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Article

Synthesis, Characterization and Preliminary Anti-Fungal study of Some New Thio-pyrimidine Derivatives of CIotrimazole

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Abstract

The diverse biological activities of chalcone derivatives with N-heterocyclic frameworks are significant in medicinal chemistry. These N-heterocyclic compounds encourage further research and analysis of molecules featuring the α,β-unsaturated carbonyl group along with an N-heterocyclic structure to achieve more targeted biological effects. These compounds exhibit a range of activities, including antibacterial, antifungal, antioxidant, anti-inflammatory, antitubercular, anticancer, antimalarial, and anti-leishmanial properties, found in both natural and synthetic forms. Chalcones have demonstrated various beneficial functions, such as chemo protective and antimicrobial effects, including antifungal activity, as well as antispasmodic effects. The antifungal properties of chalcones have been extensively studied and investigated. In this study, the chemistry and biological activity of the cyclized form of chalcones (Thio-Pyrimidine derivatives) is evaluated using preliminary techniques.

Keywords: Friedel Crafts reaction, Claisen-Schmidt condensation, Chalcones, Flavanones, Fungal infections.

1- Introduction

At present, nearly one-fourth of herbal medicines utilized in conventional medical practice contain active components derived from herbivorous sources. Importantly, the α,β-unsaturated ketone structure serves as the primary pharmacophore; any alteration to this structure leads to a reduction in biological activity. Chalcones are highly valued in medicinal chemistry for their structural variety, which plays a crucial role in drug development.[1] Chalcones **(Figure 1)** are fascinating compounds sourced from vibrant green parts of plants and botanicals, showcasing nature's wonderful heartwood, leaves, bark, fruits, and roots. [2] Such discoveries have led to the introduction of a significant number of synthetic medicines. Given that this entity remains unchanged and unexplored, biologically active chalcone-like molecules have been synthesized by incorporating alpha-substitution. Chalcone, a secondary metabolite compound, has attracted researchers' interest due to its therapeutic potential, demonstrating antifungal [3], antioxidant [4] [5] [6], antitumor [7], antimalarial [8], anticancer [9] [10] [11] [12], anti-inflammatory [13] [14] [15], and antidepressant properties. [16]

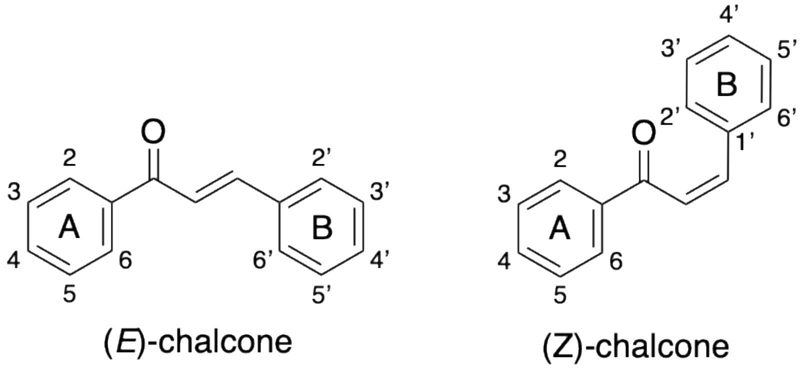


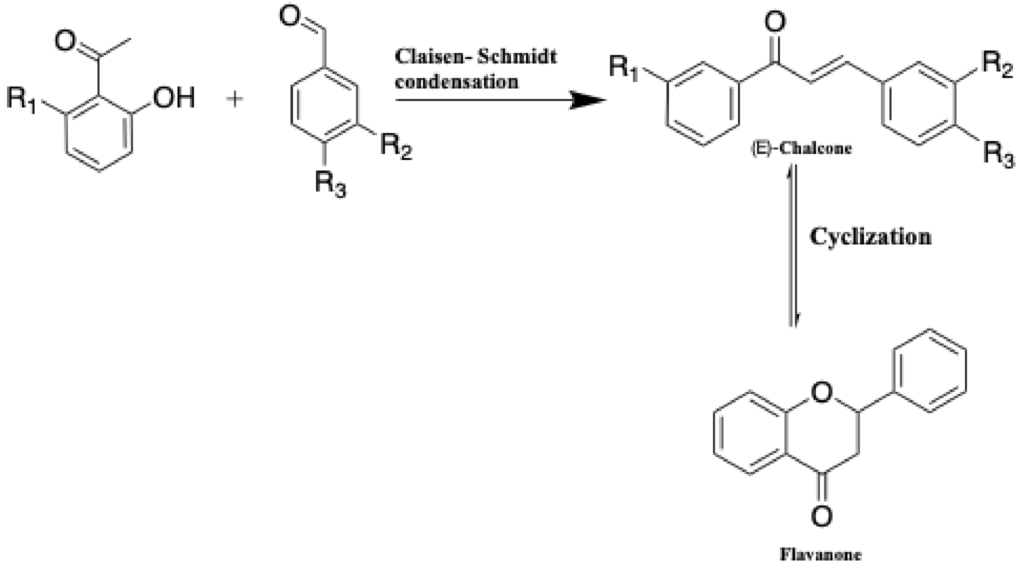
Figure 1: General structure of chalcone

Chalcones or chalconoids (1,3 diaryl-2-propen-1-one), are chemical blends usually discovered to be abundant naturally and commonly synthesised chemically. These compounds comprise aromatic residues bound by an α,β-unsaturated sequence of an electrophilic chain constructed of carbon atoms, usually three atoms. [17] Chalcones are not only exclusive to the unsaturated form; they might also be saturated, namely, dihydrochalcones, which differ in the absence of the conjugated double bond; instead of the alkenone, we have an alkane group. Also, when considering the natural form of chalcone, a phenolic hydroxyl functional group is found almost everywhere in these sources. Prenyl and Geranyl groups are also seen to a lesser extent. [18] They are coloured compounds that usually exhibit a yellow colour, resulting from the presence of a chromophore as part of the keto ethylenic group (CO-CH=CH). [19] Chalcones are a prominent category of flavonoids in the plant kingdom. Examples of herbivorous possessing chalcone components are Glycyrrhiza, Piper, Angelica, and Ruscus genus. [20]

2- A study in the steps of nature

- A collection of imidazole-chalcone hybrids generated from Clotrimazole seeks to exploit the potential antifungal properties exhibited by compounds featuring both chalcone and imidazole pharmacophores.
- The resultant hybrid compounds were meticulously assessed for their antifungal efficacy against Candida albicans, specifically evaluating fungistatic and fungicidal activities by recognized testing techniques. These discoveries may facilitate the development of improved antifungal therapies with broader therapeutic applicability.

Flavonoids are defined as a group of low molecular weight polyphenolic molecules. Chemically, the basic structure of flavenoids is composed of a C6-C3-C6 skeleton, and they commonly have a cyclic system with oxygen. [21] Flavenoids have low water solubility, so they are inactive orally and impractical for therapeutic use. [22] To distinguish between chalcones and flavenoids, it is helpful to remember that chalcones lack a C ring in their chemical skeleton; this is why they are called open-ring flavonoids [23] depicted in **(Scheme 1)**



Scheme 1: The chemical relationship between Chalcone and Flavanone

Friedel-Crafts acylation of aromatic compounds is a highly significant and practical method for preparing aromatic ketones. This electrophilic procedure entails the acylation of aromatic molecules using acid chlorides or anhydrides, typically facilitated by Lewis acids such as sulfuric acid ($H_2 SO_4$).Friedel-Crafts acylation is commonly applied to both aromatic and heterocyclic compounds, making it a widespread reaction in the production of aromatic ketones. These ketones are essential intermediates in the synthesis of various pharmaceuticals, including naproxen, dextromethorphan, and ibuprofen.[24]

The Claisen-Schmidt reaction is a classic method for synthesizing chalcones by condensing a ketone with an aldehyde using aqueous alkaline bases or alcoholic alkali. Hydrochloric acid or alkali can also act as condensing agents at concentrations of 30%, 40%, 50%, or 70%. Chalcones enable modifications of aryl rings, facilitating the creation of heteroaryl frameworks and enhancing anticancer properties [25]. In enolates formed from unsaturated ketones, electrophilic attacks can occur at three sites, with the -carbon being the preferred site for protonation and alkylation.Chalcones are essential in organic synthesis for forming enantioselective Michael adducts. The first notable enantioselective Michael addition was reported in 1978, where Wynberg and colleagues used a specific catalyst to achieve an enantiomeric excess of 23% in the addition of nitromethane to chalcone. This study paved the way for further advancements in enantioselective reactions.[26]

Thio-pyrimidine synthesis is a reversible process that converts chalcones into flavones. This transformation is illustrated in the above scheme **(Scheme 1)**. During this chemical process, we observe the relationship between unsaturated ketones and thio-pyrimidine derivatives. These chemically derived compounds have shown to be more biologically effective than the parent molecules.

Methodolgy

1- Analytical Methods and Devices:

Thin-layer chromatography was employed for chalcone derivatives.[27] Department of Chemistry, College of Pharmacy, University of Basrah. The melting temperatures of the synthesized Chalcone derivatives and complexes were determined using the SMP3 apparatus located in the Department of Chemistry, College of Science, University of Basrah. UV-visible absorption spectra are obtained using a T90+ UV-visible spectrometer. PG Instruments Ltd employs traditional quartz cells featuring an optical path length of 1 cm inside the Department of Chemistry at the College of Science, University of Thi-Qar.FT-IR spectra were obtained using a SHIMADZU/FT-IR Affinity-1 spectrophotometer and KBr or CsI discs in the Department of Chemistry at the College of Education, University of Basrah.1H-NMR (proton-nuclear magnetic spectra) and 13C-NMR (carbon nuclear magnetic resonance) spectra were acquired via a Bruker 400 MHz Avance III equipment for the 1H-NMR spectra at a frequency of 400 MHz. The 13C-NMR spectra were acquired at 125 MHz utilizing a Bruker instrument. Deuterated dimethyl sulfoxide (DMSO-D6) served as the solvent, while tetramethylsilane (TMS) was the internal standard for reference chemical shifts. University of Basrah, College of Education for Pure Sciences, Department of Chemistry. Mass spectra were obtained utilizing a 5973 Network Mass Selective Detector produced by Agilent Technology (HP), equipped with a 70 eV Electron Impact (EI) ion source, in the Department of Chemistry at the University of Tehran, Iran. The thermal analysis was conducted using the SDT Q600 V20 in the Chemistry Department at the College of Science, University of Basra. Thermogravimetric analysis of metal complexes was conducted using a TGA-50 in the Chemistry Department at the College of Science, University of Basra.

2- Evaluation of the Antifungal Activity of Chalcone Derivatives

The compounds C1-C5 and their corresponding cyclized analogs Cy1-Cy2, along with the medication Clotrimazole, were utilized, and the cultivation of the strain was preserved at −70 °C. The specimen was thawed and streaked over Sabouraud Dextrose (4%) (Agar Biolab), which contains chloramphenicol, as prepared by a specialist in the biology department. The chloramphenicol specifically isolated our strain, incubating 24 hours at 37°C. The susceptibility to Clotrimazole was assessed using the microdilution technique. A Clotrimazole stock solution of 1600 mg/L in DMSO was utilized to create a series of 2-fold ethanol dilutions with concentrations varying from 2 mg/L to 64 mg/L. The prepared solutions were distributed in a volume of 50 μL into the designated wells of 96-well microplates. A control group with three replicates for each group evaluated the impact of the chemical substances by the Microdilution spectroscopic assay utilizing a Microplate Reader (Genex MR-100).

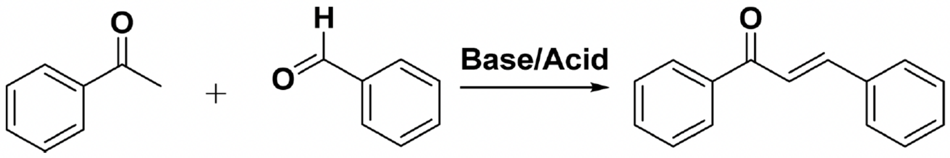
3- Statistical analysis

To assess the variance in the activities of Clotrimazole-Chalcone derivatives against fungi, a statistical significance threshold of $P \leq 0.05$ was employed using SPSS.

Chemical Preparation of Chalcone

1- Claisen-Schmidt’s condensation:

Diverse reaction methodologies and tactics can readily synthesize chalcones. The Claisen-Schmidt condensation is a widely employed method that entails the condensation of carbonyl derivatives in the presence of a base.[28] This procedure often involves the catalyzed reaction of benzaldehyde with an alkyl ketone, as illustrated in **Schemes 2**. A base catalyst is predominantly utilized for the synthesis of chalcones. This study presents a list of chalcone derivatives in **Tables 1 and 2.**



Scheme 2: Claisen−Schmidt Condensation Reaction for Chalcone synthesis

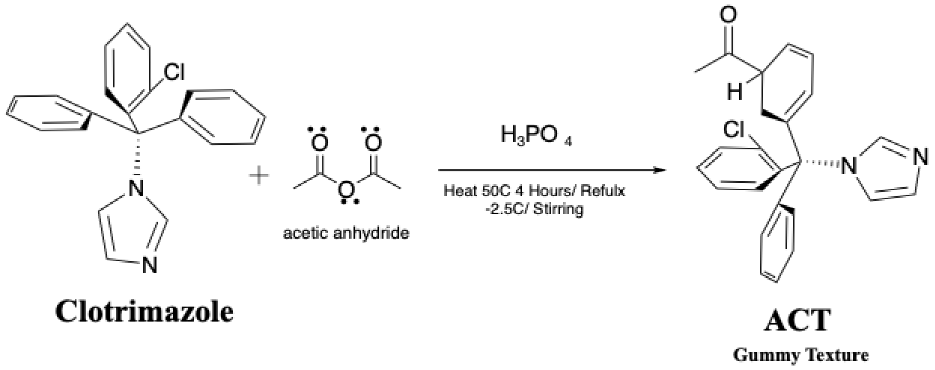
2- **Carbonylative The Heck coupling reaction** entails the vinylation of phenyl halides with styrene using a palladium catalyst under carbon monoxide conditions, leading to the synthesis of chalcone. [29].

3- **Reaction with 2-aminothiophenol:** A derivative of benzothiazepine was synthesised through the reaction of chalcone, specifically (E)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one, with 2-amino thiophenol under acidic conditions. [30].

4- **Solid acid catalyst-mediated reaction:** The addition of benzaldehyde and phenylacetylene in 1,2-dichloroethane under a microwave condition and using ion-exchange resin-like amberlyst-15, the solid acid catalyst gave chalcone [31].

Synthesis of Acylated Clotrimazole (ACT):

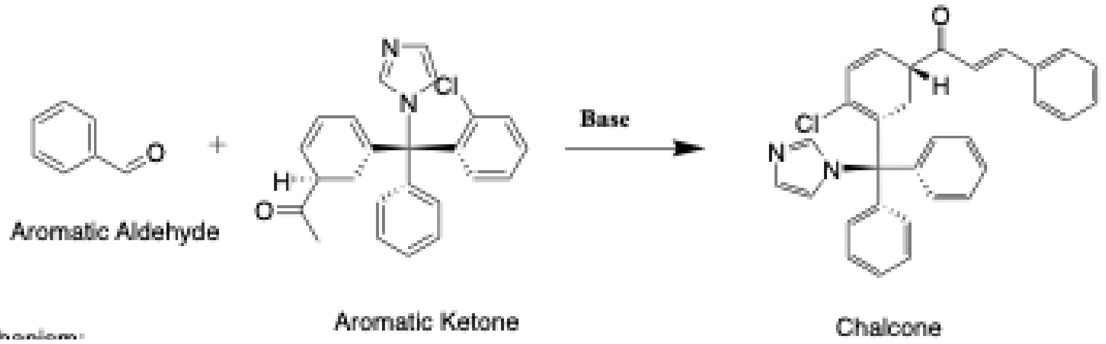
Acylated Clotrimazole was synthesised in this research by The Friedel-Crafts acylation procedure, an electrophilic aromatic substitution reaction that takes place in the ethanolic polar phase; reactions occur between 100 and 150 °C [32,33], as shown in **(Schemes 3)**.



Scheme 3: Schematic illustration for ACT synthesis

7.2 The synthesis of azole-chalcone derivatives, with C1 serving as a representative example:

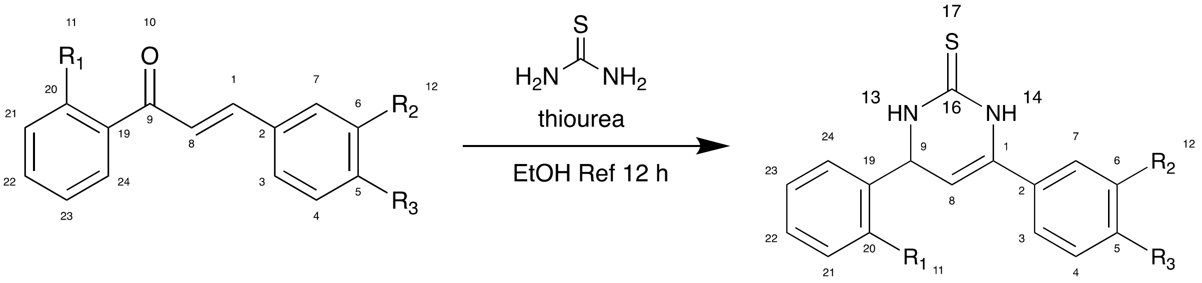
This research focused on synthesizing five azole-chalcone derivatives through a two-step process. It began with the synthesis and reaction of the acetophenone form of Clotrimazole (ACT) with various substituted benzaldehydes, as illustrated in **Scheme 4**. [34,35].



Scheme 4: Synthesis of azole-chalcone derivatives (C1)

7.3 Synthesis of Thio-Pyrimidine derivatives.

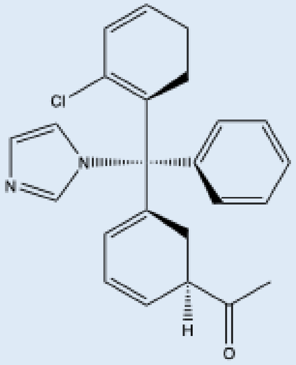
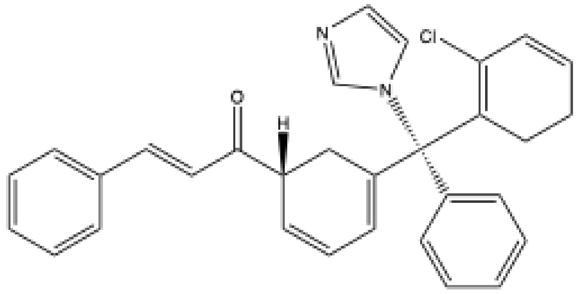
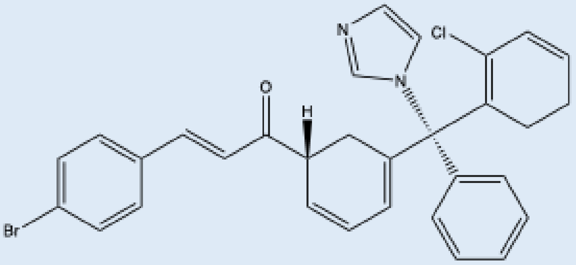
It is important to note that using a 30% v/v solution of isopropyl alcohol has a drawback, leading to an undesirable 6-endo-trig ring formation. Our study employed the Tirouflet and Corvaisier method, which offers an alternative strategy involving introducing hydroxide ions, followed by their removal through an intramolecular SN2 displacement. This method addresses the previous concern by avoiding the problematic 6-endo-trig ring closure, as 6-exo-tet processes are more favorable.The mechanism for this synthesis process is illustrated in **Scheme 5**.



Scheme 5: Cyclisation of chalcone derivatives

Chalcone derivatives synthesized in this study are presented in **Table 2.** The corresponding thiopyrimidine derivatives are presented in **Table 3.**

Table 1: Name and structure of Synthesised Chalcone Derivatives

	IUPAC Name	Chemical Structure
ACT	**1-((R)-5-((S)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)ethan-1-one**	
C1	**(E)-1-((R)-5-((S)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)-3-phenylprop-2-en-1-one**	
C2	**(E)-3-(4-bromophenyl)-1-((R)-5-((S)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)prop-2-en-1-one**	

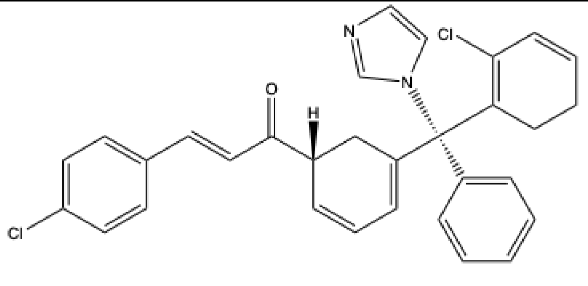
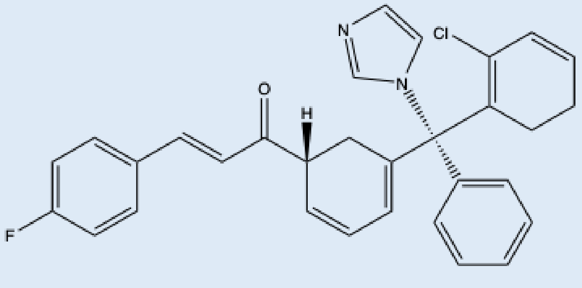
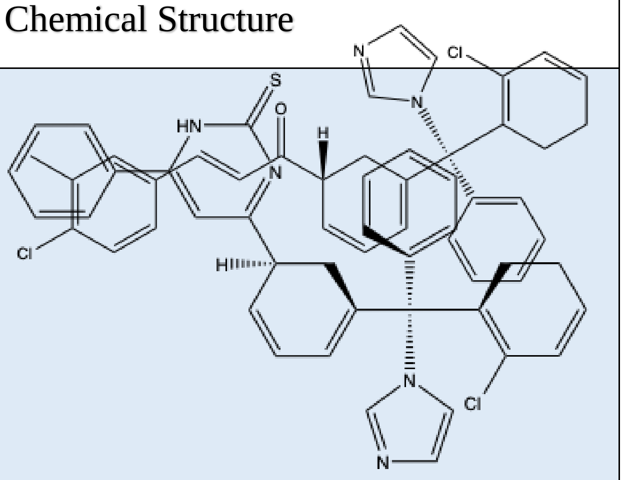
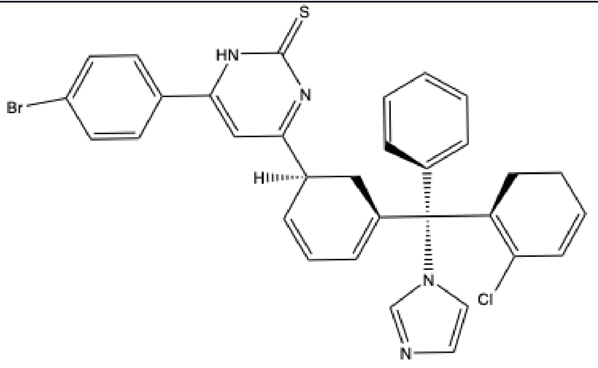
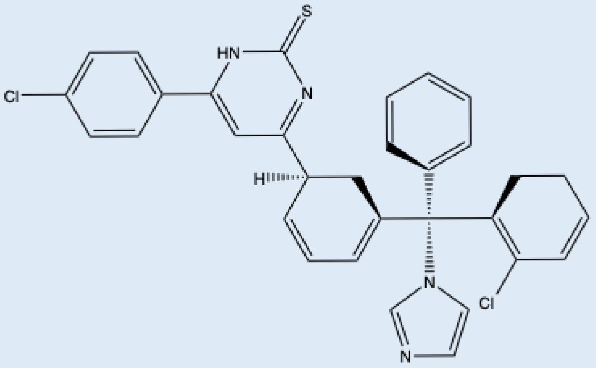
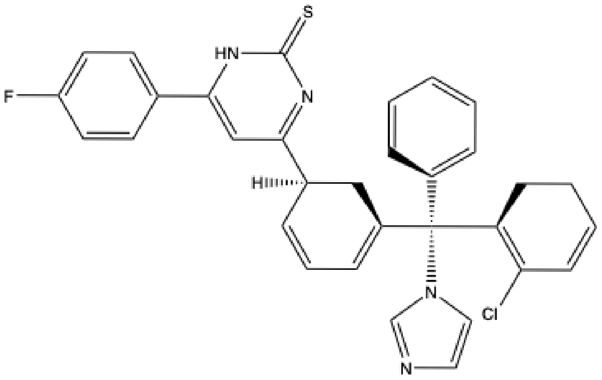
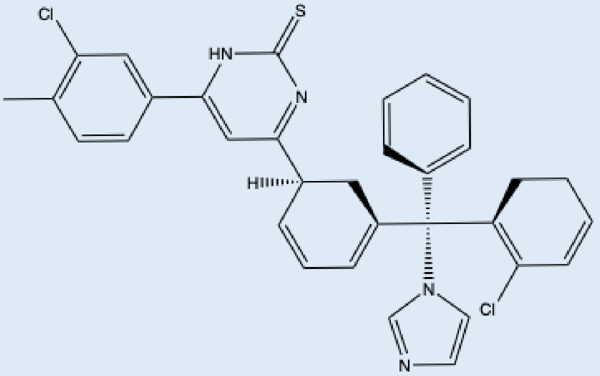
C3	**(E)-1-((R)-5-((S)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)-3-(4-chlorophenyl)prop-2-en-1-one**	
C4	**(E)-1-((R)-5-((S)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)-3-(4-fluorophenyl)prop-2-en-1-one**	

Table 2: The name and structure of Thio-Pyrimidine of Chalcone Derivatives

	IUPAC Name	Chemical Structure
Cy1	**4-((S)-5-((R)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)-6-phenylpyrimidine-2(1H)-thione**	
Cy2	**6-(4-bromophenyl)-4-((S)-5-((R)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)pyrimidine-2(1H)-thione**	
Cy3	**4-((S)-5-((R)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)-6-(4-chlorophenyl)pyrimidine-2(1H)-thione**	

Cy4	**4-((S)-5-((R)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)-6-(4-fluorophenyl)pyrimidine-2(1H)-thione**	
Cy5	**6-(3-chloro-4-methylphenyl)-4-((S)-5-((R)-(2-chlorocyclohexa-1,3-dien-1-yl)(1H-imidazol-1-yl)(phenyl)methyl)cyclohexa-2,4-dien-1-yl)pyrimidine-2(1H)-thione**	

FTIR spectra of Thio-Pyrimidine chalcone derivatives.

In Cy1-Cy5, our cyclised analogues, the IR spectrum displays characteristic bands at 1323 cm^{-1} and 1157 cm^{-1}, corresponding to the C=S and C-N groups, respectively. The IR spectrum of our derived compounds shows characteristic bands at 3328 cm^{-1} and 1500 cm^{-1}, indicating the presence of NH_2 and C=N groups, respectively. **FTIR spectra are Presented in Figures (2) - (6)**

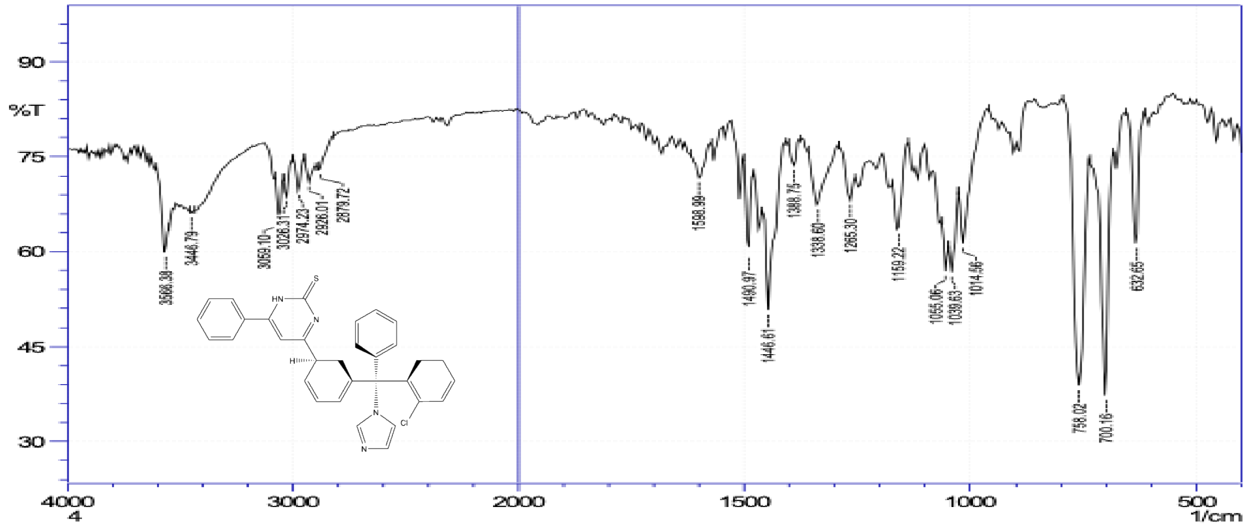


Figure 2 FTIR spectrum of Compound Cy1

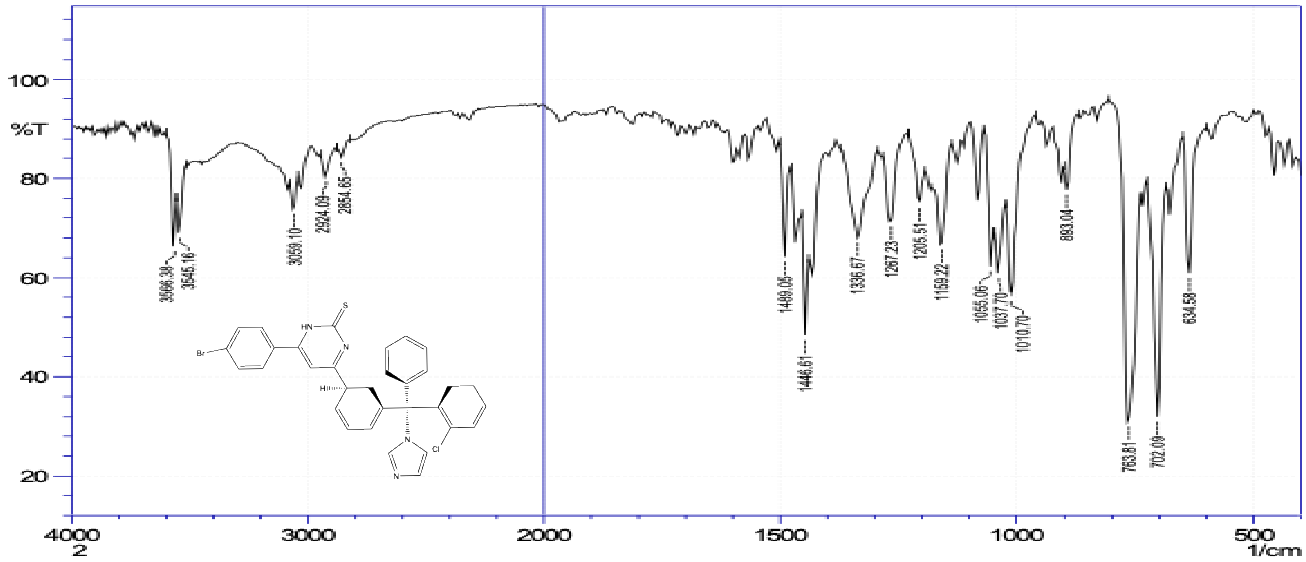


Figure 3 FTIR spectrum of Compound Cy2

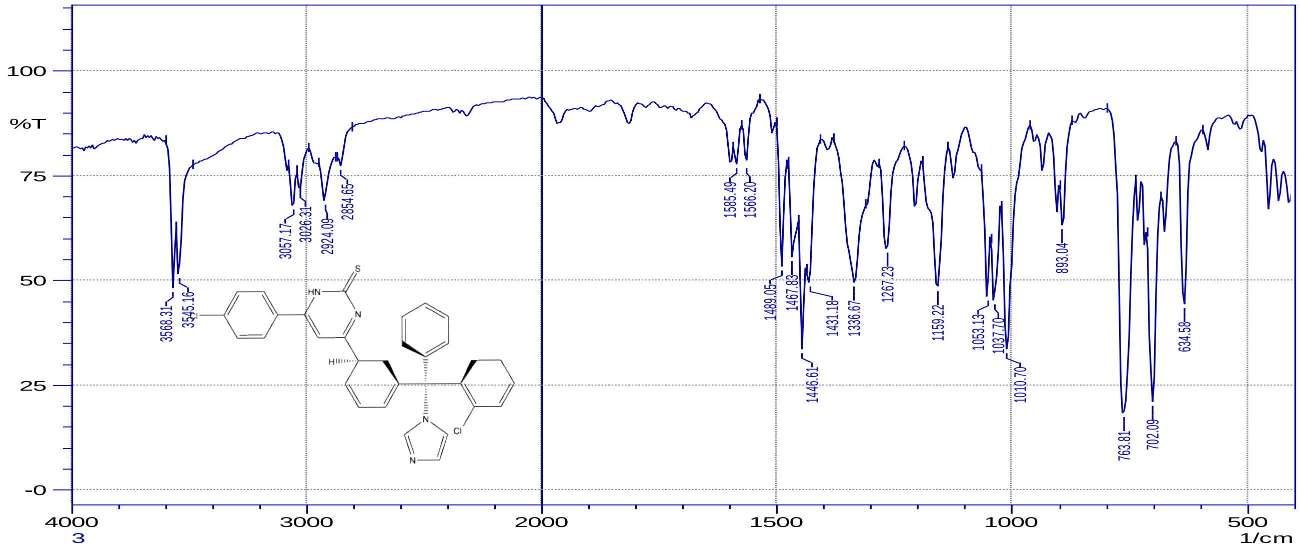


Figure 4 FTIR spectrum of Compound Cy3

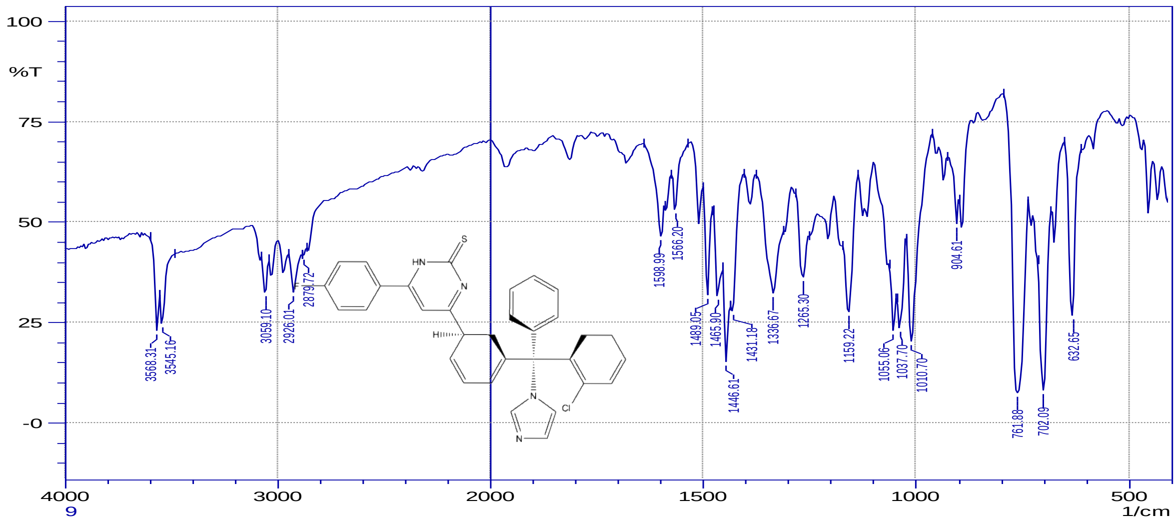


Figure 5 FTIR spectrum of Compound Cy4

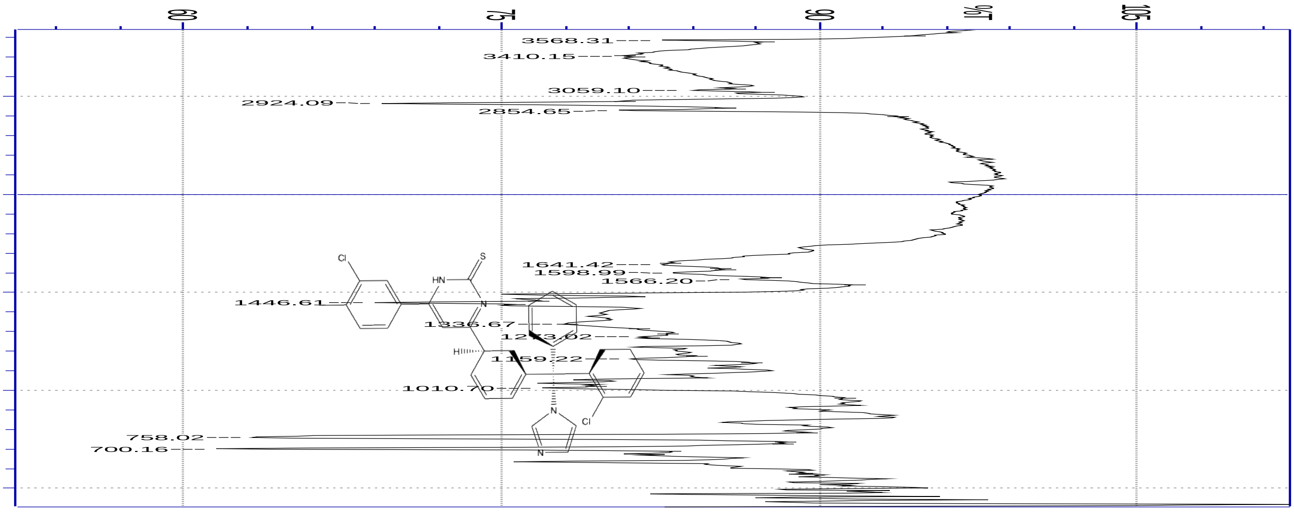


Figure 6 FTIR spectrum of Compound Cy5

^{1}H-NMR and 13C-NMR

When characterization is conducted by the ^{1}H-NMR, two singlets are clearly shown at 2.3 and 2.5 due to the closely packed doublets. The aromatic protons in the region d 6.5–7.1. The –NH groups disappeared from the original position of d 8.15 (–HN–), 8.65 (–HN–C=S), and 9.03 (–HN–C=S) and its 1H NMR spectra in $CDCl_3$ showed a characteristic septet d 3.92, integrating for a single proton at $*C_{12}$ [36,37].

Both ^{1}H-NMR and 13C-NMR spectra are Presented in Figure (7) - (16)

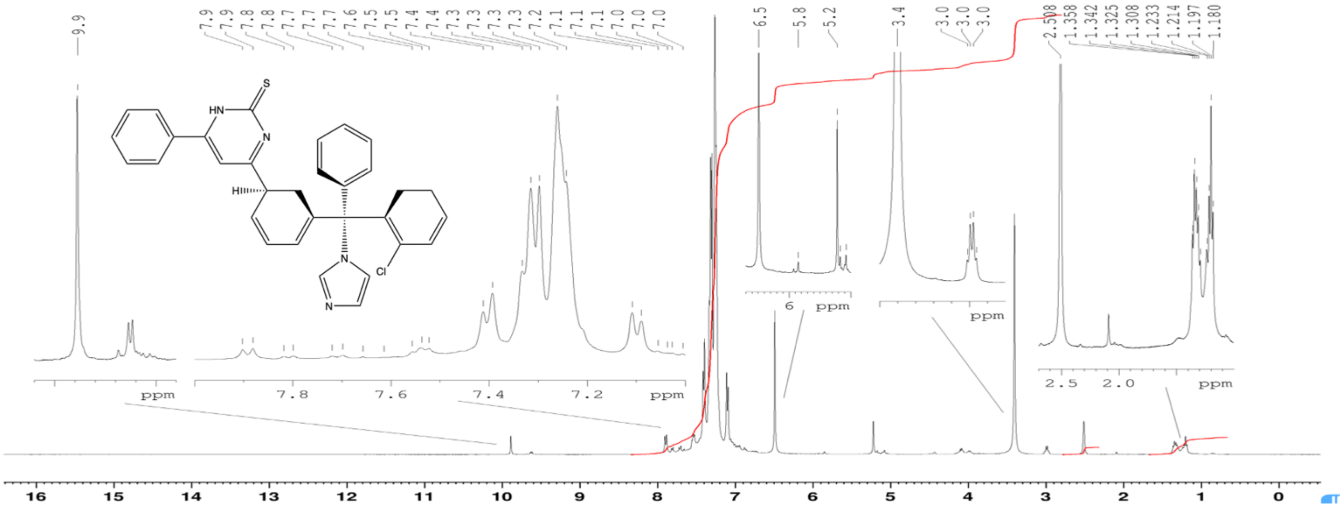


Figure 7 ^{1}H NMR Spectrum of Compound Cy1

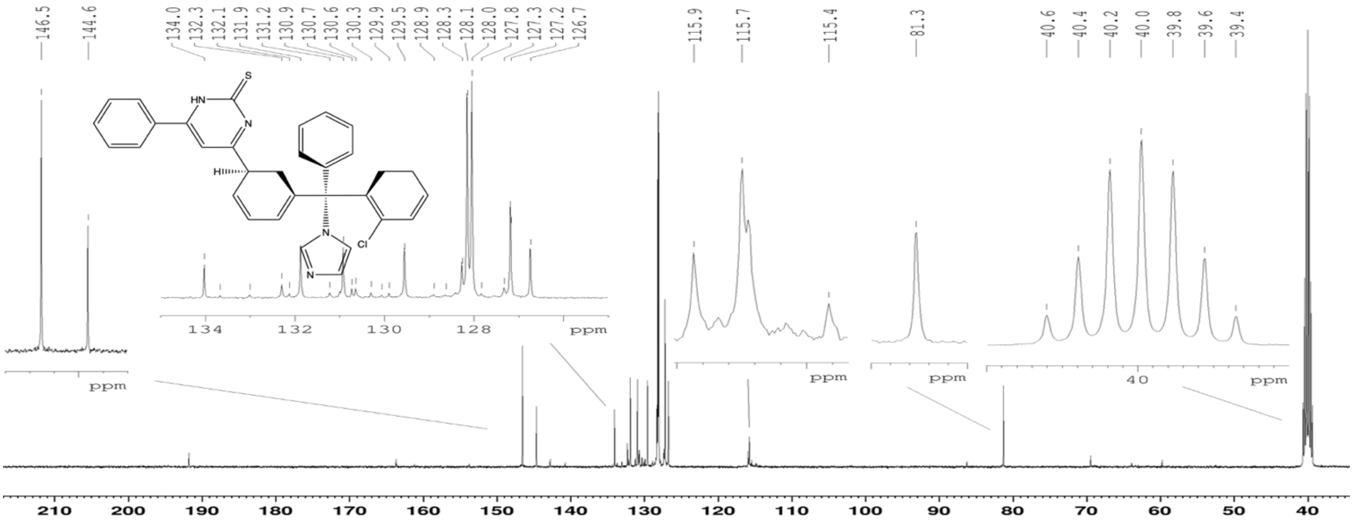


Figure 8 ^{13}C NMR spectrum of Compound Cy1

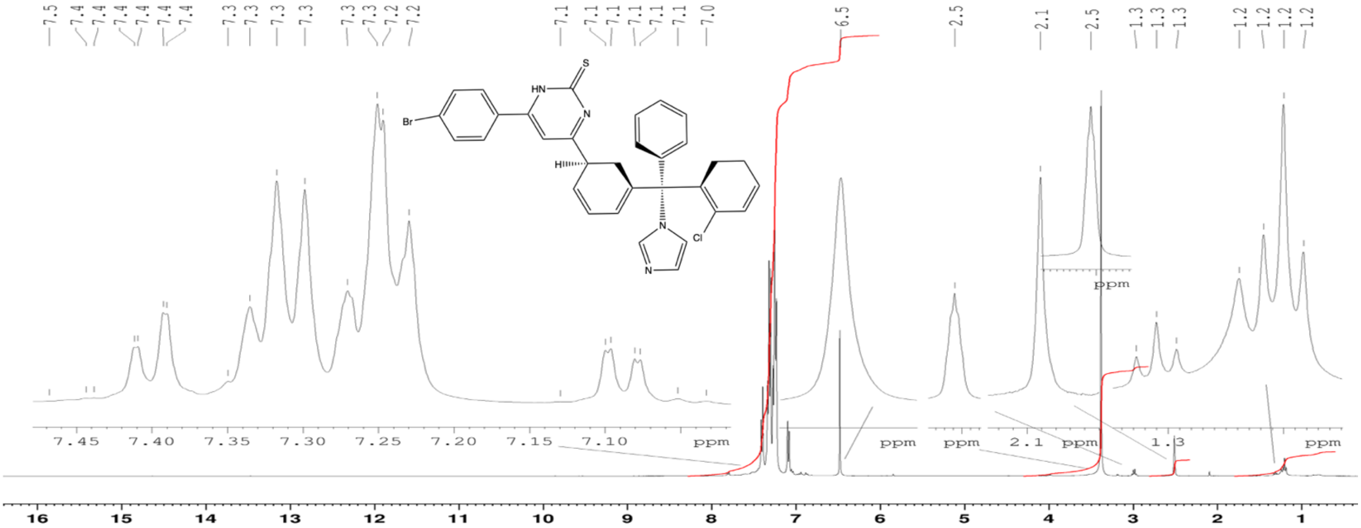


Figure 9 ^{1}H NMR Spectrum of Compound Cy2

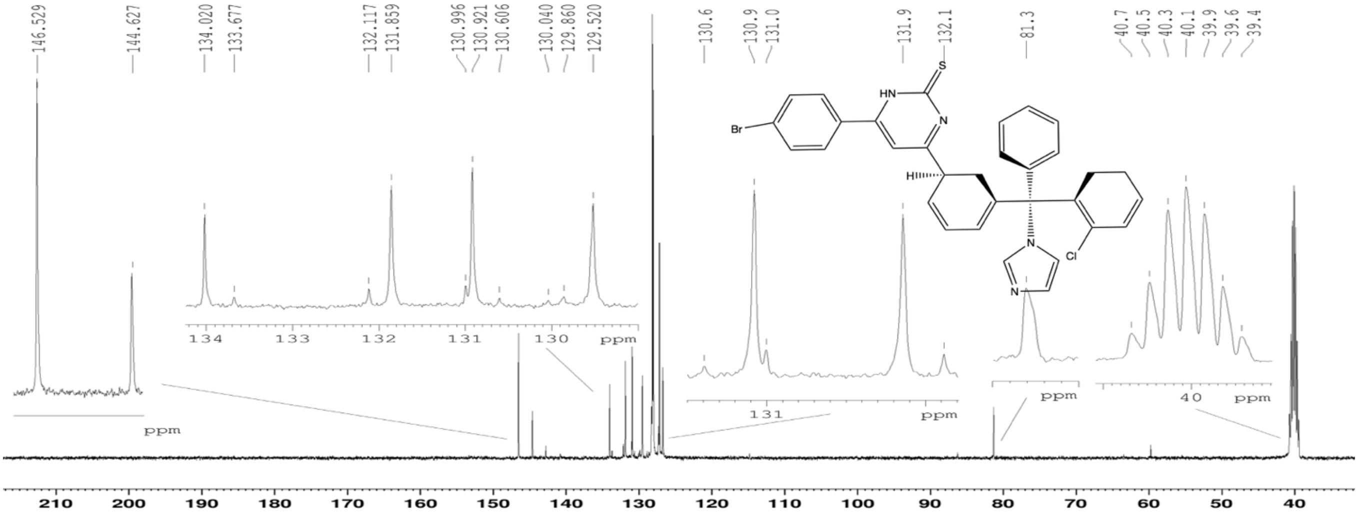


Figure 10 ^{13}C NMR spectrum of Compound Cy2

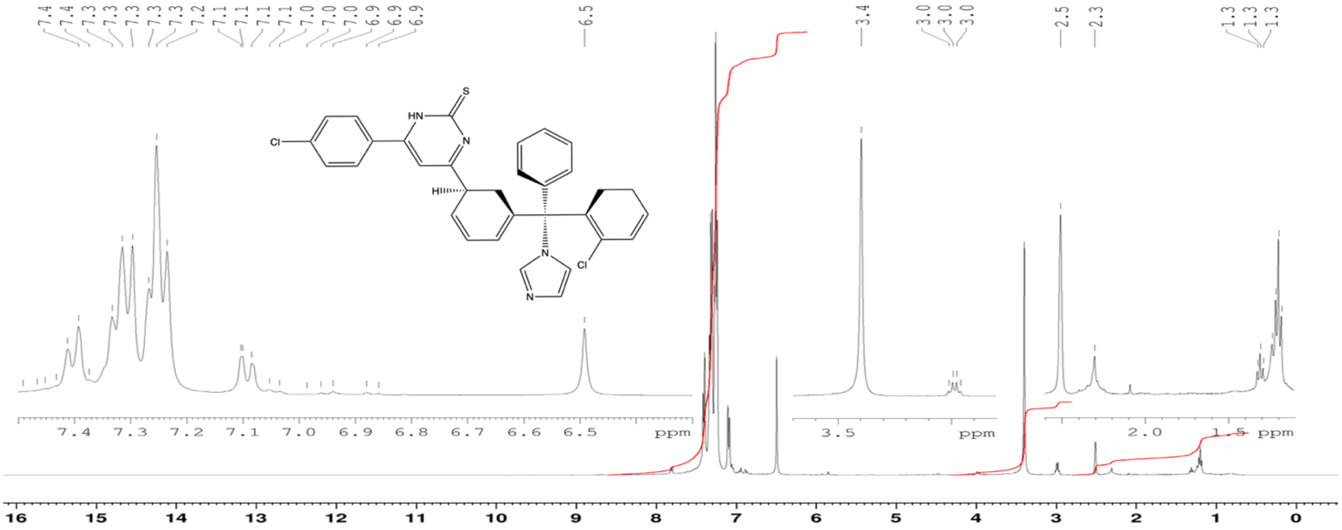


Figure 11 ^{1}H NMR Spectrum of Compound Cy3

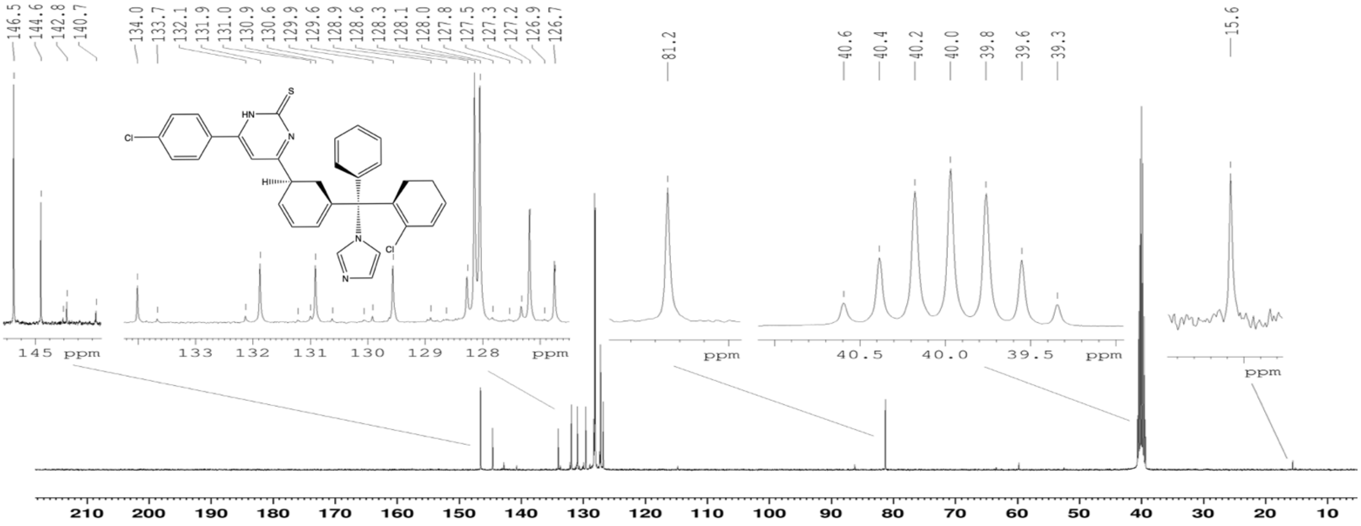


Figure 12 ^{13}C NMR spectrum of Compound Cy3

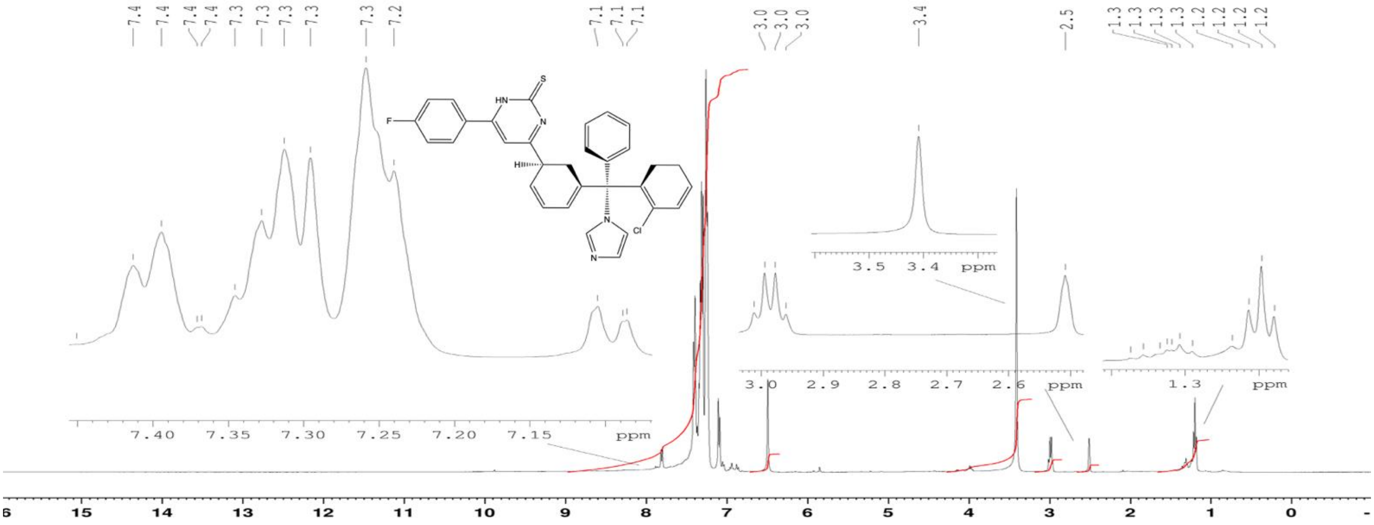


Figure 13 ^{1}H NMR Spectrum of Compound Cy4

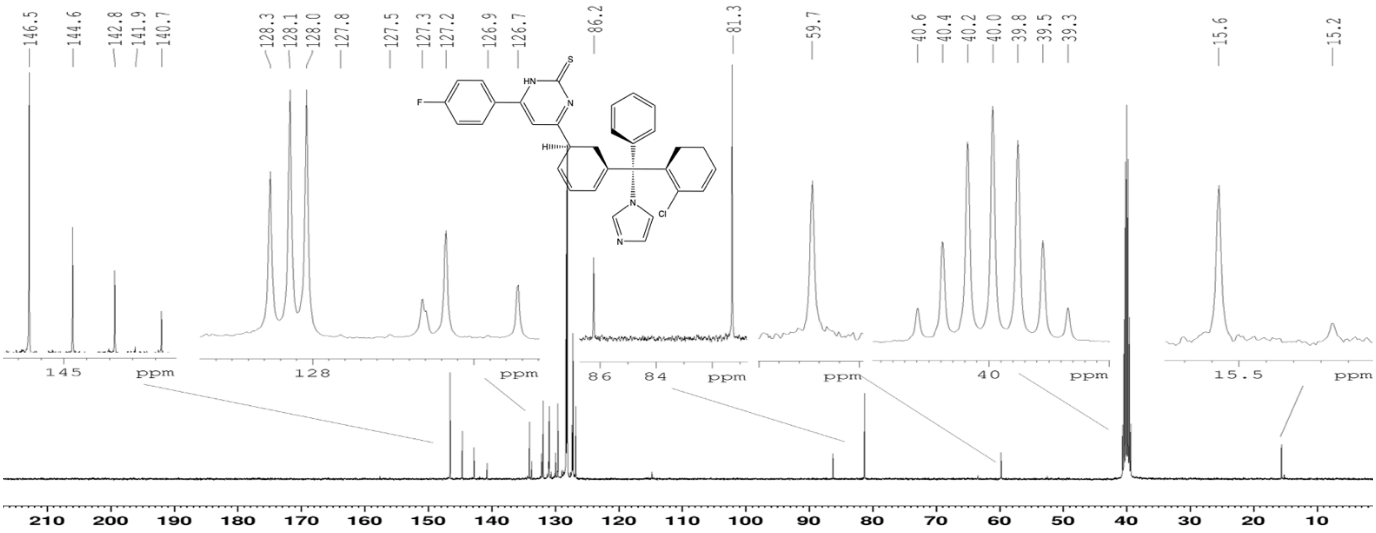


Figure 14 ^{13}C NMR spectrum of Compound Cy4

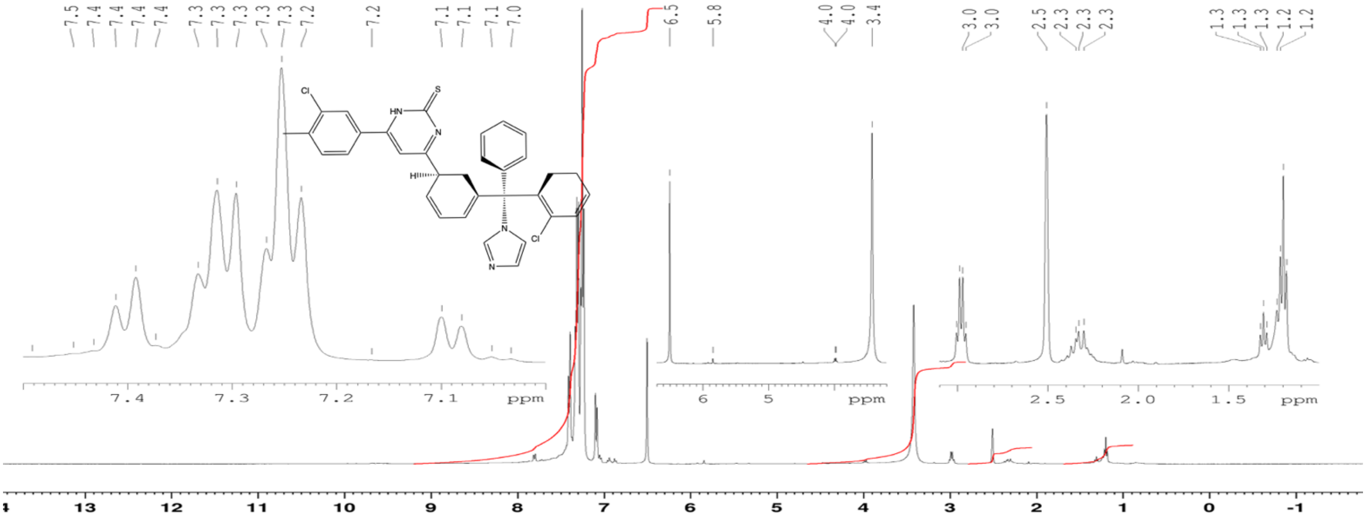


Figure 15 ^{1}H NMR Spectrum of Compound Cy5

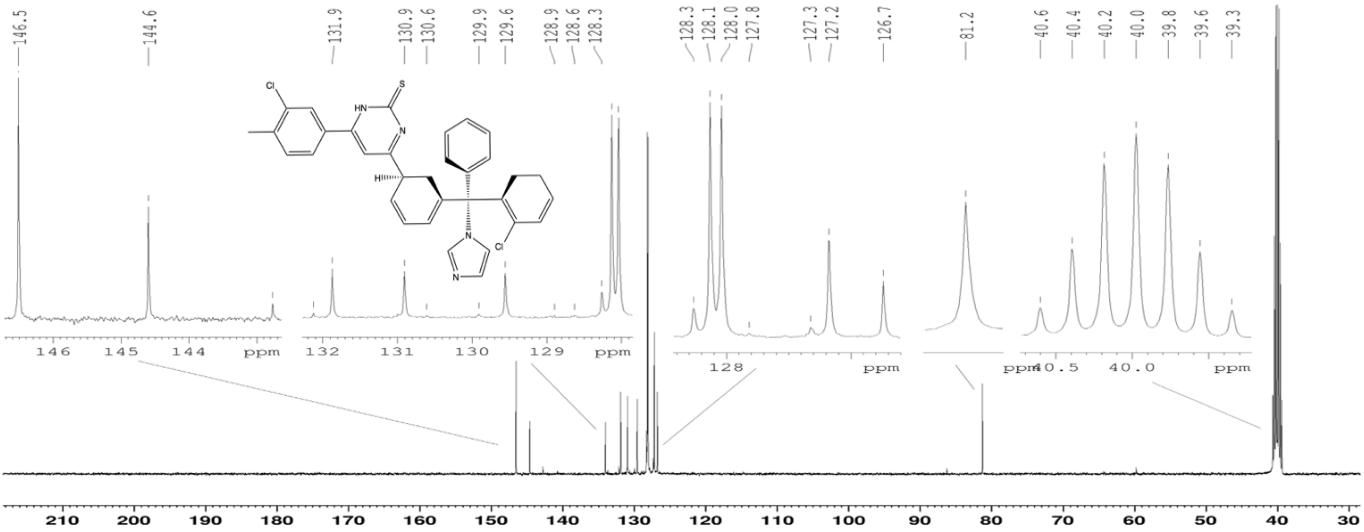


Figure 16 ^{13}C NMR spectrum of Compound Cy5

Antifungal evaluation of Thio-pyrimidine Derivatives of Clotrimazole

The antifungal activity of newly designed compounds [C1-Cy5] against Candida albicans was assessed using a microdilution assay. The study focused on isolates from skin psoriasis preserved at Thi-Qar University. [38–41]

Three derivatives, C3, C5, and Cy1, showed substantial inhibitory effects against this fungal strain, highlighting their potential as Clotrimazole alternatives in antifungal resistance management. A comparative investigation found that chalcones and their derivatives, notably cyclized forms like Cy1, have higher antifungal activity. This increased efficacy is due to extra molecular properties that selectively target fungal diseases, allowing them to outperform Clotrimazole and non-cyclized chalcone derivatives.

7. Conclusion

Chalcones are highly adaptable chemical compounds that can be easily modified and synthesized to create a variety of structural products. Despite numerous preclinical investigations, a comprehensive understanding of the action mechanisms of many chalcone derivatives remains incomplete. Chalcones are produced through Claisen–Schmidt condensation, which involves a cross-aldol condensation of suitable aldehydes and ketones via base-catalyzed reactions. The structures of the synthesized compounds were characterized using thorough spectroscopic techniques. These compounds were then tested for biological activity in vitro with a microdilution assay. The results indicated that the antifungal activity of the chalcone derivatives was significant.

Cy1, a thio-pyrimidine derivative of C1, demonstrated significant antifungal activity, achieving 100% inhibition of fungal growth after 24 hours of incubation at 37°C. Based on the current study, we conclude that compounds synthesized from acetophenones and benzaldehydes, along with their subsequent thio-pyrimidine derivatives, have shown great potential in inhibiting or eliminating fungi. These compounds may serve as therapeutic alternatives, pending further rigorous evaluation for their suitability as medicinal component

8. Notes:

Acknowledgements

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Conflict of Interest

This article offers essential insights into pharmaceutical chemistry and has the potential to help with the creation of new medicinal medicines. I state that there are no competing interests and that all authors have approved this work for submission. The material has not been previously disseminated and is not now under consideration anywhere.

Thank you for considering my manuscript. I look forward to your favourable response.

Author's Contributions

- Investigation: Experiment-based study and data collection.

- Validation: Ensuring the consistency of outcomes and research outputs.

- Resources: Providing study materials, reagents, and analytical instruments.

- Formal Analysis: Using statistical and computational methods to analyse data.

- Writing - Original Draft: Creating an original draft of the published work.

- Writing, Review, and Editing: Conducting a critical review and revision of the original research group's published work.

Limitations - Possible unintended bias during data collecting.

- Intentional use of unplanned data analysis.

- Limited access to information.

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