance, which facilitates smooth and efficient substitution.

- (2) **Compound T3b** (derived from coumarin C3), bearing a propargyl group at the 3-position, afforded a yield of 87 %. The highly reactive propargyl moiety enhances nucleophilic substitution, leading to efficient reaction progress. The high yield reflects the favorable electronic characteristics and reactivity associated with the alkyne functionality.

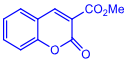
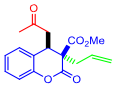
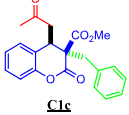
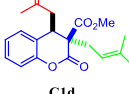
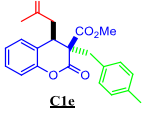
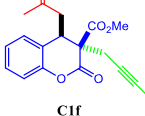
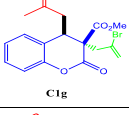
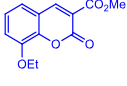
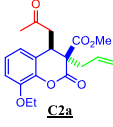
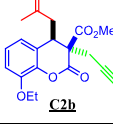
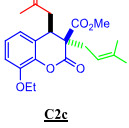
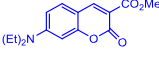
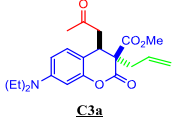
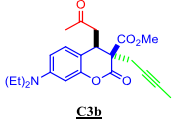
Having established the efficiency and reliability of the synthetic route, the next section explores whether these structural features translate into significant biological activities, particularly their antioxidant and anti-enzymatic potential.

3.2. Antioxidant ABTS radical activity of coumarins 1.3.4-thiadiazols derivatives

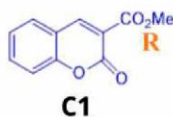
The antioxidant activity of the synthesized asymmetric coumarin–thiadiazole derivatives was investigated using the ABTS radical scavenging assay and the phenanthroline reduction assay to assess both radical quenching and reducing power. The results, summarized in

Table 1

Characteristics and yields of synthesized coumarin derivatives C1a to C3b.

Starting Coumarins	Reagent (R ₂ -X)	Products	Yields (%)
C1 	Allyl Bromide	 C1a	90
	Propargyl bromide	 C1b	91
	Benzyl chloride	 C1c	85
	1-Bromo-3-methylbut-2-ene	 C1d	88
	Methylbenzyl chloride	 C1e	83
	1-Chloro-2-butyne	 C1f	89
	2-Bromo-3-chloropropene	 C1g	87
C2 	Allyl Bromide	 C2a	91
	Propargyl bromide	 C2b	90
	Methylbenzyl chloride	 C2c	82
C3 	Allyl Bromide	 C3a	89
	1-Chloro-2-butyne	 C3b	84

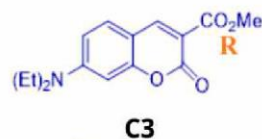
- High yield (>90%)
- Moderate (85–89%)
- Lower (<85%)



R = Methylbenzyl → 83%
 R = Benzyl → 85%
 R = 2-Bromo-3-chloroprop-1-enyl (halogenated allylic) → 87%
 R = 1-Bromo-3-methylbut-2-enyl (branched allylic) → 88%
 R = 1-Chloro-2-butylnyl (halogenated alkynyl) → 89%
 R = Allyl → 90%
 R = Propargyl → 91%



R = Methylbenzyl → 82%
 R = Propargyl → 90%
 R = Allyl → 91%



R = Allyl → 89%
 R = 1-Chloro-2-butylnyl (halogenated alkynyl) → 84%

Reaction yield increases with decreasing steric bulk and increasing linearity of the substituent at the C-3 position

Fig. 3. Structure–yield relationship (SAR) illustrating the effect of substituent nature at the C-3 position of coumarin scaffolds (C1–C3) on synthetic yield.

Table 3, are expressed as IC_{50} and $A_{0.5}$ values, and compared with the synthetic standards (BHA and BHT).

In the ABTS assay, compounds T1c and T1f exhibited the strongest antioxidant performance (IC_{50} = 24.32 ± 0.36 μ M and 35.41 ± 1.35 μ M, respectively), surpassing both BHA (42.85 ± 0.82 μ M) and BHT (47.84 ± 0.22 μ M). The high activity of these derivatives is likely due to the presence of conjugated alkenyl and alkynyl substituents, which enhance π -electron delocalization and stabilize the generated radicals. The allylic and propargylic linkages in these molecules facilitate hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms, enabling rapid neutralization of ABTS radicals.

Compounds T1d, T1b, T1a, and T2b showed comparable IC_{50} values (43–50 μ M), indicating effective radical scavenging capacities. These moderate-to-strong activities can be attributed to favorable electronic effects of substituents at the 3-position, which modulate electron density on the coumarin–thiadiazole system and enhance radical stabilization. Conversely, derivatives such as T1e, T1g, and T3b exhibited diminished activity, likely due to bulky or electron-withdrawing substituents that hinder electron delocalization or restrict radical access to reactive sites.

In the phenanthroline reduction assay, compounds T3a and T1f exhibited the highest reducing power ($A_{0.5}$ = 11.38 ± 1.38 μ M), closely approaching BHA (7.32 ± 0.83 μ M) and significantly outperforming BHT (190.32 ± 0.83 μ M). The strong performance of T3a may be attributed to the electron-rich allyl group, which enhances the molecule's ability to donate electrons to Fe^{3+} /phenanthroline complexes, thereby accelerating Fe^{2+} formation. Compound T1d also displayed good reducing ability (31.90 ± 3.61 μ M), while T2c, T1b, and T2a showed moderate performance, suggesting that optimal electronic distribution and molecular planarity contribute to efficient redox behavior.

T1c, T1d, and T1f emerged as the most potent antioxidant candidates, combining low IC_{50} and low $A_{0.5}$ values across both assays. Their superior activity likely stems from extended π -conjugation, donor substituents (alkenyl/alkynyl), and favorable HOMO–LUMO characteristics that promote facile electron and proton transfer. These findings are consistent with previously reported structure–activity trends in coumarin derivatives [35] and [36] reported that electron-donating groups (e.g., $-OH$, $-OCH_3$, $-CH_2-$) enhance antioxidant potency by increasing electron density and conjugation within the aromatic ring. Milanović et al. [37] demonstrated via DFT calculations that extended π -systems enable efficient

electron delocalization and radical stabilization. In light of these reports, the enhanced activity of T1c, T1d, and T1f can be rationalized by their conjugated substituents, which strengthen electron-donating capacity, lower ionization potential, and stabilize radical intermediates through π - π resonance. Thus, the coumarin-thiadiazole scaffold represents a promising pharmacophore for the design of novel antioxidant agents with tunable electronic and steric properties.

3.3. AChE inhibitory activities of coumarins 1,3,4-thiadiazols derivatives

AChE inhibitory activities of the synthesized coumarin-thiadiazole derivatives were evaluated and compared with the reference inhibitor galantamine ($IC_{50} = 4.14 \pm 0.07 \mu M$). The obtained IC_{50} values are summarized in Table 4. Among all tested compounds, T1g and T1f exhibited the strongest inhibition, with IC_{50} values of $20.35 \pm 0.06 \mu M$ and $38.90 \pm 0.46 \mu M$, respectively—representing approximately five- to nine-fold lower activity than galantamine but significantly higher than the rest of the series. T1e, T3a, and T2c showed moderate inhibitory effects ($IC_{50} = 76.99$ – $121.72 \mu M$), while T3b was less active ($IC_{50} = 143.71 \pm 2.53 \mu M$). All

other derivatives (T1a–T1d, T1c, T2a, T2b) displayed weak or negligible inhibition ($IC_{50} > 200 \mu M$).

The results of the present study demonstrate that several of the synthesized asymmetric coumarin-1,3,4-thiadiazole derivatives exhibit significant acetylcholinesterase (AChE) inhibitory activity, suggesting potential relevance to Alzheimer's disease (AD). Inhibition of AChE is a well-established therapeutic strategy in AD, as this enzyme regulates acetylcholine levels in the brain, and its inhibition can enhance cholinergic neurotransmission in patients with cognitive decline. Coumarin-based compounds have been widely explored for this purpose, and numerous reports have confirmed their efficacy as AChE inhibitors with potential anti-Alzheimer properties, including clinical and preclinical leads and structure-activity relationships targeting the AChE active site [38,39].

Moreover, the multifunctional design of coumarin derivatives that combines cholinesterase inhibition with antioxidant activity aligns with current trends in AD drug discovery, as oxidative stress is implicated in AD pathology and may exacerbate neurodegeneration. In this context, the observed antioxidant properties of our compounds further support

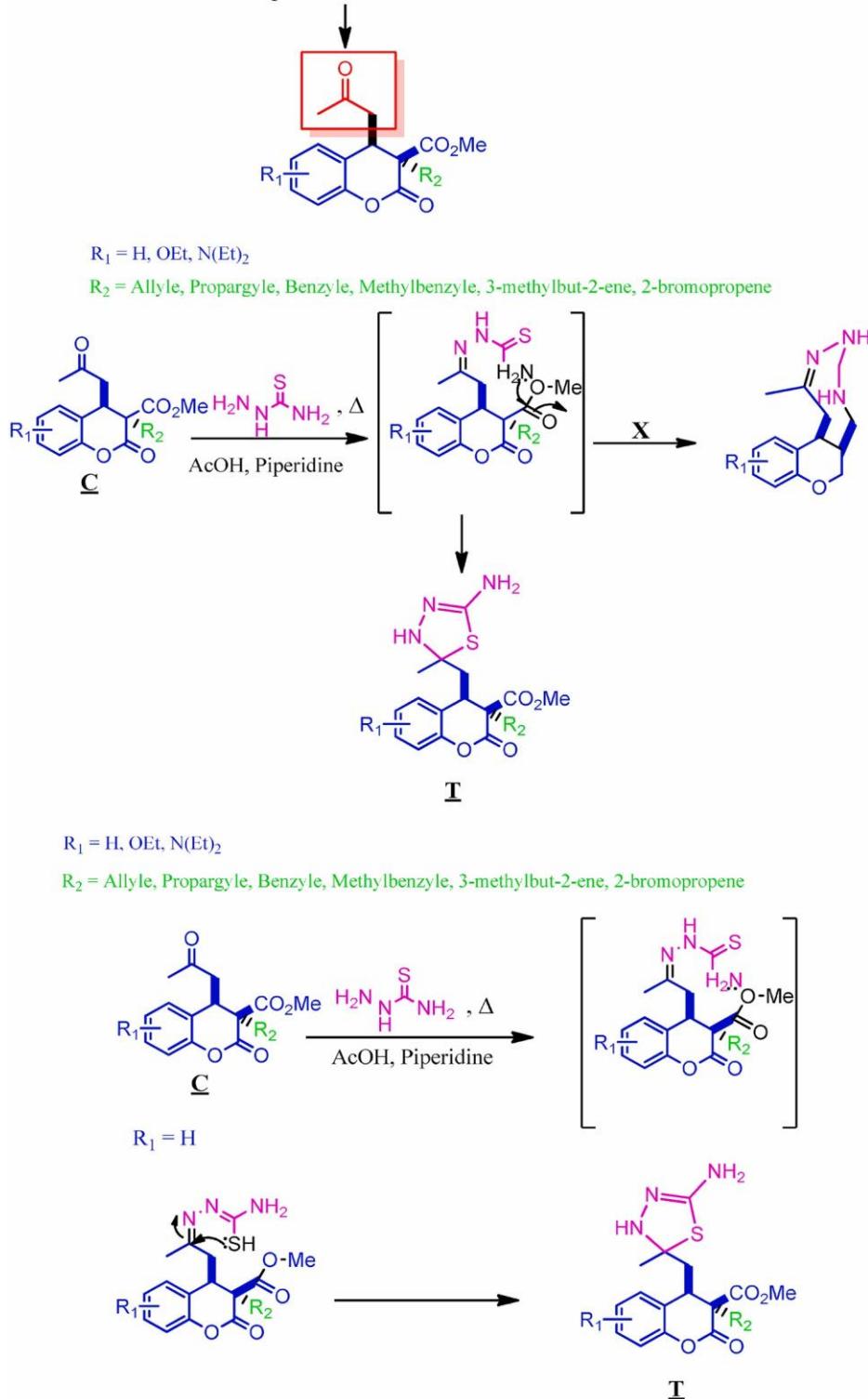


Fig. 4. Carbonyl Reactivity and Intramolecular Cyclization in Coumarin Derivatives The scheme illustrates the reactivity of the carbonyl group in coumarin derivatives, the intramolecular cyclization reaction, and its detailed mechanism. Key intermediates, bond-forming steps, and electron movements are shown to provide a comprehensive overview of the reaction pathway.

their therapeutic potential, as oxidative stress modulation complements cholinergic enhancement and may help mitigate the progression of neurodegenerative processes in Alzheimer's disease. The integration of *in vitro* AChE inhibition, antioxidant assays, and *In Silico* docking thus provides mechanistic evidence that these

derivatives warrant further investigation as multifunctional anti-Alzheimer agents.

The superior activities of T1g and T1f can be attributed to their extended conjugated π -systems and unsaturated substituents, which enhance π - π stacking and hydrogen bonding interactions with key aromatic residues (Trp84, Phe330, Tyr121) in the AChE catalytic gorge. These features increase the compounds' ability

to fit within the hydrophobic cavity and stabilize enzyme–inhibitor complexes. In contrast,

Table 2

Characteristics and yields of synthesized coumarin derivatives T3a to 13b.

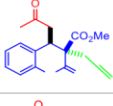
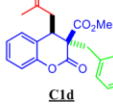
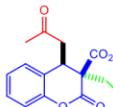
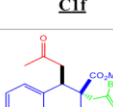
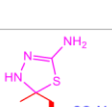
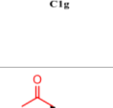
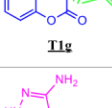
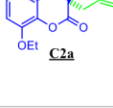
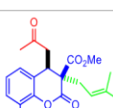
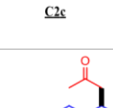
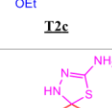
Starting Coumarins	Reagent (R ₂ -X)	Products	Yields (%)
 C1a	Thiosemicarbazide	 T1a	87
 C1b		 T1b	88
 C1d		 T1d	87
 C1e		 T1e	82
 C1f		 T1f	85
 C1g		 T1g	84
 C2a		 T2a	87
 C2b		 T2b	88
 C2c		 T2c	82
 C3a		 T3a	86
 C3b	158	 T3b	87

Table 3

Antioxidant activity of asymmetric coumarin–thiadiazole derivatives.

Compound	ABTS IC ₅₀ (μM)	Phen A ₀₋₅ (μM)
T2c	194.31 ± 0.72	94.48 ± 3.58
T1d	45.56 ± 0.89	31.90 ± 3.61
T1b	43.73 ± 0.70	92.00 ± 6.39
T2a	222.95 ± 2.59	85.90 ± 0.52
T3a	69.40 ± 3.89	11.38 ± 1.38
T1g	72.28 ± 2.11	>200
T1e	>800	>200
T3b	63.02 ± 0.36	>200
T1f	35.41 ± 1.35	11.38 ± 1.38
T1a	43.02 ± 1.16	>200
T2b	50.14 ± 0.17	>200
T1c	24.32 ± 0.36	>200
BHA	42.85 ± 0.82	7.32 ± 0.83
BHT	47.84 ± 0.22	190.32 ± 0.83

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene. both were tested as reference antioxidants under the same experimental conditions.

Table 4

Acetylcholinesterase (AChE) inhibitory activity of asymmetric coumarin–thiadiazole derivatives.

Compound	AChE IC ₅₀ (μM)
T2c	121.72 ± 3.85
T1d	>200
T1b	>200
T2a	>200
T3a	86.33 ± 3.71
T1g	20.35 ± 0.06
T1e	76.99 ± 1.17
T3b	143.71 ± 2.53
T1f	38.90 ± 0.46
T1a	>200
T2b	>200
T1c	>200
Gal (Ref.)	4.14 ± 0.07

Gal = galantamine hydrobromide (reference inhibitor).

bulky or non-conjugated substituents (e.g., in T1a, T2a, and T1e) may cause steric hindrance, limiting accessibility to the active site and reducing affinity.

Notably, T3a (allyl substituent) and T2c (alkenyl group) achieved intermediate inhibition, suggesting that moderate conjugation and limited steric bulk still support favorable interactions, albeit less efficiently than the more planar, π -rich systems of T1g and T1f. The relatively weak activity of T3b, despite the presence of a diethylamino group, likely arises from increased steric strain and altered electronic distribution, reducing its binding efficiency.

These results indicate that electronic conjugation and molecular planarity play a decisive role in modulating AChE inhibition within this series. These trends agree

with literature findings: Abu-Aisheh et al. [40] demonstrated that N¹-(coumarin-7-yl) derivatives achieve strong inhibition through π – π stacking with Trp84 and Phe330, while Sonmez et al. [41] and Kang et al. [42] reported that coumarin–thiazole and coumarin–chalcone hybrids exhibit enhanced potency via extended conjugation and aromatic interactions. Accordingly, T1g and T1f stand out as the most promising leads, combining favorable conjugation, optimal electronic density, and good inhibition potency, making them suitable scaffolds for further optimization and mechanistic exploration via molecular docking and DFT correlation.

3.4. In Silico bioactivity and electronic properties

Among the synthesized compounds, T3a, T1g, T1f, and T1c were identified as the most promising based on their acetylcholinesterase (AChE) inhibition and antioxidant activities evaluated through ABTS, Phenoltrhnil, and FRAP assays. Compound T1g exhibited the strongest AChE inhibition (IC₅₀ = 20.35 μM), approaching the potency of the standard galantamine, while T3a showed the highest reducing ability in the FRAP assay (11.38 μM), reflecting its considerable antioxidant potential. Compound T1f demonstrated a balanced bioactivity profile, combining significant AChE inhibition with FRAP activity comparable to T3a. In contrast, T1c excelled in the ABTS assay (24.32 μM), indicating an efficient free-radical scavenging capacity. These variations suggest complementary bioactive features among the selected compounds, justifying further computational investigations into their electronic and molecular properties.

Quantum chemical evaluation revealed clear distinctions in electronic reactivity and molecular stability among the compounds (Table 5). Compound T1g, with the highest HOMO energy (−4.80 eV), demonstrated strong electron-donating capability, making it well-suited for interactions with electrophilic targets. Conversely, T1c showed the lowest LUMO energy (−3.51 eV) and the smallest HOMO–LUMO gap (1.65 eV), reflecting high electrophilic character and chemical reactivity. Compounds T3a and T1f exhibited

intermediate HOMO–LUMO gaps (2.19 and 1.79 eV, respectively), indicating a balance between electronic stability and controlled reactivity. Electrostatic potential (ESP) surfaces (Fig. 5) further supported these observations, highlighting electron-rich (red) regions favorable for electrophilic attack and electron-deficient (blue) zones conducive to nucleophilic interactions. The complementary electronic characteristics of T1g and T1c suggest their suitability for diverse pharmacological applications.

3.5. Molecular docking analysis

Molecular docking against AChE (4EY7), BChE (1P0I), and catalase (1DNU) provided insights into the binding behavior of the four compounds. The heatmap of docking scores (Supplementary Figure S37) revealed that T1g and T1f had the most favorable and consistent multi-target binding affinities, with T1g showing ΔG values of -12.1 , -9.7 , and -7.7 kcal/mol for 4EY7, 1P0I, and 1DNU, respectively, and T1f displaying ΔG values of -10.1 , -8.3 , and -7.0 kcal/mol. Compounds T3a and T1c exhibited moderate binding, primarily with 1DNU ($\Delta G = -7.5$ and -7.3 kcal/mol, respectively). Detailed analyses of ligand–protein interactions (Figs. 6 and 7) highlighted hydrogen bonding, π -stacking, and hydrophobic contacts as key stabilizing factors. For instance, T1g formed hydrogen bonds with TYR72, ASP74, and HIS447 in 4EY7, complemented by π -stacking interactions with

PHE297 and TRP86, whereas T1f displayed comparable multi-target interactions. In contrast, T3a and T1c showed more selective binding, consistent with their moderate docking scores (Fig. 8).

Pharmacochemical and ADMET Properties

The pharmacochemical and ADMET assessment (Table 6) indicated favorable drug-like characteristics for all four compounds. Predicted oral absorption was high, and all compounds were P-gp substrates, which may influence intracellular concentrations. Partial inhibition of CYP2C19, CYP2C9, and CYP3A4 suggests potential for drug–drug interactions, while the absence of inhibition toward CYP1A2 and CYP2D6 is advantageous. Predicted BBB permeability was low, consistent with high TPSA values ($>128 \text{ \AA}^2$). Toxicity predictions indicated inactivity for hepatic, neural, cardiac, and immune systems, supporting the overall safety profile. Overall, compounds T1g and T1f combined strong multi-target binding with favorable ADMET properties, whereas T3a and T1c provided complementary electronic and antioxidant features, underscoring the therapeutic potential of this series.

4. Conclusion

This study demonstrates that rational modification of the coumarin–1,3,4-thiadiazole scaffold enables the development of

Table 5
A summary of the quantum parameters and physicochemical properties of compounds T3a, T1g, T1f, and T1c.

Compounds	HOMO	LUMO	Gap	IP	EA	μ	Ω	S	X	η
T3a	−4.93	−2.74	2.19	4.93	2.74	−3.84	6.72	0.46	3.84	1.10
T1g	−4.80	−3.07	1.73	4.80	3.07	−3.94	8.95	0.58	3.94	0.87
T1f	−5.20	−3.41	1.79	5.20	3.41	−4.31	10.35	0.56	4.31	0.90
T1c	−5.16	−3.51	1.65	5.16	3.51	−4.34	11.39	0.61	4.34	0.83

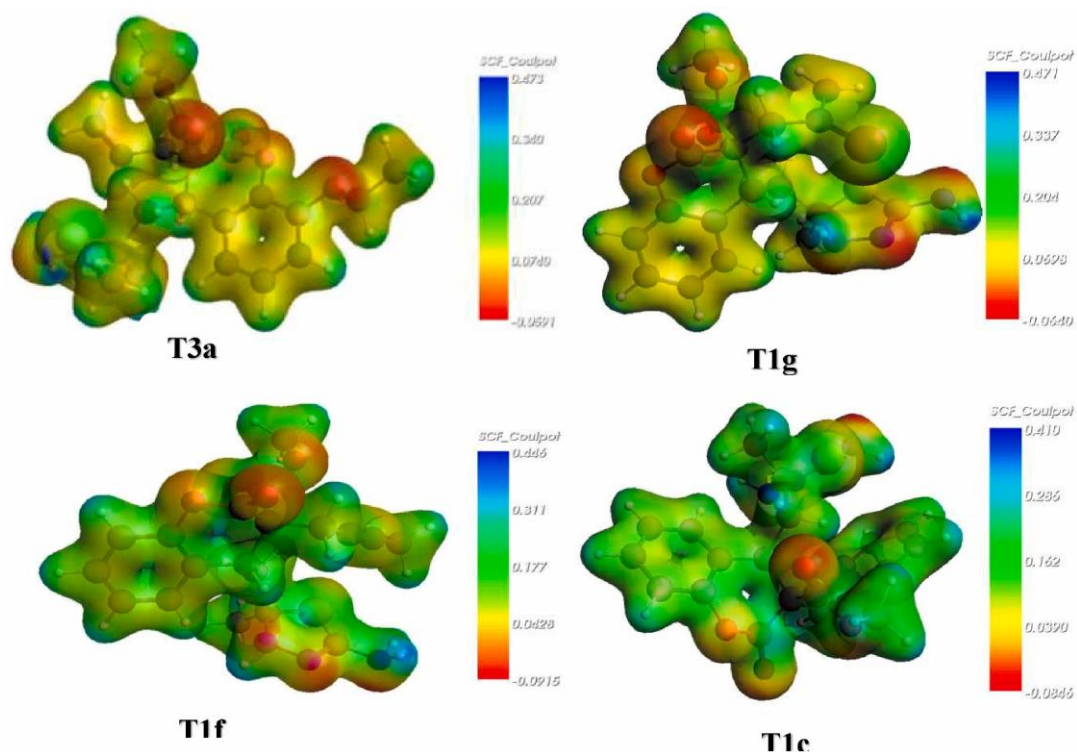


Fig. 5. Computational Mapping of the Molecular Electrostatic Potential Surfaces for Compounds T3a, T1g, T1f and T1c.

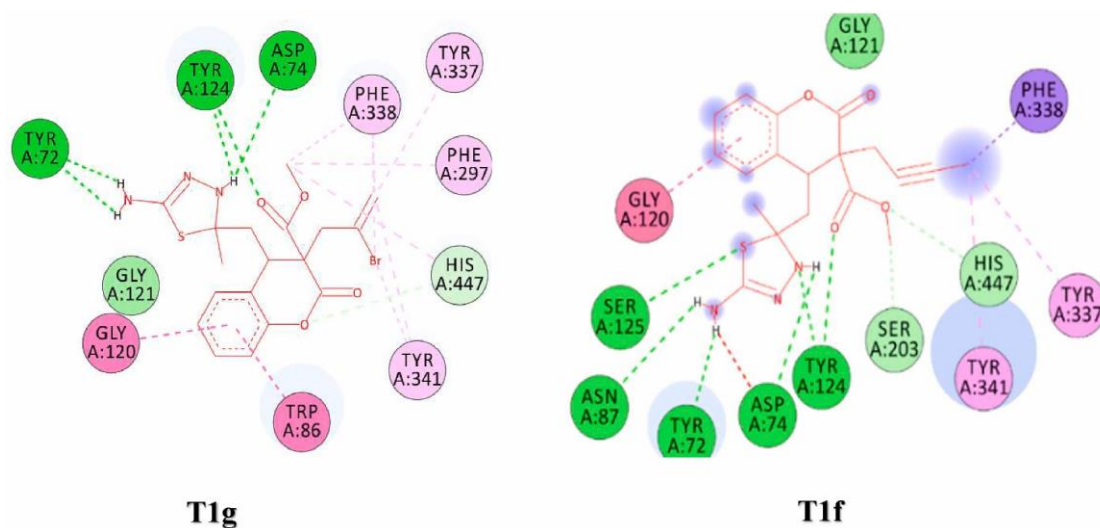


Fig. 6. Important interactions with various amino acid residue as 4EY7.

multifunctional derivatives with reproducible synthetic efficiency and Functionally, several derivatives exhibited notable antioxidant and biologically relevant activity. The alkylation strategy proved robust, acetylcholinesterase inhibitory activities, indicating that substituent- affording consistently high yields (82–88 %) and highlighting the independent electronic distribution governs both redox behaviour and fluence of electronic and steric effects on reaction efficiency. enzyme interactions. Compounds T1c and T1f showed enhanced ABTS

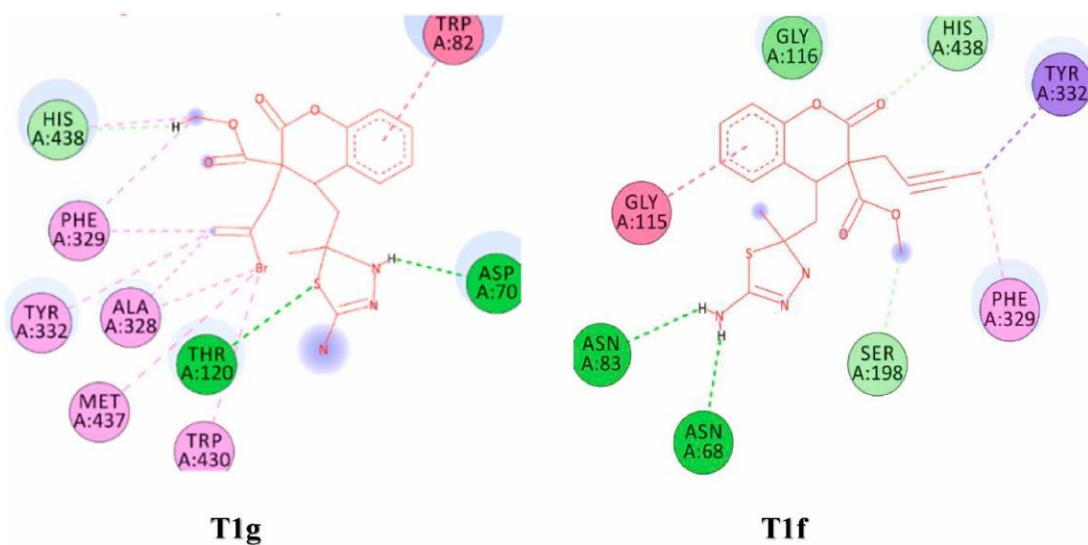


Fig. 7. Important interactions with various amino acid residue as 1DNU.

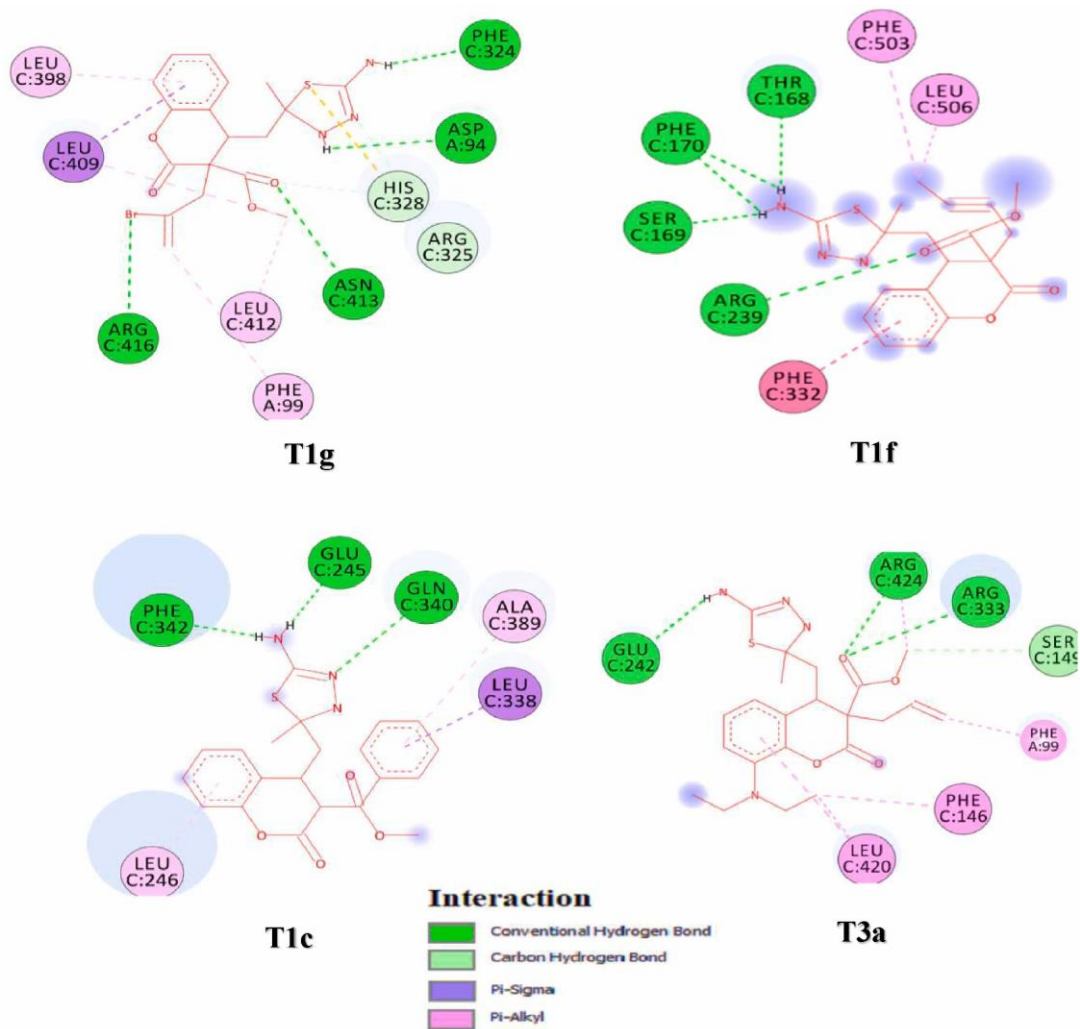


Fig. 8. Key ligand–protein interactions of compound T3a within the active site of catalase (1DNU), indicating hydrogen bonding and stabilizing hydrophobic interactions.

Table 6

The pharmacocchemical and toxicological profiles of compounds T3a, T1g, T1f, and T1c.

Compounds	T3a	T1g	T1f	T1c
MW	419.49	454.34	387.45	425.5
Fraction Csp3	0.45	0.39	0.42	0.32
NRB	8	6	5	6
NHA	6	5	5	5
NHD	2	2	2	2
TPSA	137.54	128.31	128.31	128.31
MLOGP	2.21	2.62	2.5	3.02
Solubility Class	Moderately	Moderately	Moderately	Moderately
GI absorption	High	High	High	High
BBB permeant	No	No	No	No
Pgp substrate	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	Yes	Yes
Lipinski violations	0	0	0	0
Ghose violations	0	0	0	0
Veber violations	0	0	0	0
Egan violations	1	0	0	0
Muegge violations	0	0	0	0
PAINS alerts	0	0	0	0
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive
Neurotoxicity	Inactive	Inactive	Inactive	Inactive
Cardiotoxicity	Inactive	Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Inactive	Inactive

radical scavenging activity ($IC_{50} = 24.32 \pm 0.36 \mu M$), while T1g and T1h emerged as the most potent acetylcholinesterase inhibitors ($IC_{50} = 20.35 \pm 0.06$ and $38.90 \pm 0.46 \mu M$, respectively). These experimental findings were supported by computational analyses, including favourable HOMO–LUMO energy gaps (1.65–2.19 eV) and strong docking affinities (ΔG up to -12.1 kcal/mol). Collectively, the integration of synthetic, biological, and *In Silico* approaches identifies the coumarin–thiadiazole framework as a promising platform for lead optimisation. Although preliminary, the elucidated structure–activity trends and the identification of T1f and T1g as lead candidates provide a solid basis for further structural refinement and advanced biological evaluation, particularly targeting oxidative stress and cholinergic dysfunction in Alzheimer’s disease.

Ethics statement

This manuscript does not comprise any experiments involving animals performed by any of the authors.

Statement regarding AI use

The authors had done all the text, data analysis, and interpretation of the results.

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CRedit authorship contribution statement

Fathi Benkonissa: Conceptualization. **Amar Djemoui:** Conceptualization. **Mokhtar Boualem Lahrech:** Conceptualization. **Salma Kaoutar Abdelali:** Conceptualization. **Souli Lahcene:** Conceptualization. **Ahmed Souadia:** Conceptualization. **Mohammed Messaoudi:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Investigation, Data

curation. **Chawki Bensouici:** Conceptualization. **Dems Mohamed Abdesselem:** Conceptualization.

Hatem Majdoub: Conceptualization. **Khadija Benarous:** Conceptualization. **Mohamed Yousfi:** Conceptualization. **Ahmed Barhoum:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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Data availability

Data will be made available on request.

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ملخص:

تم تحضير وتقييم سلسلة جديدة من الهجائن غير المتماثلة من الكومارين-1,3,4-ثياديازول (T2a- T1a-T1g)، T2c و T3a-T3b) بيولوجيًا بهدف استهداف بعض المسارات الكيميائية الحيوية المرتبطة بمرض الزهايمر. خُصّرت هذه المركبات انطلاقًا من ثلاثة طليعات للكومارين (C1-C3)، تلتها خطوة ألكلة للحصول على وسائط كومارينية مؤلفة، ثم تكثيفها مع الثيوسيميكاربازيد تحت ظروف قاعدية معتدلة مما أدى إلى تكوين هجائن جديدة غير متماثلة. تم الحصول على اثني عشر مركبًا بمردود مرتفع (>82%)، وتم توصيفها باستخدام مطيافية الرنين المغناطيسي النووي ^1H RMN و ^{13}C . أظهرت التقييمات البيولوجية نشاطًا مضادًا للأكسدة ملحوظًا بالإضافة إلى فعالية مثبتة لإنزيم أستيل كولين إستراز (AChE). وقد أظهرت المركبات T1a-T1d و T1f و T2b قدرة عالية على اصطياد الجذور الحرة ($\text{IC}_{50} = 24.32-50.14 \mu\text{M}$)، في حين أظهر اختبار الاختزال باستعمال o-فينانثرولين أن المركبات T1f و T3a و T1d هي الأقوى اختزالًا ($\text{A}_{0.5} = 11.38-31.90 \mu\text{M}$). أما فيما يخص تثبيط إنزيم AChE، فقد سجل المركبان T1g و T1f أفضل النتائج ($\text{IC}_{50} = 20.35-38.90 \mu\text{M}$). كما أكدت الدراسات الحاسوبية، بما في ذلك الالتحام الجزيئي وحسابات الكيمياء الكمية (DFT) وتنبؤات ADMET، وجود تفاعلات قوية مع الموقع الفعال للإنزيم وخصائص إلكترونية ودوائية حركية ملائمة. ومن بين المركبات المدروسة، برزت T1c و T1f و T1g و T3a بفضل الجمع بين نشاطها المضاد للأكسدة وقدرتها على تثبيط إنزيم AChE، مما يجعلها مرشحات واعدة لتطوير عوامل علاجية محتملة ضد مرض الزهايمر والأمراض التنكسية العصبية الأخرى المرتبطة بالإجهاد التأكسدي.

الكلمات المفتاحية: مشتقات الكومارين-ثياديازول؛ النشاط المضاد للأكسدة؛ تثبيط إنزيم أستيل كولين إستراز؛ الإرساء الجزيئي؛ نمذجة ADMET؛ توقع تشابه الدواء؛ الاضطرابات العصبية التنكسية.

Resume:

De nouveaux hybrides asymétriques coumarine–1,3,4-thiadiazole (T1a–T1g, T2a–T2c et T3a–T3b) ont été synthétisés et évalués biologiquement dans le but de cibler certaines voies biochimiques impliquées dans la maladie d'Alzheimer. Les composés ont été préparés à partir de trois précurseurs de coumarine (C1–C3), suivis d'une étape d'alkylation permettant d'obtenir des intermédiaires coumariniques alkylés, puis d'une condensation avec la thiosemicarbazide sous des conditions basiques modérées conduisant à la formation de nouveaux hybrides asymétriques. Douze composés ont été obtenus avec d'excellents rendements (>82 %) et caractérisés par spectroscopie de résonance magnétique nucléaire RMN ^1H et ^{13}C . Les évaluations biologiques ont révélé une activité antioxydante significative ainsi qu'une activité inhibitrice de l'acétylcholinestérase (AChE). Les composés T1a–T1d, T1f et T2b ont présenté une forte activité de piégeage des radicaux libres ($\text{IC}_{50} = 24,32\text{--}50,14\text{ }\mu\text{M}$), tandis que le test de réduction à l'o-phénanthroline a identifié les composés T1f, T3a et T1d comme les meilleurs agents réducteurs ($\text{A}_{0.5} = 11,38\text{--}31,90\text{ }\mu\text{M}$). En ce qui concerne l'inhibition de l'AChE, les composés T1g et T1f ont montré les meilleures activités ($\text{IC}_{50} = 20,35\text{--}38,90\text{ }\mu\text{M}$). Les études *in silico*, comprenant le docking moléculaire, les calculs de chimie quantique (DFT) et les prédictions ADMET, ont confirmé de fortes interactions avec le site actif de l'enzyme ainsi que des propriétés électroniques et pharmacocinétiques favorables. Parmi les composés étudiés, T1c, T1f, T1g et T3a se sont distingués par la combinaison de leurs activités antioxydantes et inhibitrices de l'AChE, faisant d'eux des candidats prometteurs pour le développement de nouveaux agents thérapeutiques potentiels contre la maladie d'Alzheimer et d'autres maladies neurodégénératives associées au stress oxydatif.

Mots clés: Dérivés coumarine–thiadiazole ; activité antioxydante ; inhibition de l'acétylcholinestérase ; docking moléculaire ; modélisation ADMET ; prédiction de la drug-likeness ; maladies neurodégénératives.

Abstract:

Novel asymmetric coumarin–1,3,4-thiadiazole hybrids (T1a–T1g, T2a–T2c, and T3a–T3b) were synthesized and biologically evaluated with the aim of targeting biochemical pathways involved in Alzheimer's disease. The compounds were prepared from three coumarin precursors (C1–C3), followed by an alkylation step to afford alkylated coumarin intermediates, and subsequently condensed with thiosemicarbazide under mild basic conditions to yield new asymmetric hybrids. Twelve compounds were obtained in excellent yields (>82%) and characterized by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Biological evaluation revealed significant antioxidant activity as well as acetylcholinesterase (AChE) inhibitory potential. Compounds T1a–T1d, T1f, and T2b exhibited strong free-radical scavenging activity ($\text{IC}_{50} = 24.32\text{--}50.14\ \mu\text{M}$), while the o-phenanthroline reducing power assay identified T1f, T3a, and T1d as the most potent reducing agents ($\text{A}_{0.5} = 11.38\text{--}31.90\ \mu\text{M}$). Regarding AChE inhibition, compounds T1g and T1f displayed the highest activities ($\text{IC}_{50} = 20.35\text{--}38.90\ \mu\text{M}$). In silico studies, including molecular docking, density functional theory (DFT) calculations, and ADMET predictions, confirmed strong interactions with the enzyme active site together with favorable electronic and pharmacokinetic properties. Among the investigated compounds, T1c, T1f, T1g, and T3a emerged as the most promising candidates owing to their combined antioxidant and AChE inhibitory activities, highlighting their potential as lead compounds for the development of novel therapeutic agents against Alzheimer's disease and other neurodegenerative disorders associated with oxidative stress.

Key words: Coumarin–thiadiazole derivatives; antioxidant activity; acetylcholinesterase inhibition; molecular docking; ADMET modeling; drug-likeness prediction; neurodegenerative disorders.