Synthesis of Novel Spirothiazolidinone Derivatives by Microwave Irradiation and Evaluation of Anti-Inflammatory Action

[1] Mansee Gupta, [2] Dr. Kaushelendra Mishra, [3] Dr. Parulben Mehta
[4]Dr. Abhishek Sharma

[1] M.Pharma Student , Department of Pharmaceutical Chemistry, Lakshmi Narain College of Pharmacy, Bhopal (M.P)
[2] Professor, Department of Pharmaceutical Chemistry, Lakshmi Narain College of Pharmacy, Bhopal (M.P)
[3] Principle, Department of Pharmaceutical Chemistry, Lakshmi Narain College of Pharmacy, Bhopal (M.P)
[4] Professor, Department of Pharmaceutical Chemistry, Lakshmi Narain College of Pharmacy, Bhopal (M.P)

Abstract

Spiro - oxindole derivatives have gained significant attention due to their diverse biological activities. In this study, we report a concise and efficient synthesis of novel Spirothiazolidinone derivatives through a one-pot, three-component reaction involving isatins, thiazolidine-2,4-dione, and various aromatic aldehydes under microwave irradiation. The reaction proceeds smoothly, affording the desired products in good yields and short reaction times. The synthesized compounds were characterized by spectroscopic techniques such as IR, ^{1}H NMR, and ^{13}C NMR. The anti-inflammatory activity of these compounds was evaluated using the carrageenan-induced paw edema model in rats. Several compounds exhibited promising anti-inflammatory activity, comparable to standard drugs like diclofenac sodium. The structure-activity relationship (SAR) studies revealed that the nature of the substituents on the aromatic ring significantly influenced the anti-inflammatory activity. These findings highlight the potential of Spirothiazolidinone derivatives as promising lead compounds for the development of novel anti-inflammatory agents.

Keywords: - Spirooxindole derivatives, Thiazolidinone, Microwave irradiation, One-pot synthesis, Anti-inflammatory activity, Carrageenan-induced paw edema, Structure-activity relationship (SAR), Drug discovery, Medicinal chemistry, Heterocyclic compounds

1. Introduction

Inflammation, a complex biological response to harmful stimuli, is a vital defense mechanism. It involves a cascade of events, including the release of inflammatory mediators, recruitment of immune cells, and tissue repair. While acute inflammation is essential for combating infections and healing wounds, chronic inflammation can lead to various diseases, such as arthritis, cardiovascular disease, and neurodegenerative disorders.

The Role of Anti-inflammatory Drugs

Anti-inflammatory drugs play a crucial role in managing inflammatory conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to alleviate pain and reduce inflammation. However, their long-term use can lead to adverse effects, such as gastrointestinal ulcers and renal impairment. Therefore, there is a continuous search for novel anti-inflammatory agents with improved efficacy and reduced side effects.

Spirooxindole Derivatives: A Promising Class of Compounds

Spirooxindole alkaloids are natural compounds with a distinct spirocyclic structure, commonly found in plants like Rubiaceae and Apocynaceae. This unique structure features an oxindole moiety fused to a ring, enabling diverse biological activities. These alkaloids exhibit a wide range of pharmacological properties, including anticancer, antimicrobial, anti-inflammatory, analgesic, and antioxidant effects. They also show promise in treating malaria, viral infections, atherosclerosis, and diabetes, and have insecticidal properties.

Specific examples highlight their therapeutic potential. Trigolute A, from Trigonostemon lutescens, may help treat kidney diseases. Gelsemine, from Gelsemium sempervirens, is being studied for its potential to address anxiety, oxidative stress, inflammation, and cancer. (-)-Horsfiline, from Horsfieldia Superba, traditionally used for pain relief, has a simple structure amenable to synthesis. Spindomycins A and B, isolated from a Streptomyces species, act as tyrosine kinase inhibitors and antitumor agents. Finally, compounds like Corynoxine A and B, Rhyncophylline, and Corynoxeine, found in Uncaria tormentosa (cat's claw), contribute to th

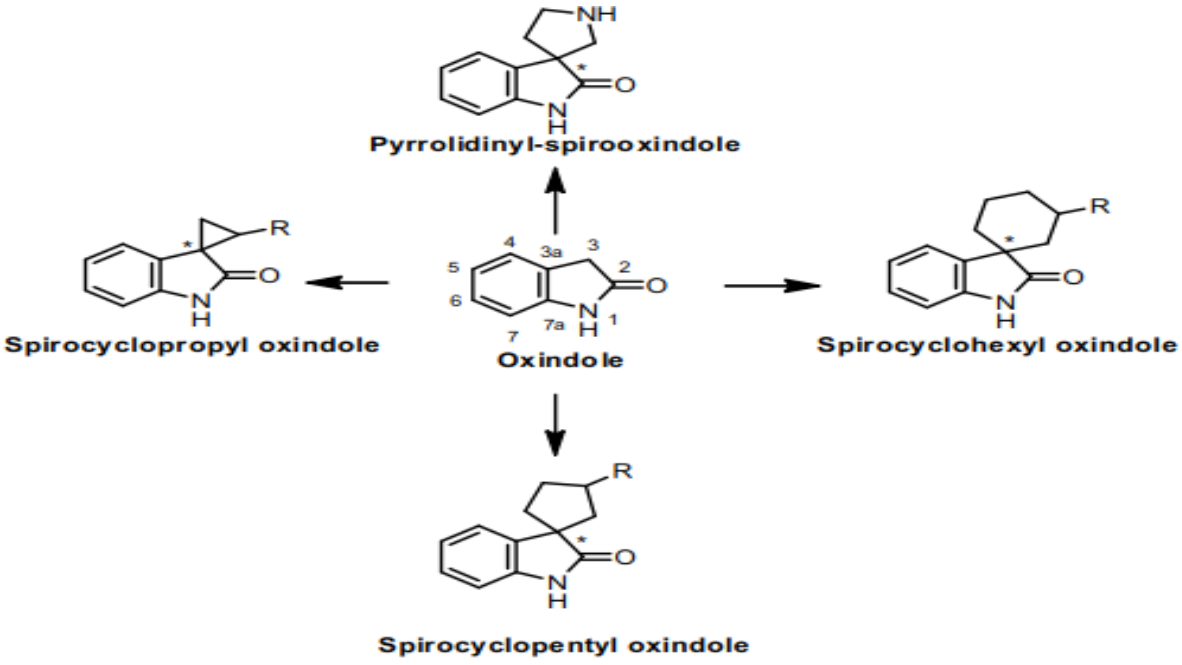


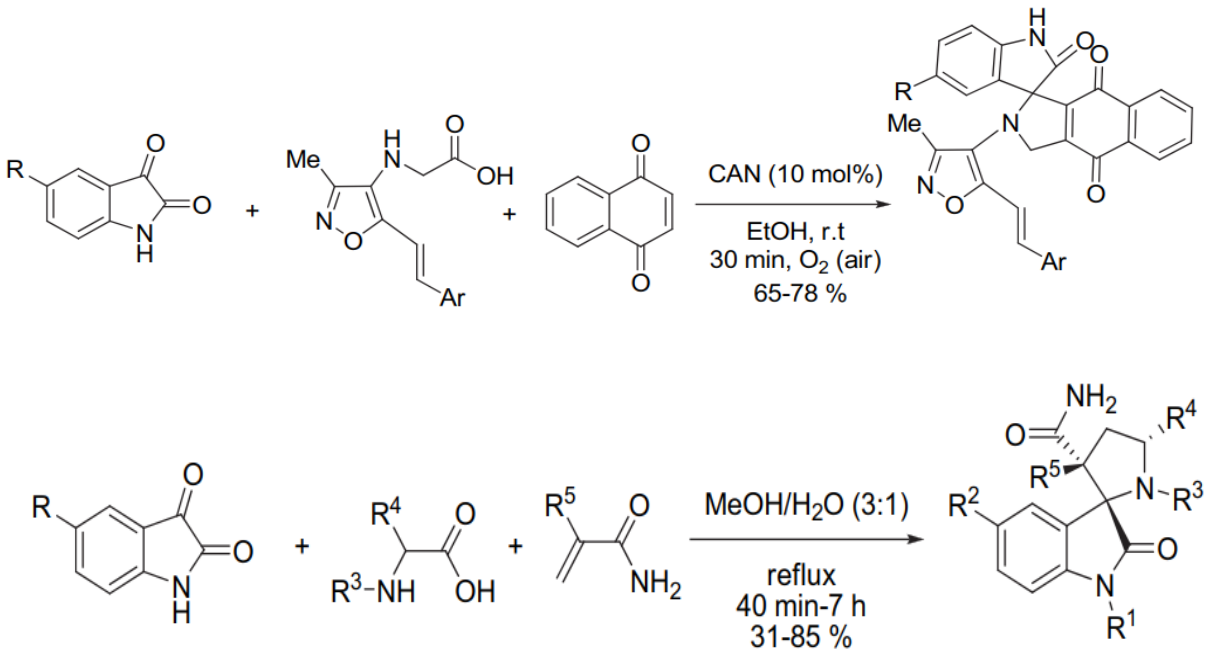
Figure 1.1 Chiral Spirooxindole with a quaternary stereogenic center

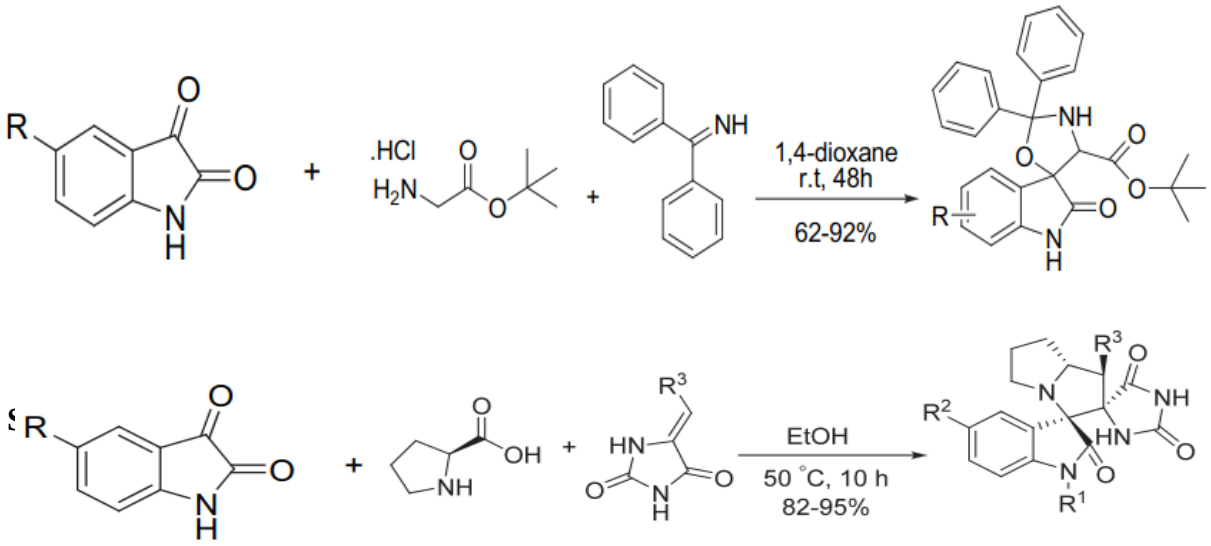
In Vivo Models for Anti-inflammatory Activity: Paw edema model in rats' measures paw volume after carrageenan injection to assess inflammation, while UV-erythema in guinea pigs evaluates erythema post-UV exposure to determine anti-inflammatory effects. Oxazolone-induced ear edema in mice measures ear weight differences following oxazolone application to assess edema, and vascular permeability tests in rats evaluate dye leakage to measure inflammation. The croton-oil ear edema model assesses ear plug weight differences after croton oil application in rats and mice to measure edema. Pleurisy tests measure exudation and leukocyte counts in the pleural space post-carrageenan injection, and the granuloma pouch technique analyzes exudate for leukocyte counts and inflammatory markers in a pouch created under the rat's skin.

Synthesis of spiro compounds

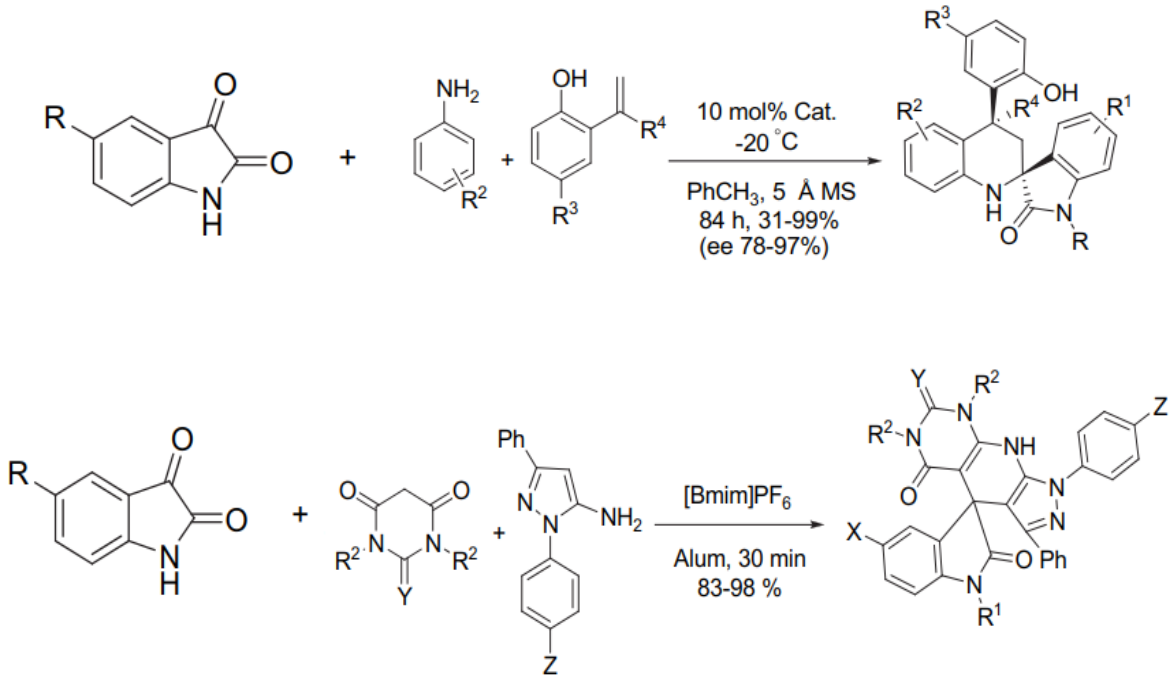
Five-membered spiro-fused compounds: Researchers have explored various methods for synthesizing spirooxindoles. Rajanarendar's group employed a three-component reaction using isoxazole acetic acid, isatins, and 1,4-naphthoquinone to create isoxazoles with anti-inflammatory and analgesic activity. Lipson et al. focused on synthesizing spirooxindolopyrrolidines and spiropyrrolizidines via a 1,3-dipolar cycloaddition of acrylamides and aroylacrylic acids with azomethine ylides.

Meanwhile, Huang et al. developed a catalyst-free, three-component method for synthesizing unnatural amino acids containing a 3-hydrooxindole skeleton. Another approach involved the preparation of dispirooxindoles through a 1,3-dipolar cycloaddition of azomethine ylides with 5-benzylideneimidazolidine-2,4-dione. These diverse synthetic strategies highlight the ongoing interest in developing efficient methods for accessing spirooxindole derivatives with potential therapeutic applications.



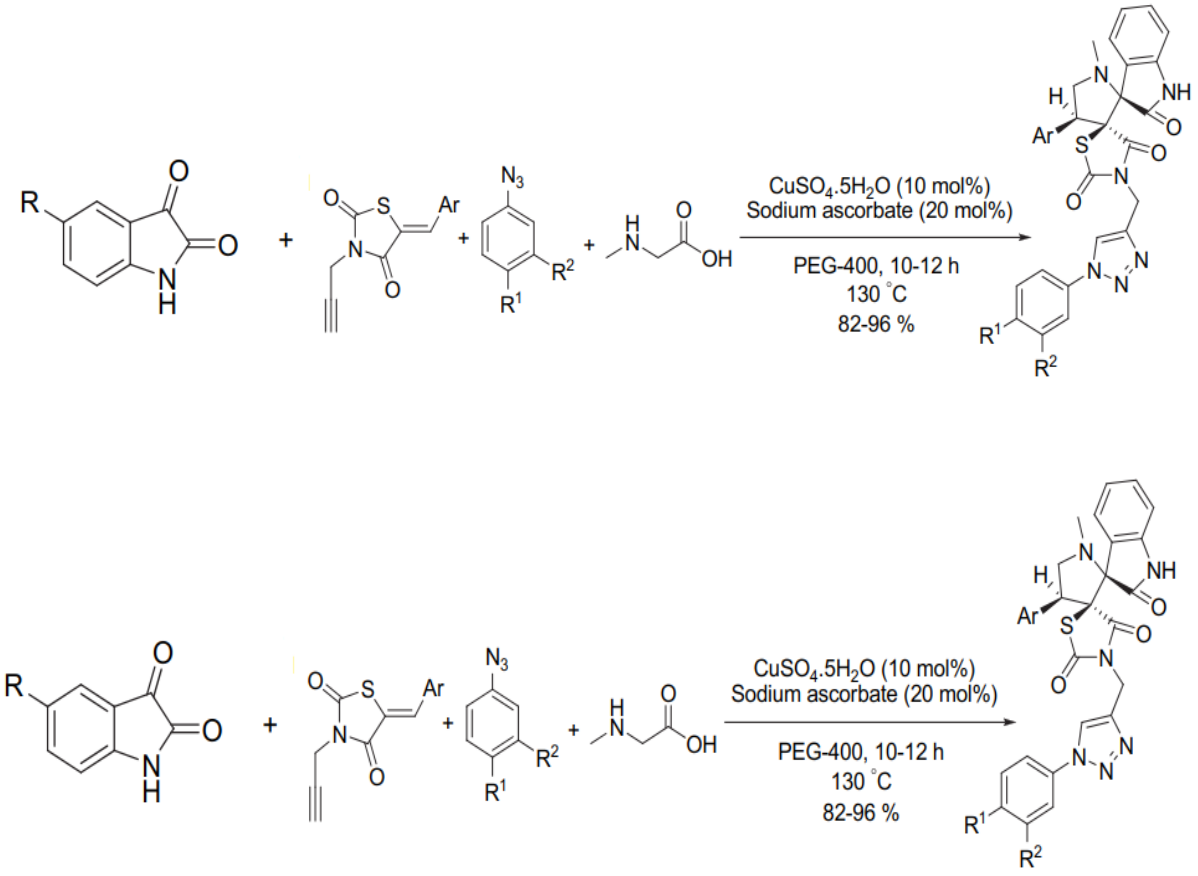


Scientists are exploring diverse methods to synthesize complex spirooxindoles. One approach uses an enantioselective Povarov reaction with isatins, anilines, and α-alkyl o-hydroxystyrenes to create spiro-[indolin-3,2′-quinolines] with excellent stereoselectivity. Another method involves a cyclocondensation reaction of isatins, barbituric acids, and 1,3-diphenyl-1H-pyrazol-5-amines to produce spiro[indoline-pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidine]trione derivatives. Finally, a simple method utilizing isatins, 5-phenyl-1H-pyrazol-3-amine, and 1,3-dicarbonyl compounds in the presence of sulfamic acid provides access to pyrazolopyridine-based spirooxindoles.



Synthesis involving four-component reactions.

Researchers have developed new ways to synthesize complex spirooxindoles. One method uses a four-component reaction with a copper catalyst and a green solvent to create dispiropyrrolidine-linked 1,2,3-triazole derivatives. Another approach employs a four-component reaction with isatins, barbituric acids, phenyl hydrazines, and phenacyl cyanide in a green reaction medium to generate diverse spirooxindole structures.



Microwave-Assisted Synthesis: A Green Approach

Microwave irradiation has revolutionized organic synthesis by providing a rapid and efficient method for carrying out reactions. By applying microwave energy directly to the reaction mixture, it is possible to significantly reduce reaction times, minimize the use of solvents, and enhance energy efficiency, making the synthesis process more sustainable and cost-effective.

2. Scope of the Study

This research aims to synthesize new spirothiazolidinone derivatives and evaluate their potential as antimicrobial agents. The study focuses on these compounds because incorporating a spiro moiety, particularly at the indole-3 carbon atom, is known to enhance biological activity. The research plan involves designing a synthetic scheme, procuring necessary chemicals, synthesizing the compounds, characterizing them using techniques like melting point and NMR, and finally, evaluating their antimicrobial activity in vitro using the albumin denaturation method.

3. Material and Methods

This research uses readily available chemicals and instruments from various sources (Loba Chemie, CDH, Avra, etc.) for the synthesis and analysis of novel spirothiazolidinone compounds. The synthesis will involve standard laboratory equipment like heating mantles, magnetic stirrers, and ovens. Analysis of the synthesized compounds will be done using NMR, FT-IR spectroscopy, and melting point apparatus to determine their properties and purity.

4. Synthesis, Chemical characterization and Anti-inflammatory activity analysis

Preparation of Compounds

3,4-thiazolidinedione (3): Chloroacetic acid and thiourea are reacted under microwave irradiation to form a precipitate, which is then recrystallized to obtain the pure product.

N-substituted thiazolidinedione (4a-e): 2,4-Thiaolidinedione is dissolved in ethanol with potassium hydroxide and reacted with an alkyl halide under microwave irradiation. The resulting product is separated and recrystallized.

Hydrazine carbothioamide derivative (5a-e): 3-alkyl-thiazolidine-3,4-dione is reacted with thiosemicarbazide and glacial acetic acid under microwave irradiation. The product is isolated and recrystallized.

Triazaspiro derivative (6a-e): Compound 5a-e is reacted with acetic anhydride under microwave irradiation. The resulting product is isolated and recrystallized.

Characterization of Compounds

Synthesized compounds are characterized by melting point, solubility, yield, and spectroscopic analysis (NMR, Mass, and IR). Melting points are determined using an electrically heated melting point apparatus, thin layer chromatography is used to assess purity. Solubility is determined in various solvents.

Anti-inflammatory Activity

Inhibition of albumin denaturation: Synthesized molecules are tested for their ability to inhibit albumin denaturation at different concentrations. Absorbance readings are taken to calculate the percentage inhibition.

Antiprotease action method: The antiprotease activity of the compounds is evaluated using trypsin and casein. The percentage inhibition of protease activity is calculated based on absorbance readings.

Percentage inhibition = (Abs control –Abs sample) x 100/ Abs control

5. Results and Discussion

This study aimed to create and evaluate new spirothiazolidinone compounds for their anti-inflammatory effects. The compounds were synthesized in a four-step process, starting with the preparation of thiazolidine-3,4-dione. This was then modified with different alkyl groups and further reacted to form the final spirothiazolidinone compounds (6a-e). Each compound was analyzed to determine its yield, melting point, solubility, and how it moved on a TLC plate, providing information about its properties and purity.

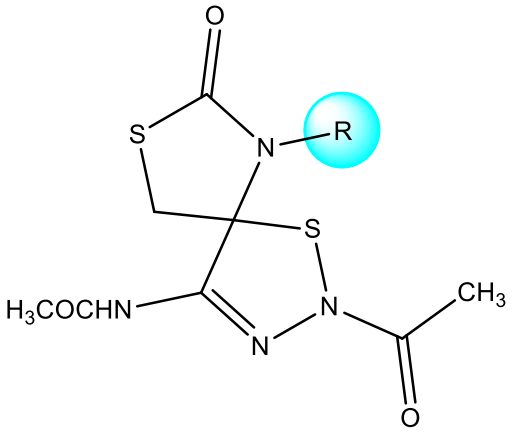


Table 5.1 List of substitution used for preparing 6a-e

Compound	Alkyl Group
4a	H
4b	CH_3
4c	C_2H_5
4d	C_3H_7
4e	C_4H_9

Table 5.2 Physicochemical features of 6a-e

Compound	Color	Yield (%)	Melting Point (°C)
6a	White	71	166-168
6b	White	74	173-175
6c	Pale White	68	155-157
6d	Pale Yellow	65	147-149
6e	Yellow	67	138-140

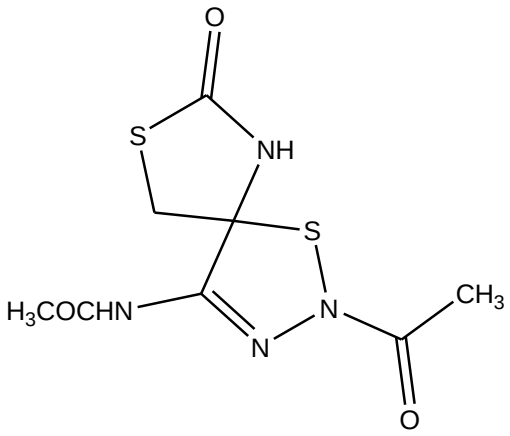
The solubility of the synthesized compounds 6a-e was assessed in water, methanol, chloroform and DMSO. All the compounds were soluble in chloroform and methanol (Table 5.4).

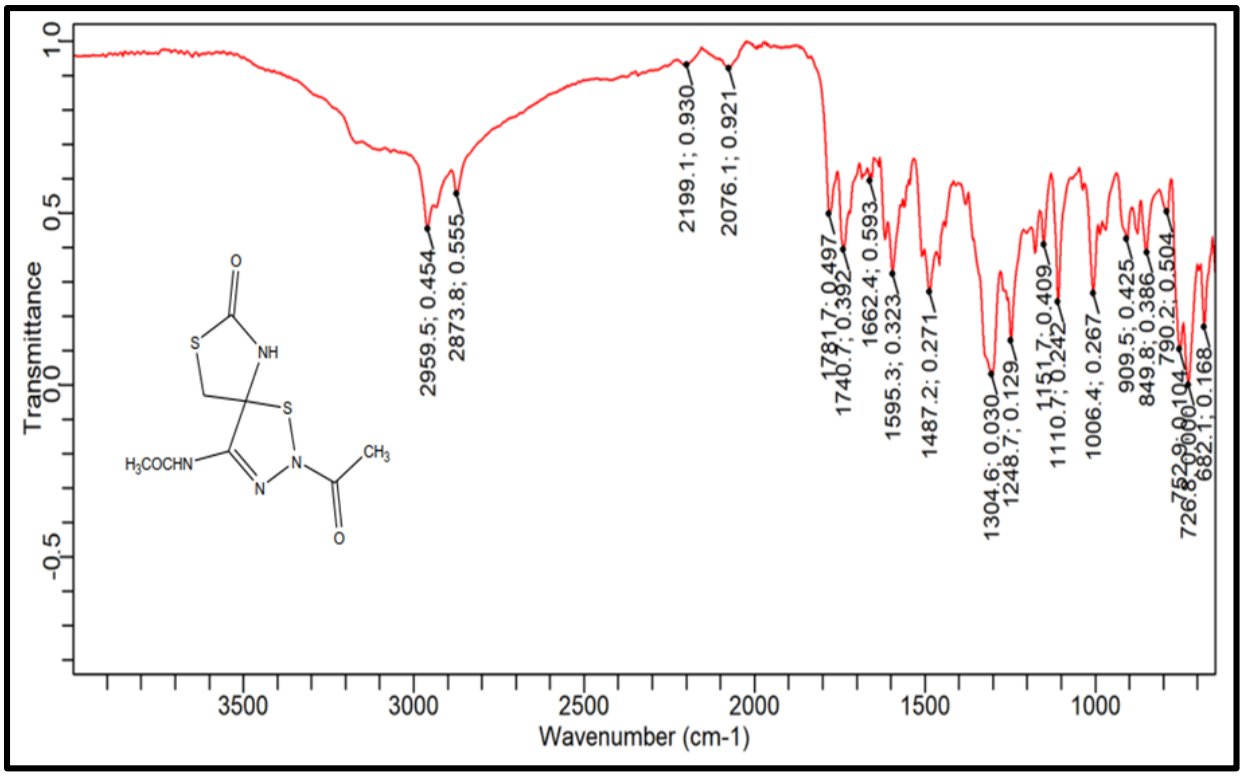
Table 5.3 Solubility of compounds 6a-e

Compound	Water	Methanol	Chloroform	DMSO
6a	Soluble	Soluble	Slightly Soluble	Insoluble
6b	Soluble	Soluble	Slightly Soluble	Insoluble
6c	Soluble	Soluble	Slightly Soluble	Insoluble
6d	Soluble	Soluble	Soluble	Insoluble
6e	Slightly Soluble	Soluble	Soluble	Insoluble

The structure of the compounds was determined using proton NMR in $CDCl_3$ solvent, IR and Mass study.

Spectral features of 6a - IUPAC: N-(2-acetyl-7-oxo-1,8-dithia-2,3,6-triazaspiro[4.4]non-3-en-4-yl)acetamide

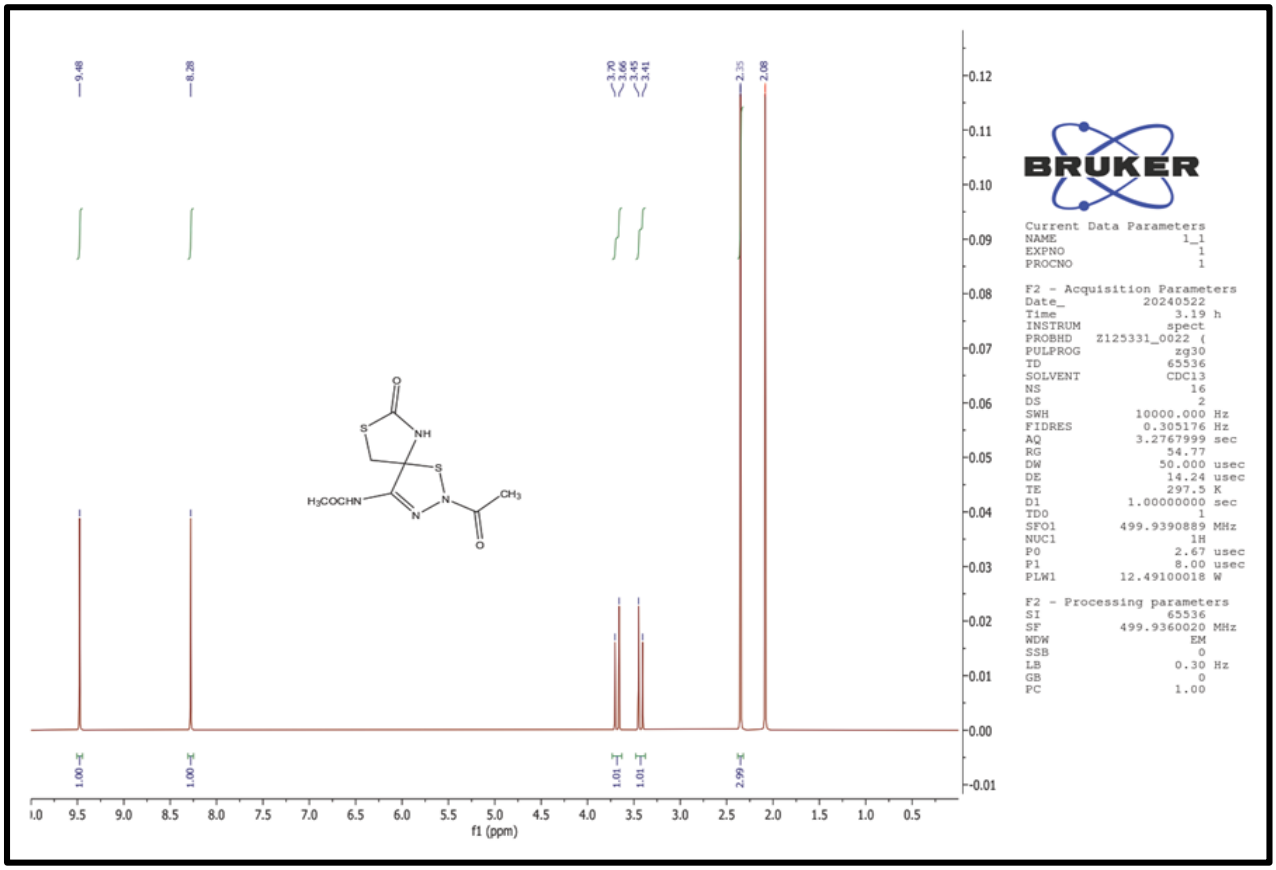




Graph 1 FTIR spectra of 6a

Table 5.4 Interpretation of FTIR spectra of 6a

S. No.	Peak due to	Reference Range (cm^{-1})	Peak obtained at wave number (cm^{-1})
1	C-H stretching	3000-2840	2959.50
2	C=O stretching	1818-1705	1781.66
3	N-H bending	1650-1580	1595.30
4	C-C stretching	1300-800	1248.65
5	C-N bending	1250-1020	1151.74
6	C-S stretching	950-850	909.47

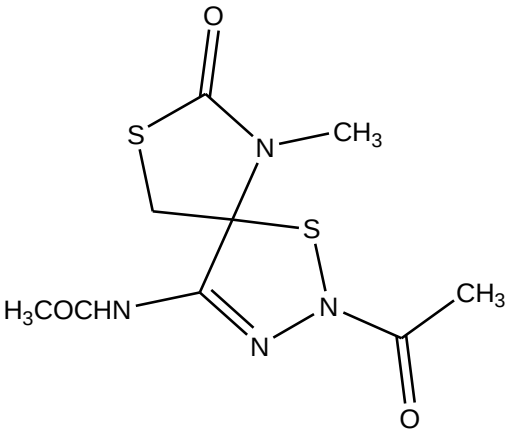


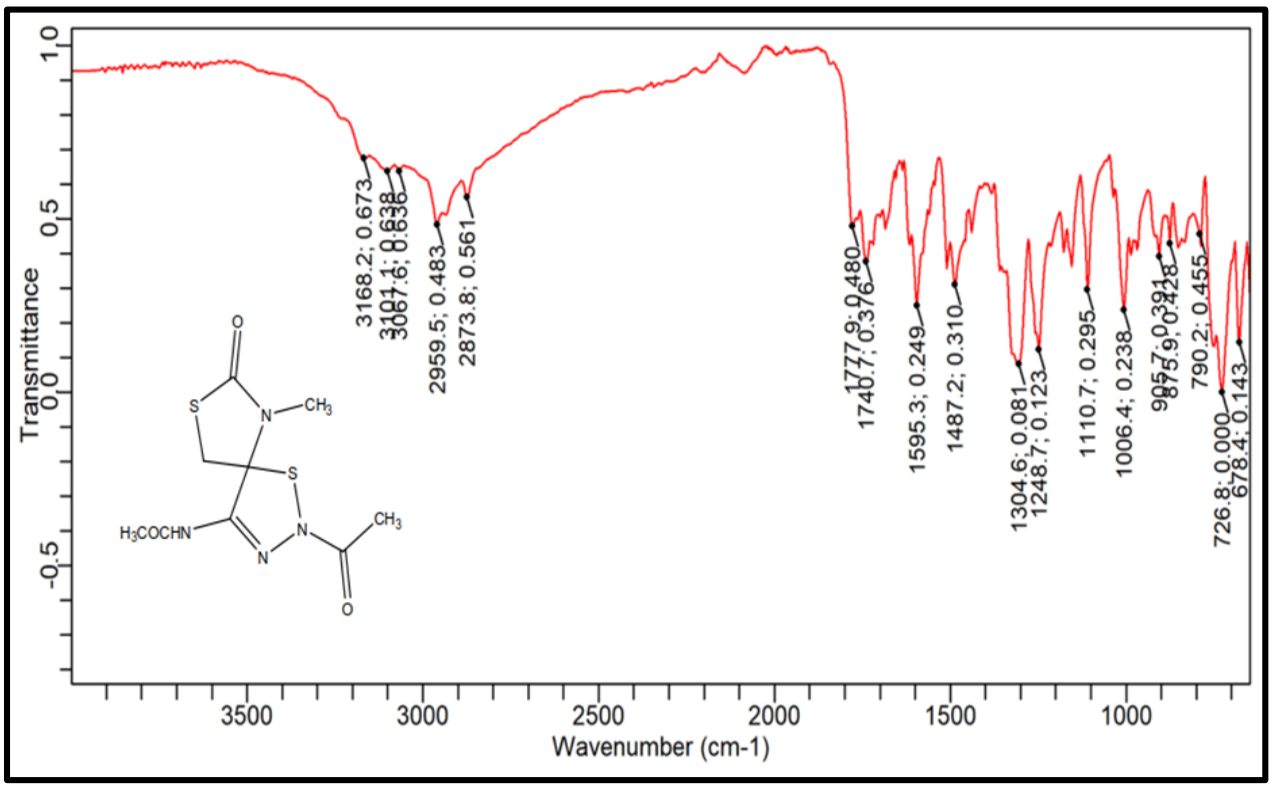
Graph 2 1H NMR spectra of 6a

Table 5.7 Interpretation of 1HNMR spectra of 6a

S. No	Peak at chemical shift (ppm)	Peak due to proton from
1	9.48	NH (thiazolidine)
2	8.28	NH (acetamide)
3	3.68, 34.3	CH_2 (thiazolidine)
4	2.35	CH_3 (acetamide)
5	2.08	CH_3 (acetyl)

Spectral features of 6b IUPAC: N-(2-acetyl-6-methyl-7-oxo-1,8-dithia-2,3,6-triazaspiro[4.4]non-3-en-4-yl)acetamide

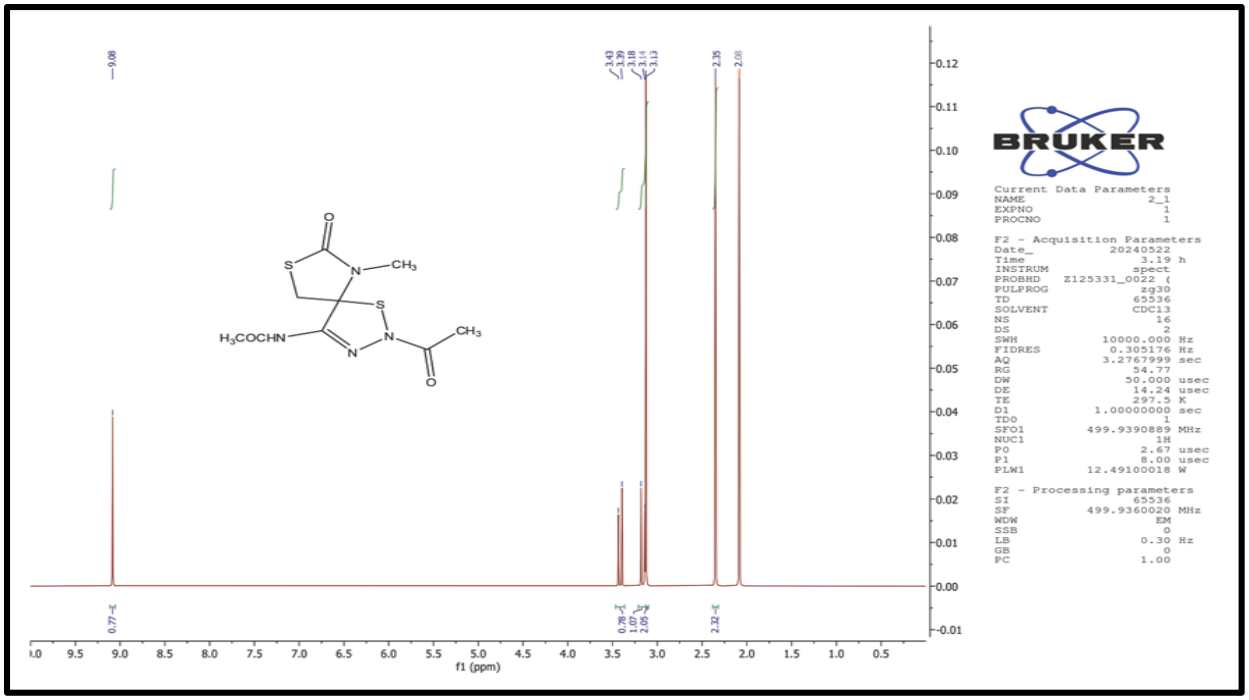




Graph 3 FTIR spectra of 6b

Table 5.8 Interpretation of FTIR spectra of 6b

S. No.	Peak due to	Reference Range (cm^{-1})	Peak obtained at wave number (cm^{-1})
1	C-H stretching	3000-2840	2959.50, 3067.59
2	C=O stretching	1818-1705	1777.94
3	N-H bending	1650-1580	1595.30
4	C-C stretching	1300-800	1248.65
5	C-N bending	1250-1020	1110.74
6	C-S stretching	950-850	905.74

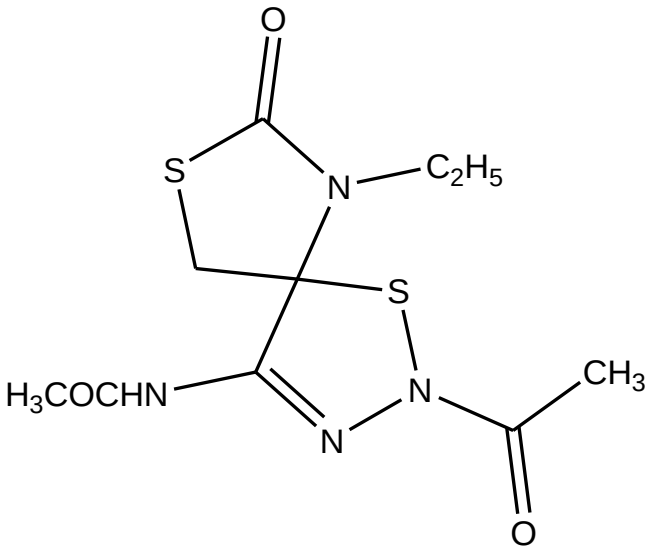


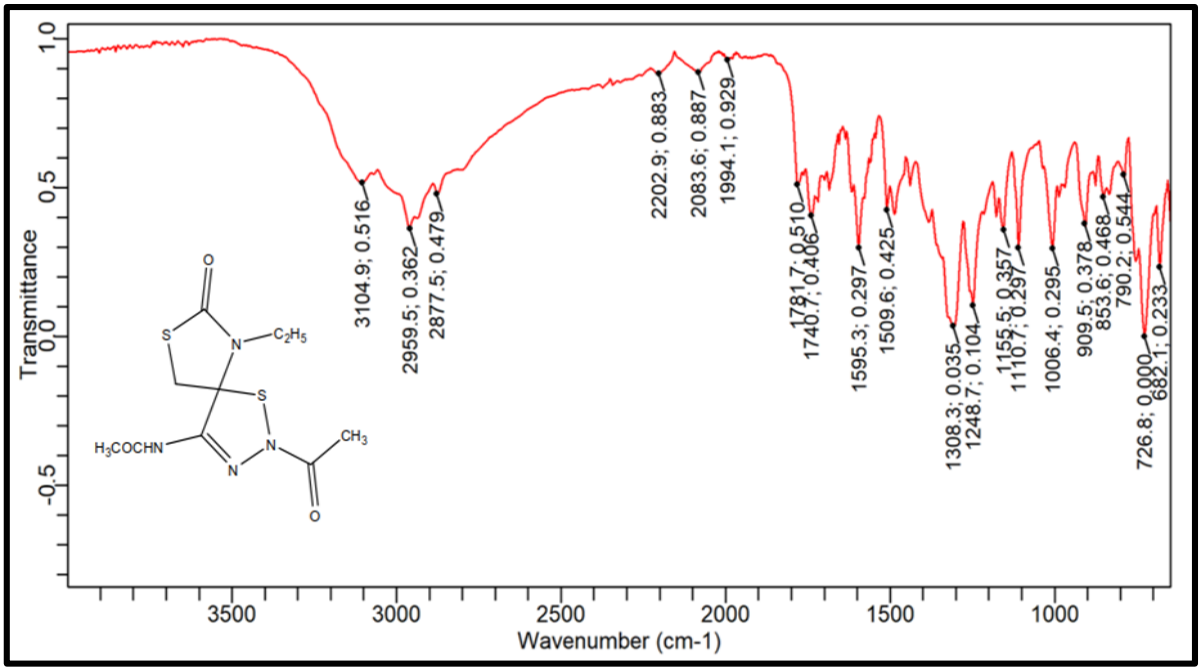
Graph 4 1H NMR spectra of 6b

Table 5.9 Interpretation of 1HNMR spectra of 6b

S. No	Peak at chemical shift (ppm)	Peak due to proton from
1	9.08	NH (thiazolidine)
2	3.41, 3.16	CH_2 (thiazolidine)
3	3.13	CH_3 (methyl)
4	2.35	CH_3 (acetamide)
5	2.08	CH_3 (acetyl)

Spectral features of 6c – IUPAC : N-(2-acetyl-6-ethyl-7-oxo-1,8-dithia-2,3,6-triazaspiro[4.4]non-3-en-4-yl)acetamide

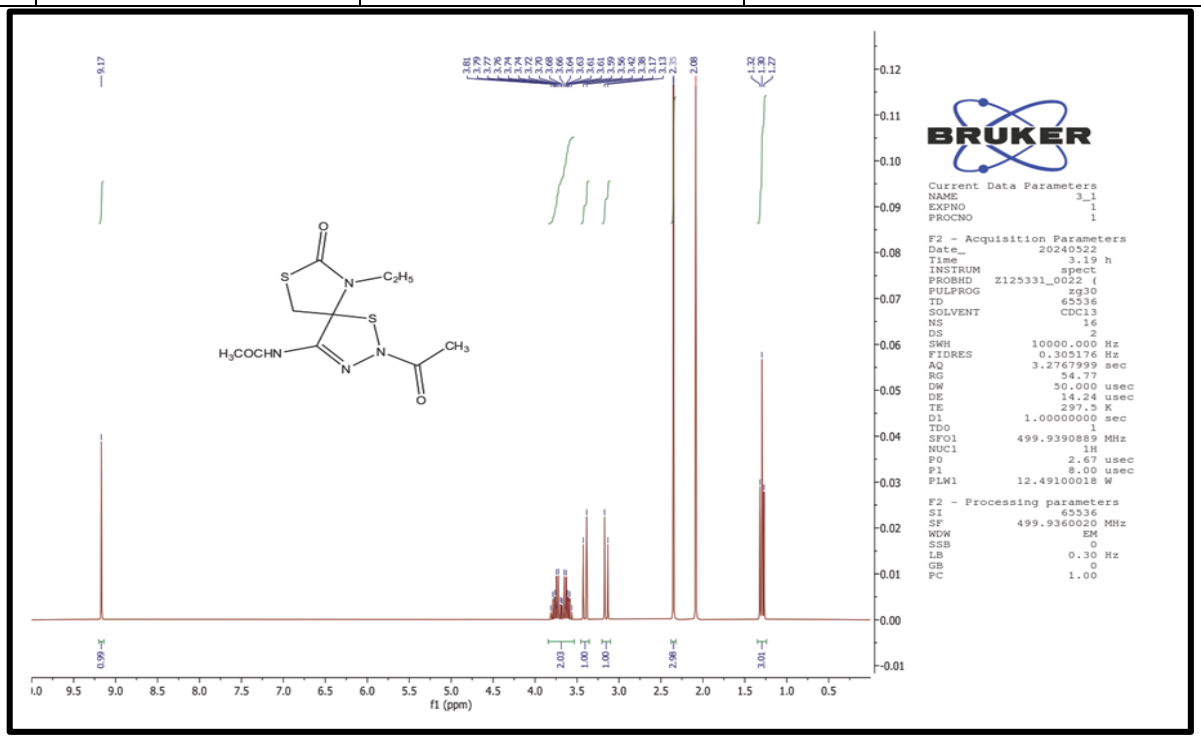




Graph 5 FTIR spectra of 6c

Table 5.10 Interpretation of FTIR spectra of 6c

S. No.	Peak due to	Reference Range (cm^{-1})	Peak obtained at wave number (cm^{-1})
1	C-H stretching	3000-2840	2959.50
2	C=O stretching	1818-1705	1781.66
3	N-H bending	1650-1580	1595.30
4	C-C stretching	1300-800	1248.65
5	C-N bending	1250-1020	1110.74
6	C-S stretching	950-850	909.47

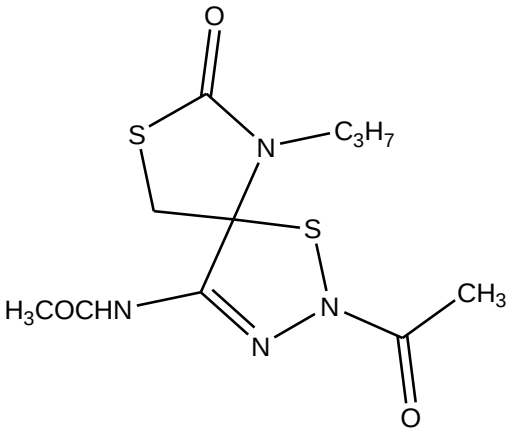


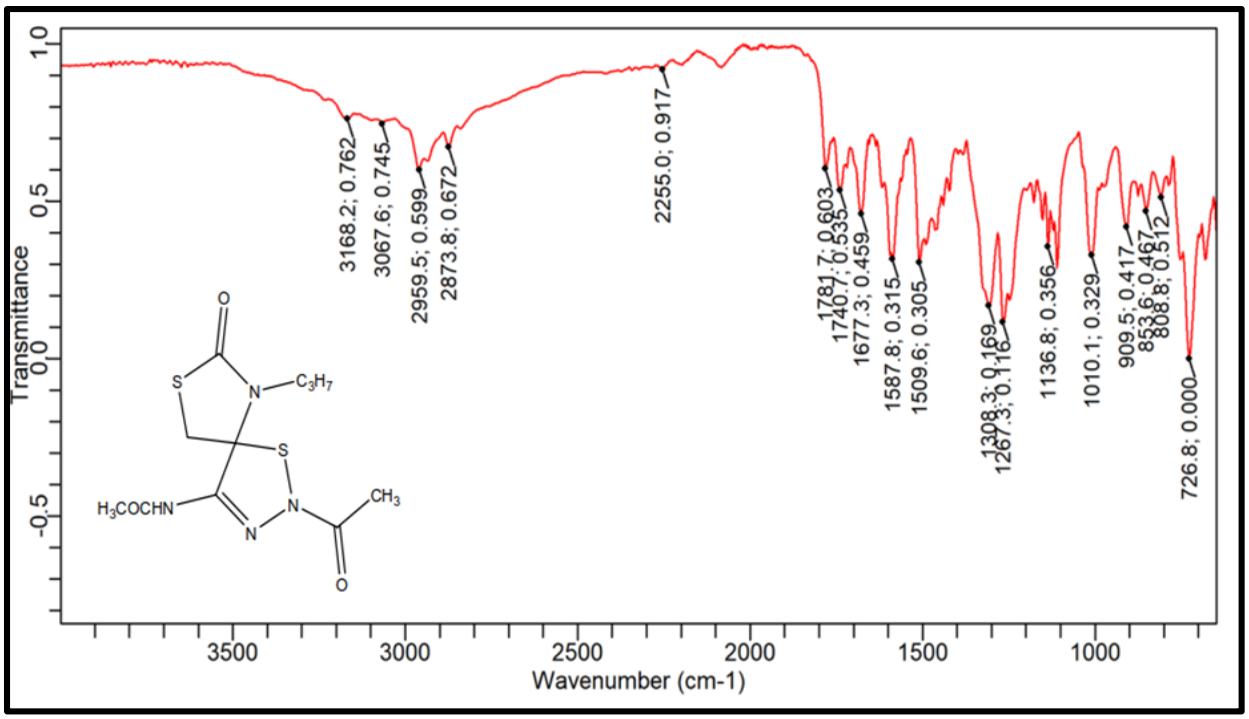
Graph 6 1H NMR spectra of 6c

Table 5.11 Interpretation of 1HNMR spectra of 6c

S. No	Peak at chemical shift (ppm)	Peak due to proton from
1	9.17	NH (thiazolidine)
2	3.40, 3.15	CH_2 (thiazolidine)
3	3.62, 3.75	CH_2 (ethyl)
4	2.35	CH_3 (acetamide)
5	2.08	CH_3 (acetyl)
6	1.30	CH_3 (ethyl)

Spectral features of 6d - IUPAC: N-(2-acetyl-7-oxo-6-propyl-1,8-dithia-2,3,6-triazaspiro[4.4]non-3-en-4-yl)acetamide

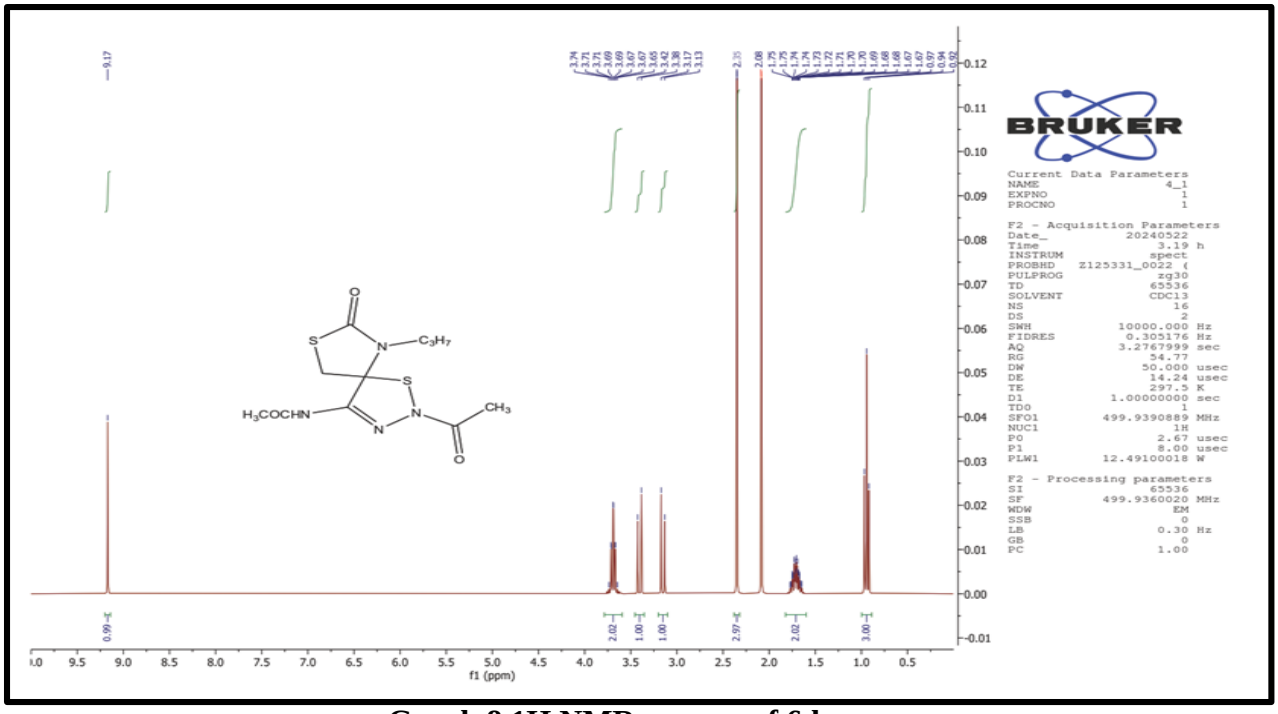




Graph 7 FTIR spectra of 6d

Table 5.12 Interpretation of FTIR spectra of 6d

S. No.	Peak due to	Reference Range (cm^{-1})	Peak obtained at wave number (cm^{-1})
1	C-H stretching	3000-2840	2959.50
2	C=O stretching	1818-1705	1781.66
3	N-H bending	1650-1580	1587.84
4	C-C stretching	1300-800	1267.29
5	C-N bending	1250-1020	1010.10
6	C-S stretching	950-850	909.47

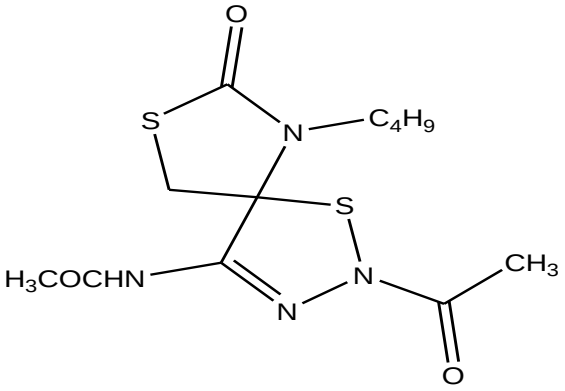


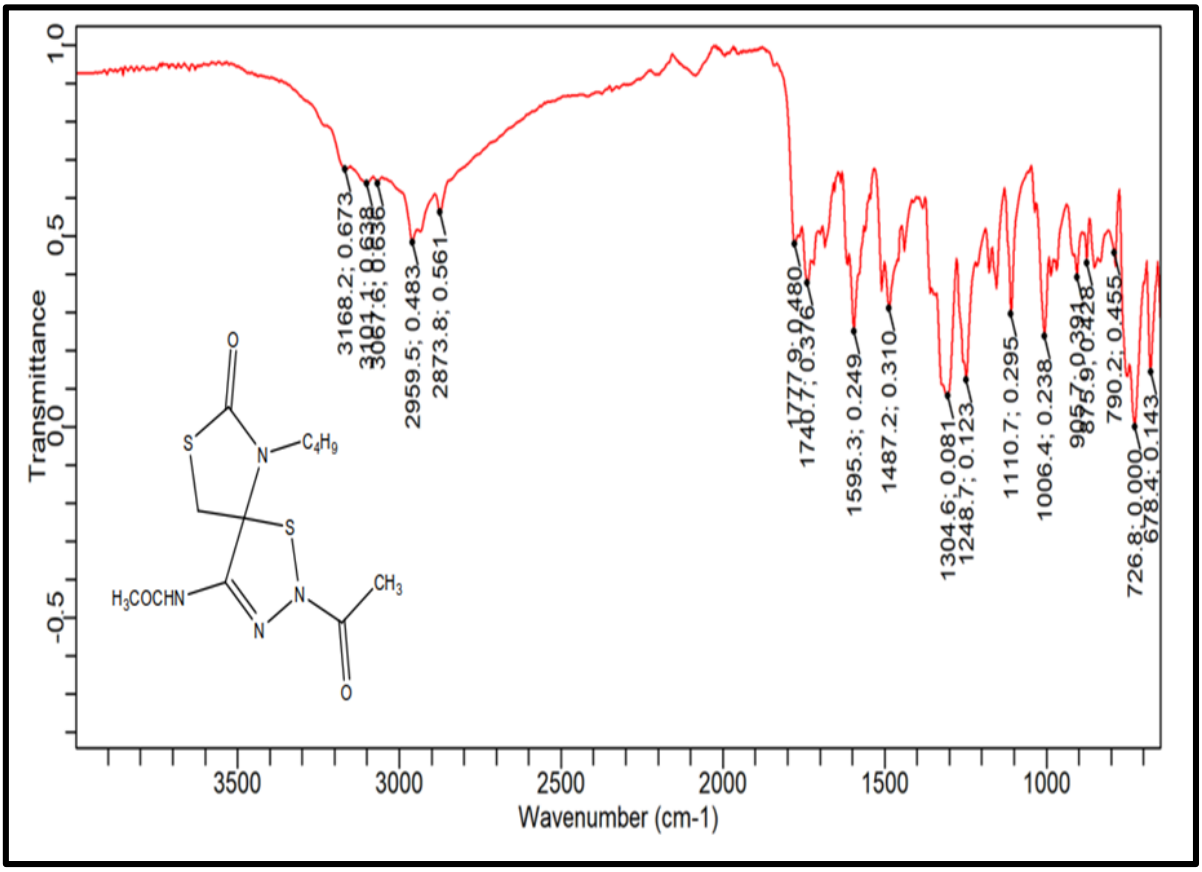
Graph 8 1H NMR spectra of 6d

Table 5.13 Interpretation of 1HNMR spectra of 6d

S. No	Peak at chemical shift (ppm)	Peak due to proton from
1	9.17	NH (thiazolidine)
2	3.40, 3.15	CH_2 (thiazolidine)
3	3.70, 3.68	CH_2 (propyl)
4	2.35	CH_3 (acetamide)
5	2.08	CH_3 (acetyl)
6	1.73, 1.69	CH_2 (propyl)
7	0.94	CH_3 (propyl)

Spectral features of 6e IUPAC: N-(2-acetyl-6-butyl-7-oxo-1,8-dithia-2,3,6-triazaspiro[4.4]non-3-en-4-yl)acetamide

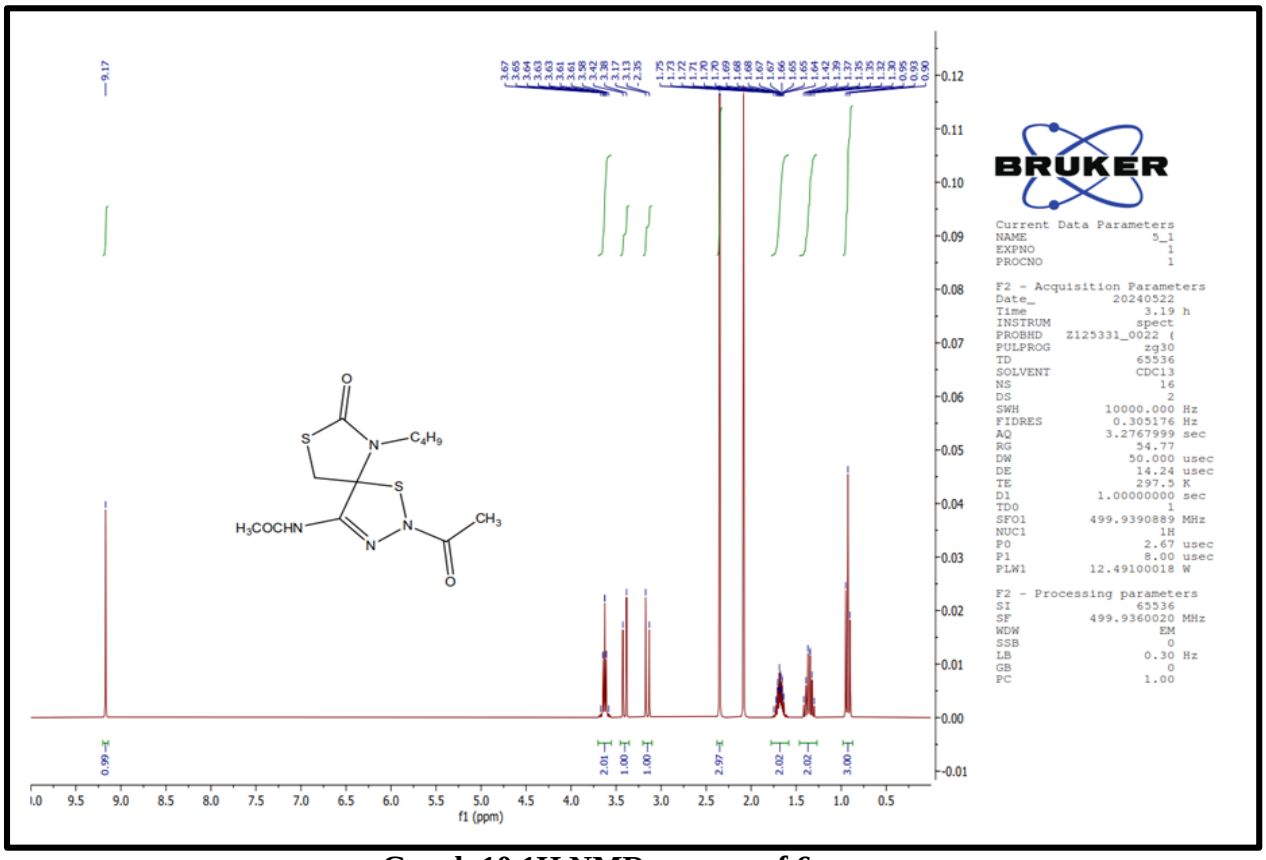




Graph 9 FTIR spectra of 6e

Table 5.14 Interpretation of FTIR spectra of 6e

S. No.	Peak due to	Reference Range (cm^{-1})	Peak obtained at wave number (cm^{-1})
1	C-H stretching	3000-2840	2959.50
2	C=O stretching	1818-1705	1777.94
3	N-H bending	1650-1580	1595.30
4	C-C stretching	1300-800	1248.65
5	C-N bending	1250-1020	1110.74
6	C-S stretching	950-850	905.74



Graph 10 1H NMR spectra of 6e

Table 5.15 Interpretation of 1HNMR spectra of 6e

S. No	Peak at chemical shift (ppm)	Peak due to proton from
1	9.17	NH (thiazolidine)
2	3.63	CH_2 (butyl)
3	3.40, 3.15	CH_2 (thiazolidine)
4	2.35	CH_3 (acetamide)
5	2.08	CH_3 (acetyl)
6	1.70, 1.66	CH_2 (butyl)
7	0.93	CH_3 (butyl)

5.1.2 Anti-inflammatory potential

The anti-inflammatory action of the synthesized compounds was evaluated using two of the well-established in vitro methods viz., protease inhibition activity and inhibition of albumin denaturation. The results are presented in table 5.14 and 5.15 respectively.

Table 5.16 Inhibition of albumin denaturation by test compounds

Treatment	Inhibition of albumin denaturation (%)					
	100 µg/mL	**200 µg/mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**	**10 µg/mL**
6a	6.1±0.391	11.9±1.246	21.7±1.044	29.8±2.217	39.3±2.759	ND
6b	5.26±1.163	10.25±1.196	17.88±1.695	22.24±2.068	29.66±3.162	ND
6c	9.70±1.169	18.68±2.016	32.04±2.032	42.84±2.116	51.96±0.066	ND
6d	13.86±2.105	23.57±2.004	33.28±3.036	52.04±3.101	62.47±1.033	ND
6e	4.22±2.002	10.64±2.139	19.44±2.058	27.54±2.189	37.91±2.534	ND
Ibuprofen	ND	ND	ND	ND	ND	52.38±2.516

ND-Not Determined

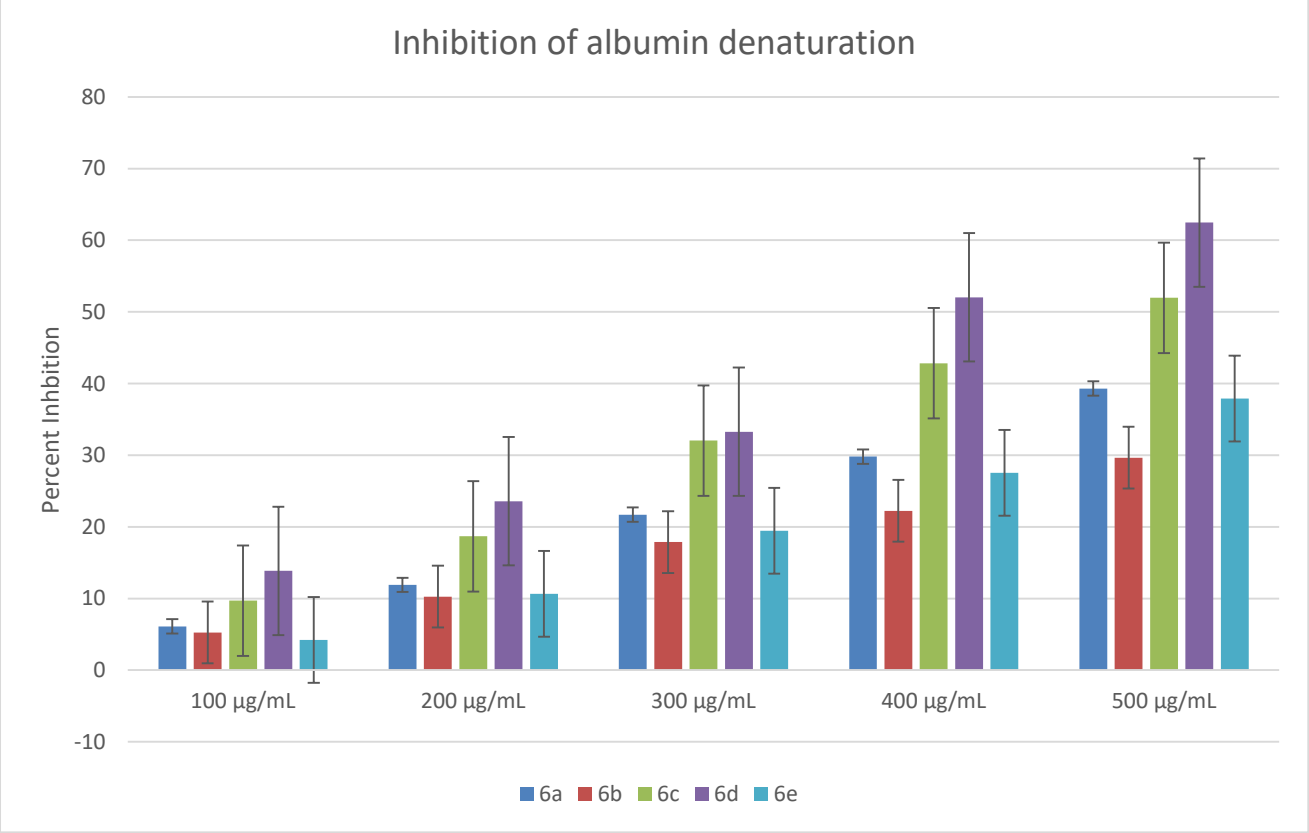


Figure 5.1 Inhibition of albumin denaturation

Table 5.15 Percent inhibition of protease action by test compounds

Treatment	Inhibition of Protease Action (%)					
	10 µg/mL	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**
Ibuprofen	52.26±1.066	ND	ND	ND	ND	ND
6a	ND	5.08±0.066	8.33±0.033	11.90±1.033	20.12±1.033	23.86±3.011
6b	ND	4.64±0.613	7.96±0.869	11.12±1.135	17.57±1.135	21.06±1.139
6c	ND	7.26±1.039	14.44±0.911	19.36±2.136	23.19±1.299	33.04±3.113
6d	ND	9.49±1.066	16.05±1.038	21.84±2.111	33.40±2.036	47.43±3.011
6e	ND	3.56±0.925	7.32±0.663	10.15±0.966	16.39±2.033	20.27±2.123

ND-Not Determined

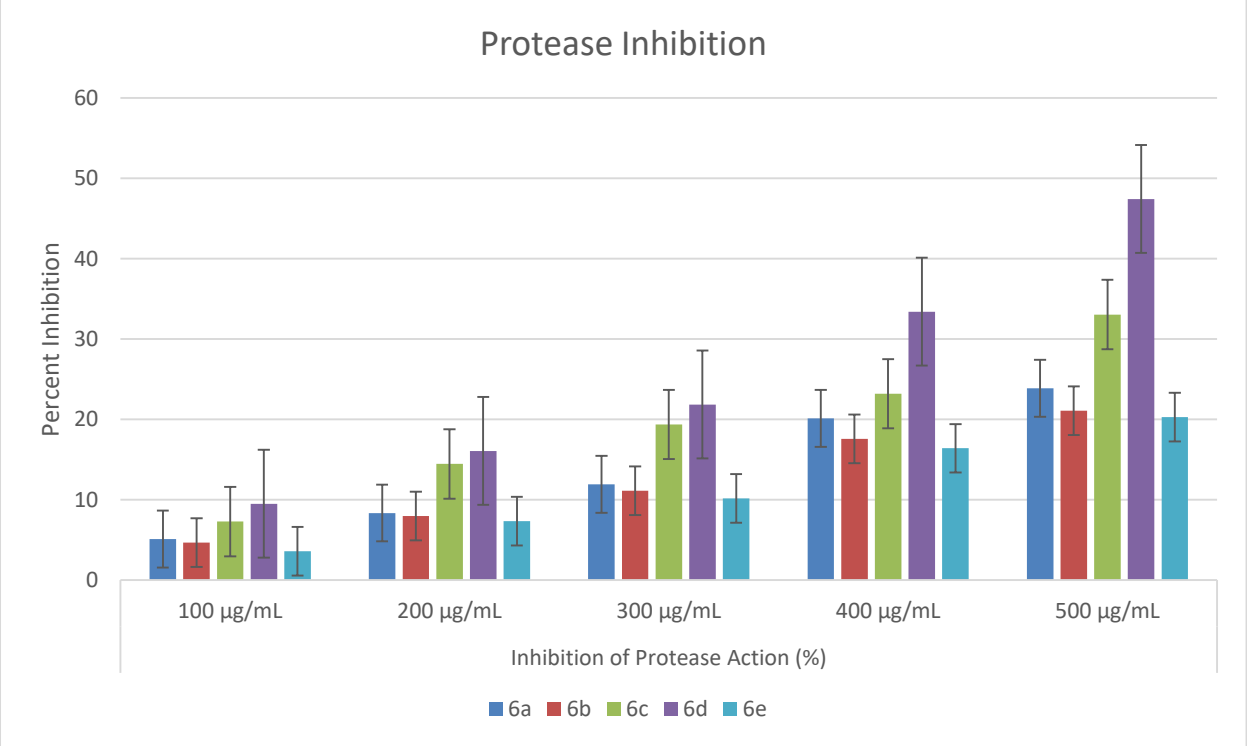


Figure 5.2 Inhibition of protease action

6. Summary and Conclusion

This study focused on creating new spirothiazolidinone compounds and testing their ability to reduce inflammation. They made these compounds by using microwaves to speed up the reaction between existing chemicals. They confirmed the structure of these new compounds by using techniques like IR and NMR spectroscopy, which help identify the different chemical bonds and atoms present.

To see if the compounds could reduce inflammation, they used two tests. The first test looked at how well the compounds prevented a protein (albumin) from changing its structure (denaturation), which is linked to inflammation. The second test examined how well the compounds blocked the activity of protease, an enzyme that can cause tissue damage during inflammation.

The results showed that the compounds were effective at reducing inflammation in both tests, and their effectiveness varied depending on the length of the alkyl chain in their structure. A compound with a three-carbon alkyl chain (6d) was the most effective, while a compound with a four-carbon chain (6e) was the least effective. This suggests that the length of the alkyl chain plays a role in how these compounds work to reduce inflammation. The order of activity was 6d>6c>6a>6b>6e.

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