2026 Volume: 02 Issue: 08 www.ijrpa.com ISSN 2456-9995 Research Article

International Journal Research Publication Analysis

Page: 01-26

SYNTHESIS AND CHARACTERIZATION OF NEW DERIVATIVES OF PYRAZOLE AND SCREENED FOR THEIR ANTITUBERCULAR POTENTIALS

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Article Received: 30 June 2026
Article Revised: 20 July 2026
Published on: 07 August 2026

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DOI: https://doi-doi.org/101555/ijrpa.3563

ABSTRACT

Tuberculosis (TB) remains one of the leading infectious diseases worldwide, with the increasing prevalence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* posing a major challenge to current chemotherapy. In the present study, a new series of pyrazole-thiazole coupled chromenone derivatives (CH-1 to CH-14) were synthesized through a two-step synthetic route involving thiosemicarbazide and 3-(2-bromoacetyl)-2H-chromen-2-one. The synthesized compounds were purified and characterized by IR, ^1H NMR, FAB-mass spectroscopy, and elemental analysis, confirming their proposed chemical structures. The antitubercular activity of the compounds was evaluated against *Mycobacterium tuberculosis* H37Rv and multidrug-resistant (MDR) clinical isolates using the Microplate Alamar Blue Assay (MABA) and Luciferase Reporter Phage (LRP) assay. Several compounds exhibited promising activity, with CH-3 and CH-8 showing the lowest MIC values (1.75 µg/mL) against H37Rv, while CH-1, CH-3, CH-8, CH-10, and CH-11 demonstrated significant inhibition in the LRP assay, producing more than 50% reduction in relative light units. Among all derivatives, CH-10 exhibited the highest reduction in bacterial viability at higher concentrations, indicating potent antitubercular activity. These findings suggest that pyrazole-thiazole-chromenone hybrids represent promising lead molecules for the development of new antitubercular agents against both drug-sensitive and multidrug-resistant *M. tuberculosis* strains.

KEYWORDS: *Pyrazole derivatives; Thiazole derivatives; Chromenone; Heterocyclic compounds; Antitubercular activity; Mycobacterium tuberculosis.*

1. INTRODUCTION

Organic compounds possessing at least one heteroatom other than carbon inside a ring structure are known as heterocyclic compounds. Oxygen, nitrogen, and sulphur are examples of hetero atoms [1]. They have become more important in our everyday lives in recent years as a result of their diverse pharmacological effects and other applications. Heterocyclic compounds are found in many vitamins, alkaloids, synthetic medications, and antibiotics. In nature, there are many heterocyclic compounds. Biological processes convey a range of heterocyclic active chemicals among these many elements of nature [2].

Heterocyclic compounds make up the majority of biological molecules like deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The most important macromolecule in life is DNA. In addition, heterocyclic compounds include Pyridoxal (Vitamin B6), Niacin (Vitamin B3), Riboflavin (Vitamin B2), Thiamin (Vitamin B1), and Ascorbic acid (Vitamin C). Many notable known heterocyclic core structures include haemoglobin (oxygen transporters in the blood), chlorophyll, and enzymes. Antibacterial, antibiotic, antidiabetic, antidepressant, antifungal, anti-HIV, anti-inflammatory, antimalarial, antimicrobial, antitumor, antiviral, herbicidal, fungicidal, and insecticidal agents are among the many drugs, natural products, biomolecules, and biologically active compounds that contain them. agents, corrosion inhibitors, dyestuff, fluorescence sensor, data storage, and plastics are only a few examples [3,4]. Analytical reagents, brightening agents, corrosion inhibitors, dyestuff, fluorescence sensor, information storage, and plastics are only a few of the heterocycles' uses in material science.

1.1. Pyrazoles

Pyrazoles are a white liquid with a five-membered two-nitrogen heterocyclic molecule with the formula C3H4N2. Pyrazoles are chemicals with tautomeric structures that vary. Unsubstituted pyrazole may be represented in two tautomeric forms, whereas pyrazole derivatives with various substituents on two carbon atoms next to the nitrogen atoms on the ring can be represented in five tautomeric structures. When replacements are made on the first, third, and fourth positions of this ring, it becomes highly active and responds quickly to produce different moieties. [5,6]

1.2. Thiazole

In medicinal chemistry, thiazole, or 1,3-thiazole, is a heterocyclic molecule that includes both sulphur and nitrogen and has a vast family of derivatives. Thiazole has the chemical formula C3H3NS and is a transparent to light yellow liquid with a pyridine-like odour. Many natural compounds have thiazole as the primary structural component. The thiazole ring is well-

known as part of the vitamin thiamine (B1). Thiazoles belong to the azole family, which also includes imidazole, triazole, and oxazole. The functional group thiazole can also be considered. The intriguing pharmacological characteristics of thiazole derivatives in response to various structural alterations are worth investigating in order to construct high-effective drugs with minimal side effects [7,8].

1.3. Antimicrobials

Antimicrobials are substances that kill or prevent hazardous microorganisms from multiplying or growing. Because microbes are not visible to the naked eye, a strong microscope is required. Some of these microorganisms are beneficial, while others are dangerous. There are three sorts of microbes. Bacteria, viruses, and fungus are the three types. Viruses are the tiniest and most primitive forms of life, measuring 10 to 100 times smaller than bacteria. The primary distinction between viruses and bacteria is that viruses require a live host, such as a plant or animal, to reproduce, whereas most bacteria may grow on nonliving surfaces. Although certain bacteria are beneficial, viruses are invariably destructive. Bacteria are divided into three types: small balls (-cocci), rods (-bacilli), and spirals (-bacilli) (-boriella) [9-11].

1.3.1. Tuberculosis (TB)

Despite the availability of more than ten anti-TB medications and the Bacille Calmette Guerin (BCG) anti-TB vaccination, tuberculosis remains one of the leading causes of mortality in humans. The advent of extensively drug-resistant tuberculosis (XDR-TB) and multidrug-resistant tuberculosis (MDR-TB), both of which have shown low efficacy against existing anti-TB medications, has exacerbated the challenge of TB control. According to the WHO's Global Tuberculosis Report 2021, multidrug-resistant TB patients account for 3.5 percent of new cases and 20.5 percent of previously treated cases worldwide. As a result, a lot of effort is being put into identifying novel molecular entities that are effective against bacterial strains. Furthermore, several currently accessible medications have been demonstrated to have certain side effects [12-14].

The existing TB medication regimen's complexity and toxicity require quick attention. Similarly, the important issue of tuberculosis medication resistance requires promCH attention. As a result, new tuberculosis medications are required.

2. MATERIALS AND METHOD

Different substituted 1H-pyrazole-4-carbaldehyde was purchased from Merck, India. The Sodium acetate, Thio-semicarbazide, ethanol and 3-(2-bromoacetyl)-2H-chromen-2-one were

purchased from sigma Aldrich. All the chemicals were purchased from Sigma Aldrich and Merck India. Commercial grade solvents used for the reactions were distilled before use.

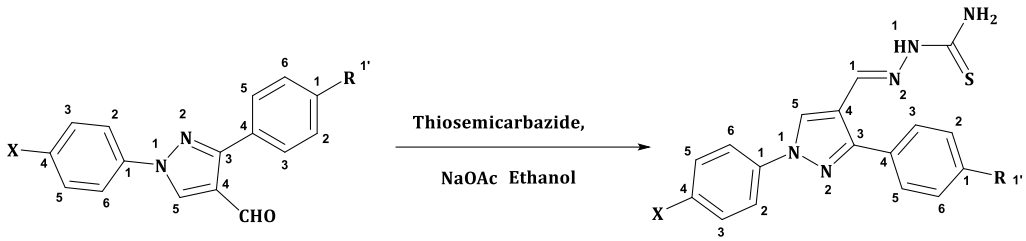
2.1. Synthesis scheme

Synthesis of new pyrazole derivatives involves the following two steps.

Step 1: Synthesis of Compound B with the reaction of compound A with thiosemicarbazide and sodium acetate

Step 2: Reaction of Compound B with 3-(2-bromoacetyl)-2H-chromen-2-one to synthesize compound C.

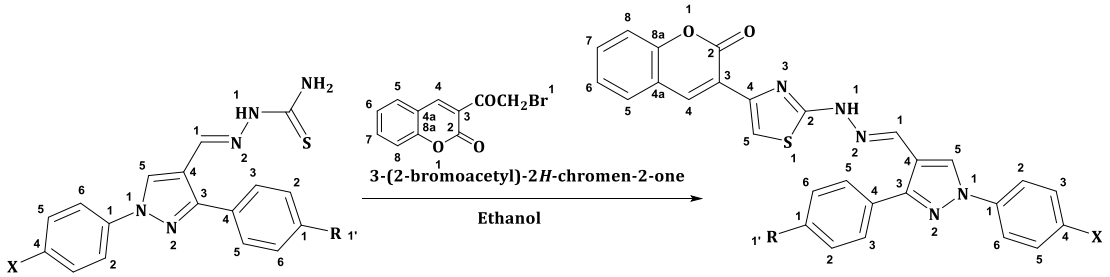
STEP 1: Synthesis of Compound B with the reaction of compound A with thio-semicarbazide and NaOAc.



3-([1,1'-bisubstitued phenyl]-4-yl)-1-(4-substitutedphenyl)-1*H*-pyrazole-4-carbaldehyde (Compound A)

(*E*)-2-((3-([1,1'-bisubstitued phenyl]-4-yl)-1-(4-substitutedphenyl)-1*H*-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (Compound B)

STEP 2: Reaction of Compound B with 3-(2-bromoacetyl)-2H-chromen-2-one to synthesize compound C.



Compound B

(*E*)-3-(2-(2-((3-([1,1'-bisubstitued phenyl]-4-yl)-1-(4-substitutedphenyl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-4-yl)-2 *H*-chromen-2-one (Compound C, TP-1 to TP-14)

2.2. CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

List of Synthesized compounds: In this study, a total of 14 substances were synthesized and purified using column chromatography. The IR, Proton NMR spectroscopy, mass, and elemental analyses were used to analyze all compounds.

Table 1: List of Final synthesized compounds.

SN	Code	Chemical name
1.	CH-1	(E)-3-(2-(2-((1,3-bis(4-chlorophenyl)-1H-pyrazol-4-yl)methylene) hydrazineyl) thiazol-4-yl)-2H-chromen-2-one
2.	CH-2	(E)-3-(2-(2-((1-(4-bromophenyl)-3-(4-chlorophenyl)-1H-pyrazol-4yl) methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
3.	CH-3	(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
4.	CH-4	(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(p-tolyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
5.	CH-5	(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-methoxyphenyl)-1H-pyrazol-4-yl) methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
6.	CH-6	(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-ethylphenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
7.	CH-7	(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-ethoxyphenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
8.	CH-8	(E)-3-(2-(2-((1-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
9.	CH-9	(E)-3-(2-(2-((1-(4-bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
10.	CH-10	(E)-3-(2-(2-((1,3-bis(4-nitrophenyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
11.	CH-11	(E)-3-(2-(2-((3-(4-nitrophenyl)-1-(p-tolyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
12.	CH-12	(E)-3-(2-(2-((1-(4-methoxyphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
13.	CH-13	(E)-3-(2-(2-((1-(4-ethylphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one
14.	CH-14	(E)-3-(2-(2-((1-(4-ethoxyphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Table 2: Physicochemical properties of the synthesized compounds.

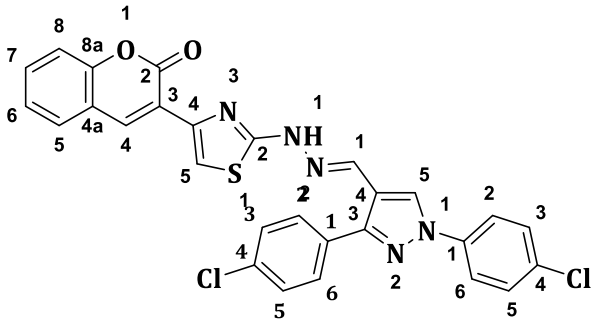
SN	Code	Chemical formula	Mol. Weight	Percent Yield	Melting point
1.	CH-1	$C_{28}H_{17}C_{l2}N_5O_2S$	558.44	78	242-244°C
2.	CH-2	$C_{28}H_{17}BrClN_5O_2S$	602.89	82	236-238°C
3.	CH-3	$C_{28}H_{17}ClN_6O_4S$	568.99	76	255-257°C
4.	CH-4	$C_{29}H_{20}ClN_5O_2S$	537.10	75	233-235°C
5.	CH-5	$C_{29}H_{20}ClN_5O_3S$	554.02	73	214-216°C
6.	CH-6	$C_{30}H_{22}ClN_5O_2S$	552.05	68	220-222°C
7.	CH-7	$C_{30}H_{22}ClN_5O_3S$	568.05	62	215-217°C
8.	CH-8	$C_{28}H_{17}ClN_6O_4S$	568.99	80	232-234°C
9.	CH-9	$C_{28}H_{17}BrN_6O_4S$	613.45	85	242-244°C
10.	CH-10	$C_{28}H_{17}N_7O_6S$	579.55	80	262-268°C
11.	CH-11	$C_{29}H_{20}N_6O_4S$	548.58	75	224-226°C
12.	CH-12	$C_{29}H_{20}N_6O_5S$	564.58	78	218-220°C
13.	CH-13	$C_{30}H_{22}N_6O_4S$	562.60	62	232-234°C
14.	CH-14	$C_{30}H_{22}N_6O_5S$	578.60	60	210-212°C

Table 3: Solubility Data of Synthesized Compounds.

SN	Code No.	Cool water	Hot water	Ethyl alcohol	Ether	Acetone	Chloroform	DMSO
1.	CH-1	-	-	+++	++	-	-	+++
2.	CH-2	-	-	+++	++	+	-	+++
3.	CH-3	-	-	+++	++	+	+	+++
4.	CH-4	-	-	+++	++	++	-	+++
5.	CH-5	-	-	+++	++	++	-	+++
6.	CH-6	-	-	+++	++	+	-	+++
7.	CH-7	-	-	+++	++	+	+	+++
8.	CH-8	-	-	+++	++	-	-	+++
9.	CH-9	-	-	+++	++	+	-	+++
10.	CH-10	-	-	+++	++	++	-	+++
11.	CH-11	-	-	+++	++	-	-	+++
12.	CH-12	-	-	+++	++	+	-	+++
13.	CH-13	-	-	+++	++	++	-	+++
14.	CH-14	-	-	+++	++	-	-	+++

2.3. CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS BY IR, NMR, MASS AND ELEMENTARY ANALYSIS

1. COMPOUND CODE: - CH-1



IUPAC Name: (E)-3-(2-(2-((1,3-bis(4-chlorophenyl)-1H-pyrazol-4-yl)methylene)-hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{28}H_{17}Cl_2N_5O_2S$;

Molecular Weight: 558.44

Elemental Analysis

Elements	C	N	O	S
Calculated	60.24	12.56	5.73	5.78
Found	60.22	12.54	5.75	5.76

IR (cm^{-1})

SN	Propagation number (cm^{-1})	Mode of vibration
1.	3415.15	(N-H)
2.	3240.56	Ar-H,

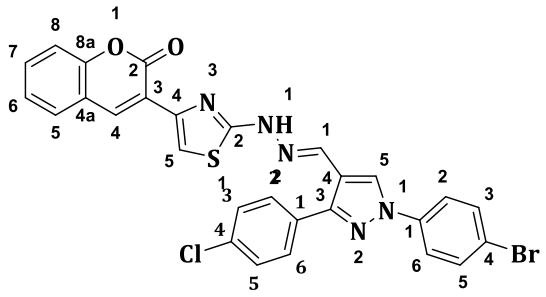
3.	1720.33	(C=O)
4.	1627.55	(C=N)
5.	1497.23	(C=C)
6.	1094.32	(C-O)
7.	850.22	(C-Cl)

1HNMR (ppm)

δ 7.36-7.40 (m, 2H, Ar-H), 7.44-7.50 (m, 2H, Ar-H), 7.52- 7.56 (m, 4H, Ar-H), 7.60-7.63 (m, 1H, Ar-H), 7.76 (s, 1H), 7.77-7.79 (d, 2H, Ar-H), 7.85-7.87 (d, 1H), 7.98-8.00 (d, 2H, Ar-H), 8.19 (s, 1H, -CH=N), 8.53 (s, 1H), 8.92 (s, 1H), 12.03 (s, 1H, -NH)

FAB Mass (m/z): 557.05

2. COMPOUND CODE: CH-2



IUPAC NAME

(E)-3-(2-(2-((1-(4-bromophenyl)-3-(4-chlorophenyl)-1H-pyrazol-4yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{28}H_{17}BrClN_5O_2S$

Molecular Weight: 602.89

Elemental Analysis

Elements	**C**	**N**	**O**	**S**
Calculated	55.82	11.65	5.32	5.34
Found	55.78	11.62	5.32	5.32

IR (cm^{-1})

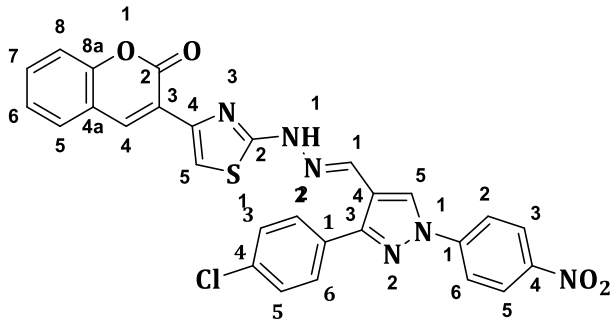
SN	**Propagation number (cm^{-1})**	Mode of vibration
1.	3415.32	(N-H)
2.	3238.24	Ar-H,
3.	1718.56	(C=O)
4.	1625.23	(C=N)
5.	1492.30	(C=C)
6.	1092.35	(C-O)
7.	848.56	(C-Cl)
8.	1020.37	**(C-Br)**

1HNMR (ppm)

δ 7.38-7.42 (m, 2H), 7.75 (s, 1H), 7.76-7.78 (d, 2H), 7.83-7.85 (d, 1H), 7.98-8.02 (d, 2H), 8.21 (s, 1H), 8.53 (s, 1H), 8.92 (s, 1H), 12.03 (s, 1H, -NH);

FAB Mass (m/z): 601.00

3. COMPOUND CODE: - CH-3



IUPAC name: (E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{28}H_{17}ClN_6O_4S$

Molecular Weight: 568.99

Elemental Analysis

Elements	C	N	O	S
Calculated	59.15	14.75	11.26	5.65
Found	59.11	14.77	11.25	5.63

IR (cm^{-1})

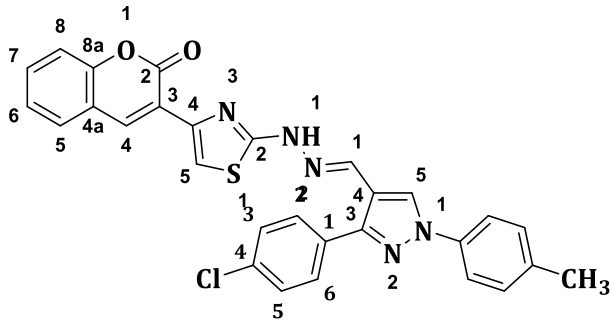
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3430.32	(N-H)
2.	3238.56	Ar-H,
3.	1721.23	(C=O)
4.	1625.32	(C=N)
5.	1495.23	(C=C)
6.	1092.28	(C-O)
7.	850.22	(C-Cl)
8.	1365.32	(N-O)
9.	1569.22	(N=0)

1HNMR (ppm)

δ 7.35-7.39 (m, 2H), (m, 1H, Ar-H), 7.75 (s, 1H,), 7.76-7.79 (d, 2H), 7.82-7.86 (d, 1H), 7.95-7.99. (d, 2H), 8.18 (s, 1H, -CH=N), 8.52 (s, 1H), 8.91 (s, 1H,), 11.57 (s, 1H, -NH)

FAB Mass (m/z): 568.07

4. COMPOUND CODE: - CH-4



IUPAC name

(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(p-tolyl)-1H-pyrazol-4-yl)methylene)hydrazineyl) thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{29}H_{20}ClN_5O_2S$

Molecular Weight: 538.02

Elemental Analysis

Elements	**C**	**N**	**O**	**S**
Calculated	64.78	13.02	5.96	5.98
Found	64.74	13.02	5.95	5.96

IR (cm^{-1})

SN	**Propagation number (cm^{-1})**	Mode of vibration
1.	3410.12	(N-H)
2.	3238.21	Ar-H,
3.	1721.13	(C=O)
4.	1625.15	(C=N)
5.	1496.28	(C=C)
6.	1092.30	(C-O)
7.	850.12	(C-Cl)

1HNMR (ppm)

δ 7.38-7.42 (m, 2H, Ar-H), 7.60-7.62 (m, 1H, Ar-H), 7.75 (s, 1H), 7.98-8.01 (d, 2H), 7.84-7.86 (d, 1H), 7.96-8.00 (d, 2H), 8.21 (s, 1H, -CH=N), 8.52 (s, 1H), 8.92 (s, 1H), 11.57 (s, 1H, -NH), 3.38 (s, 3H, CH3)

FAB Mass (m/z): 537.10

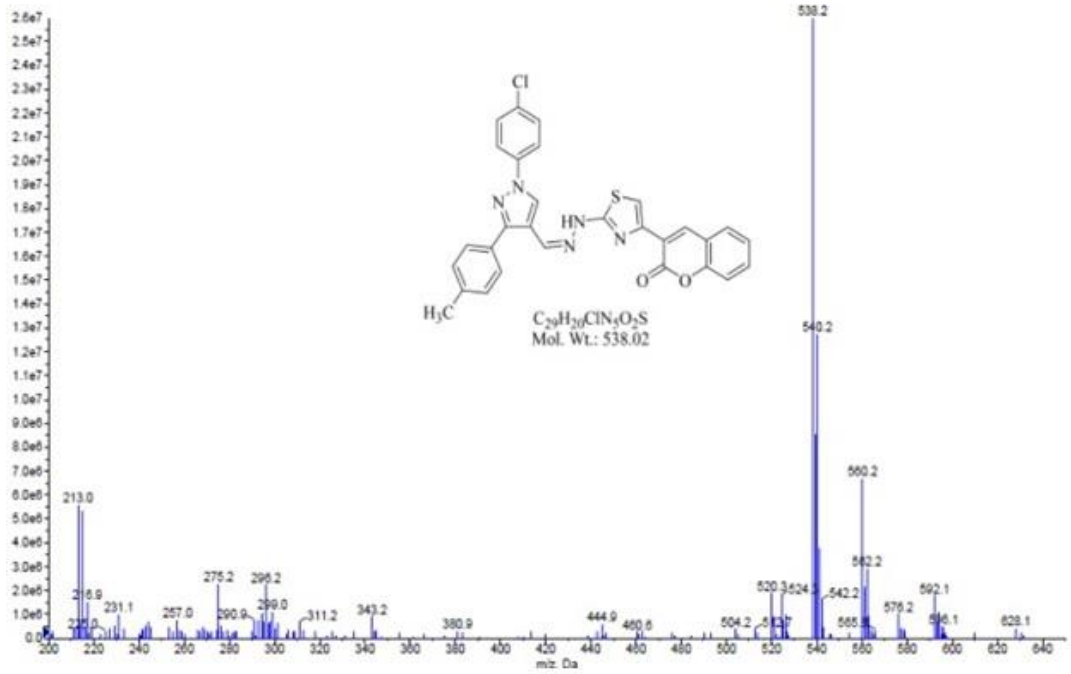


Figure 1: Mass Spectrum of compound CH-4.

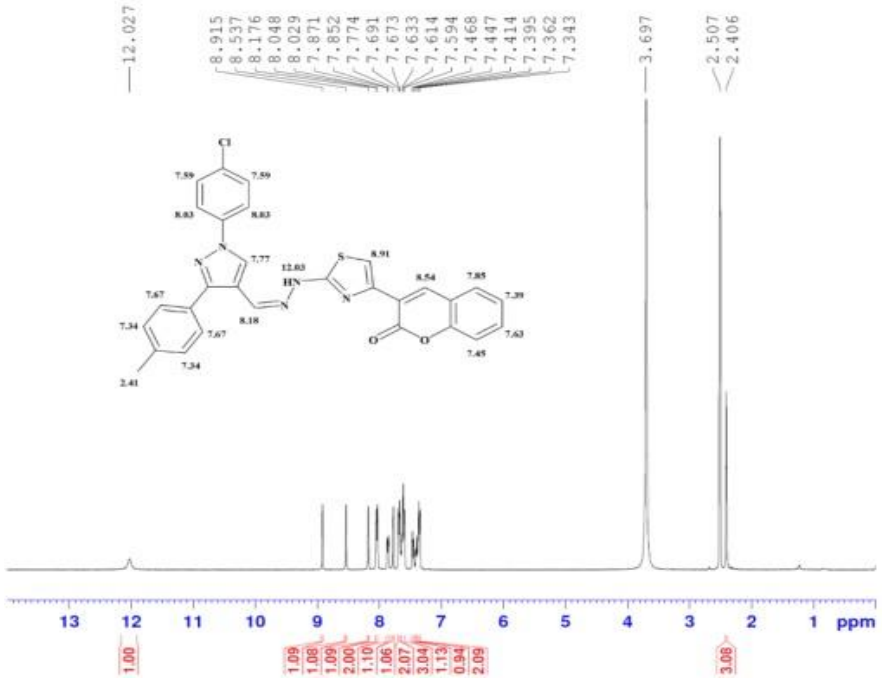
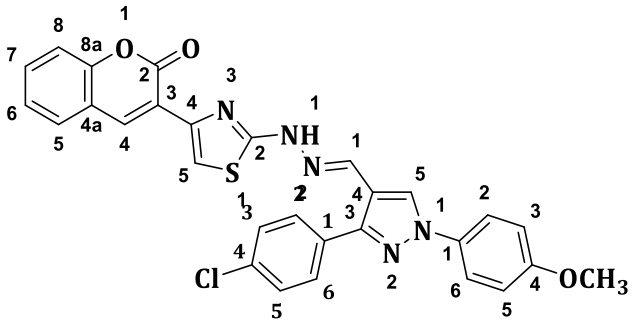


Figure 2: 1HNMR spectrum of Compound CH-4.

5. COMPOUND CODE: - CH-5



IUPAC Name

(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{29}H_{20}ClN_5O_3S$

Molecular Weight: 554.02

Elemental Analysis

Elements	C	N	O	S
Calculated	62.88	12.68	8.68	5.82
Found	62.87	12.64	8.66	5.79

IR (cm^{-1})

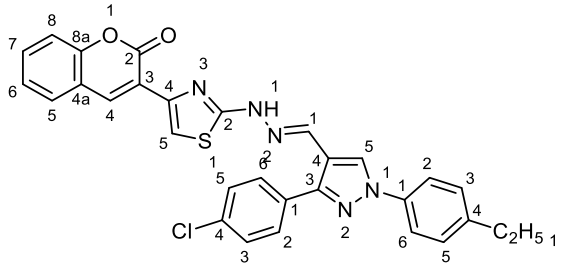
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3411.22	(N-H)
2.	3239.25	Ar-H,
3.	1720.21	(C=O)
4.	1626.24	(C=N)
5.	1496.32	(C=C)
6.	1095.22	(C-O)
7.	848.32	(C-Cl)

1HNMR (ppm)

δ 7.36-7.40 (m, 2H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.78 (s, 1H), 7.79-8.02 (d, 2H), 7.85-7.87 (d, 1H), 7.98-8.00 (d, 2H), 8.17 (s, 1H, -CH=N), 8.53 (s, 1H), 8.92 (s, 1H), 11.57 (s, 1H, -NH); 3H, 3.82 (s, -OCH3)

FAB Mass (m/z): 553.10

6. COMPOUND CODE: - CH-6



IUPAC NAME

(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-ethylphenyl)-1H-pyrazol-4-yl)methylene)hydrazine -yl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{30}H_{22}ClN_5O_2S$

Molecular Weight: 552.05

Elemental Analysis

Elements	**C**	**N**	**O**	**S**
Calculated	65.27	12.72	5.82	5.81
Found	65.27	12.69	5.80	5.81

IR (cm^{-1})

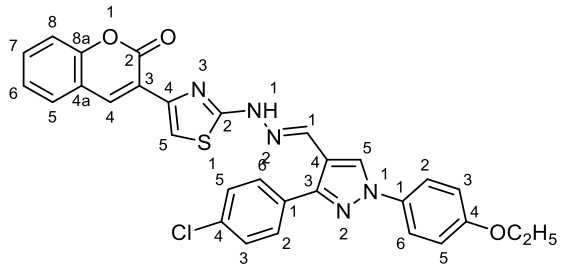
SN	**Propagation number (cm^{-1})**	Mode of vibration
1.	3411.23	(N-H)
2.	3242.22	Ar-H,
3.	1718.25	(C=O)
4.	1622.32	(C=N)
5.	1496.23	(C=C)
6.	1092.23	(C-O)
7.	851.12	(C-Cl)

1HNMR (ppm)

δ 7.36-7.40 (m, 2H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.75 (s, 1H), 7.77-7.79 (d, 2H), 7.86-7.88 (d, 1H), 7.98-8.02 (d, 2H), 8.21 (s, 1H, -CH=N), 8.53 (s, 1H), 8.90 (s, 1H), 11.57 (s, 1H, -NH); δ 2.29 (s, 6H, CH3), 4.16 (s, 2H, CH2).

FAB Mass (m/z): 551.12

7. COMPOUND CODE: - CH-7



IUPAC name

(E)-3-(2-(2-((3-(4-chlorophenyl)-1-(4-ethoxyphenyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{30}H_{22}ClN_5O_3S$

Molecular Weight: 568.05

Elemental Analysis

Elements	C	N	O	S
Calculated	63.45	12.35	8.46	5.68
Found	63.43	12.33	8.45	5.64

IR (CM^{-1})

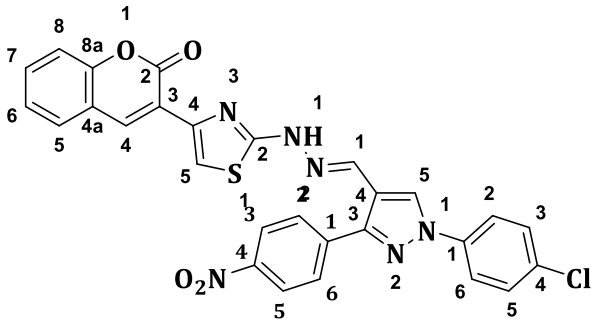
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3410.32	(N-H)
2.	3239.26	Ar-H,
3.	1721.12	(C=O)
4.	1629.15	(C=N)
5.	1499.14	(C=C)
6.	1094.12	(C-O)
7.	851.02	(C-Cl)

1HNMR (ppm)

δ 7.38-7.40 (m, 2H, Ar-H), 7.78 (s, 1H), 7.78-8.02 (d, 2H), 7.85-7.87 (d, 1H), 7.98-8.10 (d, 2H), 8.19 (s, 1H, -CH=N), 8.52 (s, 1H), 8.90 (s, 1H), 11.57 (s, 1H, -NH); δ 2.29 (s, 6H, CH3), 3.37 (s, 6H, CH3), 4.16 (s, 2H, CH2),

FAB Mass (m/z): 567.11

8. COMPOUND CODE: - CH-8



IUPAC name

(E)-3-(2-(2-((1-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazine yl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{28}H_{17}ClN_6O_4S$

Molecular Weight: 568.99

Elemental Analysis

Elements	**C**	**N**	**O**	**S**
Calculated	59.12	14.78	11.28	5.65
Found	59.11	14.77	11.25	5.63

IR (cm^{-1})

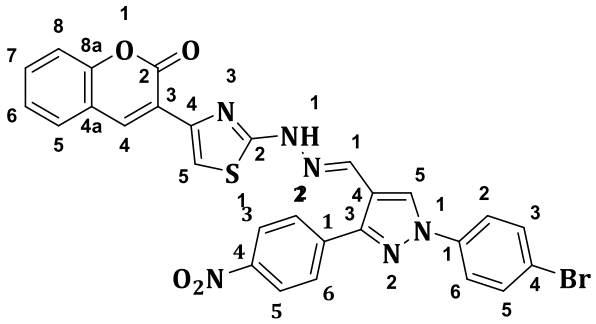
SN	**Propagation number (cm^{-1})**	Mode of vibration
1.	3418.35	(N-H)
2.	3245.56	Ar-H,
3.	1717.33	(C=O)
4.	1624.55	(C=N)
5.	1496.23	(C=C)
6.	1097.32	(C-O)
7.	850.22	(C-Cl)
8.	1364.11	(N-O)
9.	1570.24	(N=0)

1HNMR (ppm)

δ 7.37-7.40 (m, 2H, Ar-H), 7.58-7.62 (m, 1H, Ar-H), 7.78 (s, 1H), 7.75-7.78 (d, 2H), 7.82-7.84 (d, 1H), 7.98-8.02 (d, 2H), 8.18 (s, 1H, -CH=N), 8.52 (s, 1H), 8.90 (s, 1H), 12.03 (s, 1H, -NH); *δ* 3.35 (s, 6H, CH3), 4.21 (s, 2H, CH2),

FAB Mass (m/z): 568.07

9. COMPOUND CODE: - CH-9



IUPAC name

(E)-3-(2-(2-((1-(4-bromophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazine yl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{28}H_{17}BrN_6O_4S$

Molecular Weight: 613.45

Elemental Analysis

Elements	C	N	O	S
Calculated	54.85	13.70	10.46	5.25
Found	54.82	13.70	10.43	5.23

IR (cm^{-1})

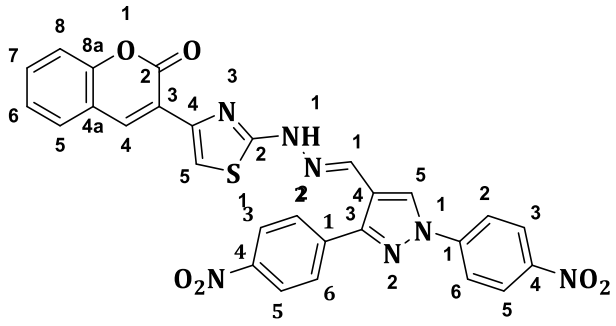
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3422.35	(N-H)
2.	3247.26	Ar-H,
3.	1721.13	(C=O)
4.	1625.15	(C=N)
5.	1498.23	(C=C)
6.	1097.22	(C-O)
7.	851.28	(C-Cl)
8.	1019.37	(C-Br)
9.	1365.18	(N-O)
10.	1571.24	(N=0)

1HNMR (ppm)

δ 7.38-7.42 (m, 2H, Ar-H), 7.62-7.65 (m, 1H, Ar-H), 7.78 (s, 1H), 7.76-7.79 (d, 2H), 7.85-7.87 (d, 1H), 7.98-8.04 (d, 2H), 8.21 (s, 1H, -CH=N), 8.56 (s, 1H), 8.90 (s, 1H), 11.57 (s, 1H, -NH); δ 2.27 (s, 6H, CH3), 4.12 (s, 2H, CH2),

FAB Mass (m/z): 611.57

10. COMPOUND CODE: - CH-10



IUPAC name

(E)-3-(2-(2-((1,3-bis(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{28}H_{17}N_7O_6S$

Molecular Weight: 579.55

Elemental Analysis

Elements	C	N	O	S
Calculated	58.04	16.94	16.58	5.53
Found	58.03	16.92	16.56	5.53

IR (cm^{-1})

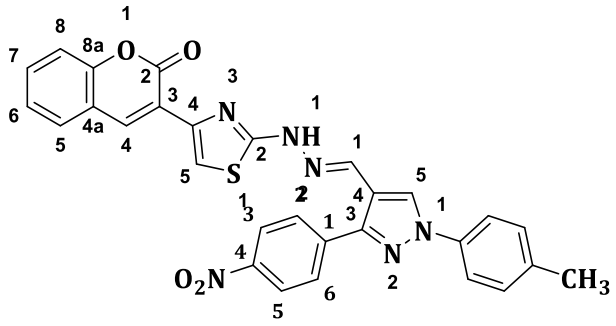
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3420.35	(N-H)
2.	3243.26	Ar-H,
3.	1721.13	(C=O)
4.	1625.25	(C=N)
5.	1496.23	(C=C)
6.	1092.32	(C-O)
7.	1364.28	(N-O)
8.	1571.24	(N=0)

1HNMR (ppm)

δ 7.36-7.40 (m, 2H), 7.62-7.63 (m, 1H), 7.78 (s, 1H, pyrazol-5H), 7.77-7.79 (d, 2H), 7.88-7.90 (d, 1H), 7.98-8.04 (d, 2H), 8.21 (s, 1H, -CH=N), 8.56 (s, 1H), 8.91 (s, 1H), 11.57 (s, 1H, -NH); 3.35 (s, 6H, CH3), 4.21 (s, 2H, CH2),

FAB Mass (m/z): 579.10

11. COMPOUND CODE: - CH-11



IUPAC name

(E)-3-(2-(2-((3-(4-nitrophenyl)-1-(p-tolyl)-1H-pyrazol-4-yl)methylene)hydrazineyl)thiazo l-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{29}H_{20}N_6O_4S$

Molecular Weight: 548.58

Elemental Analysis

Elements	C	N	O	S
Calculated	63.52	15.32	11.68	5.86
Found	63.50	15.32	11.67	5.84

IR (cm^{-1})

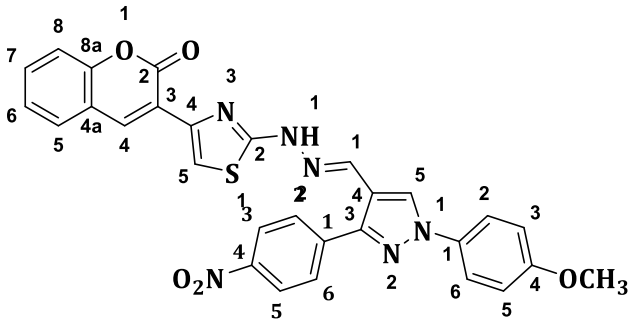
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3419.25	(N-H)
2.	3245.22	Ar-H,
3.	1721.13	(C=O)
4.	1625.15	(C=N)
5.	1496.32	(C=C)
6.	1097.28	(C-O)
7.	1364.28	(N-O)
8.	1572.42	(N=0)

1HNMR (ppm)

δ 7.37-7.42 (m, 2H, Ar-H); 7.75 (s, 1H), 7.77-7.79 (d, 2H), 7.82-7.85 (d, 1H), 7.98-8.04 (d, 2H), 8.17 (s, 1H, -CH=N), 8.52 (s, 1H), 8.91 (s, 1HH), 11.57 (s, 1H, -NH); δ 2.27 (s, 6H, CH3), 4.21 (s, 2H, CH2).

FAB Mass (m/z): 548.13

12. COMPOUND CODE: - CH-12



IUPAC Name

(E)-3-(2-(2-((1-(4-methoxyphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{29}H_{20}N_6O_5S$

Molecular Weight: 564.58

Elemental Analysis

Elements	C	N	O	S
Calculated	61.75	14.90	14.19	5.68
Found	61.70	14.89	14.17	5.68

IR (cm^{-1})

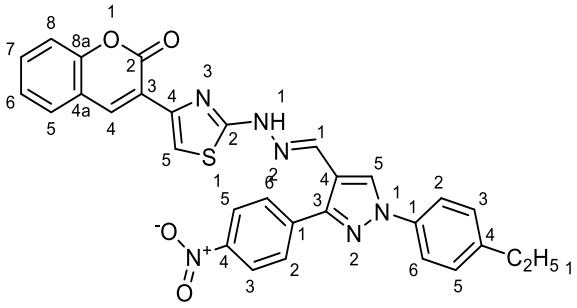
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3415.15	(N-H)
2.	3245.26	Ar-H,
3.	1721.33	(C=O)
4.	1624.21	(C=N)
5.	1495.13	(C=C)
6.	1098.22	(C-O)
7.	1364.28	(N-O)
8.	1572.25	(N=0)

1HNMR (ppm)

δ 7.38-7.42 (m, 2H, Ar-H); 7.78 (s, 1H), 7.79-8.01 (d, 2H), 7.85-7.87 (d, 1H), 7.98-8.02 (d, 2H), 8.18 (s, 1H, -CH=N), 8.52 (s, 1H), 8.90 (s, 1H), 11.57 (s, 1H, -NH); δ 2.25 (s, 6H, CH3), 4.12 (s, 2H, CH2), 3.86 (s, 3H, OCH3),

FAB Mass (m/z): 564.12

13. COMPOUND CODE: - CH-13



IUPAC NAME

(E)-3-(2-(2-((1-(4-ethylphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazine yl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{30}H_{22}N_6O_4S$

Molecular weight: 562.60

Elemental Analysis

Elements	C	N	O	S
Calculated	64.05	14.96	11.38	5.72
Found	64.05	14.94	11.37	5.70

IR (cm^{-1})

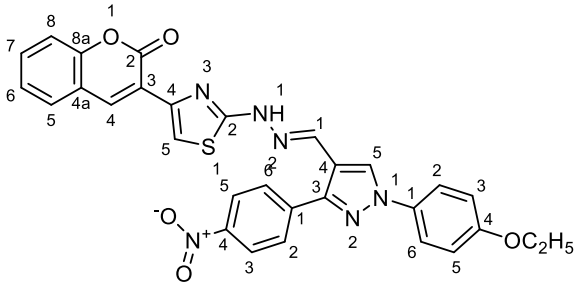
SN	Propagation number (cm^{-1})	Mode of vibration
1.	3419.15	(N-H)
2.	3242.26	Ar-H,
3.	1721.23	(C=O)
4.	1625.25	(C=N)
5.	1494.23	(C=C)
6.	1096.32	(C-O)
7.	1365.28	(N-O)
8.	1571.24	(N=0)

1HNMR (ppm)

δ 7.38-7.40 (m, 2H, Ar-H), 7.78 (s, 1H), 7.77-7.79 (d, 2H), 7.86-7.89 (d, 1H), 7.98-8.02 (d, 2H), 8.21 (s, 1H, -CH=N), 8.52 (s, 1H), 8.90 (s, 1H), 12.03 (s, 1H, -NH); δ 2.27 (s, 6H, CH3), 4.21 (s, 2H, CH2),

FAB Mass (m/z): 562.14

14. COMPOUND CODE: - CH-14



IUPAC name

(E)-3-(2-(2-((1-(4-ethoxyphenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)thiazol-4-yl)-2H-chromen-2-one

Chemical Formula: $C_{30}H_{22}N_6O_5S$

Molecular Weight: 578.60

Elemental Analysis

Elements	C	N	O	S
Calculated	62.32	14.55	13.85	5.56
Found	62.28	14.52	13.83	5.54

IR (cm^{-1})

SN	Propagation number (cm^{-1})	Mode of vibration
1.	3420.35	(N-H)
2.	3242.56	Ar-H,
3.	1721.33	(C=O)
4.	1625.55	(C=N)
5.	1495.23	(C=C)
6.	1092.32	(C-O)
7.	1360.38	(N-O)
8.	1572.14	(N=0)

1HNMR (ppm)

δ 7.37-7.40 (m, 2H, Ar-H), 7.76 (s, 1H), 7.76-7.79 (d, 2H), 7.84-7.87 (d, 1H), 7.98-8.02 (d, 2H), 8.21 (s, 1H, -CH=N), 8.52 (s, 1H), 8.91 (s, 1H), 12.03 (s, 1H, -NH); δ 2.27 (s, 6H, CH3), 4.21 (s, 2H, CH2).

FAB Mass (m/z): 578.14

2.4. Evaluation of antimicrobial activity

There should be reliable and repeatable procedures for evaluating antimicrobial activity in order to investigate and assess it. Agar-streak dilution technique, Serial dilution method, and Agar-diffusion method are the several antimicrobial activity assessment methods accessible.

[53].

***In vitro* Antitubercular activity –Microplate Alamar Blue Assay (MABA)method**

The Middle brook 7H9 broth was used to assess antitubercular screening for the newly synthesised compounds CH1-CH14 against Mycobacterium TB H37Rv strain (ATCC-27294). The MABA technique was used to calculate the Minimum Inhibitory Concentration (MIC) of each produced chemical [15]

Luciferase Reporter Phase (LRP) assay

The antitubercular potential of the fourteen generated compounds was evaluated using the LRP test for Mycobacterium TB H37Rv strains and Clinical isolate: S, H, R, and E resistant M. tuberculosis (MDR) [16].

3. RESULT AND DISCUSSION

Microplate Alamar Blue Assay (MABA)method

The antitubercular potential of the newly synthesized compounds CH1-CH14 was investigated. The MIC value of the synthesized compounds against the standard antitubercular medication is determined using the serial dilution based MABA technique. Figure 1 shows the MIC of the produced compounds against the pathogenic microorganisms Mycobacterium TB H37Rv. All of the chemicals were evaluated against the pathogenic Mycobacterium TB H37Rv strain at doses ranging from 0.78 to 100 g/mL. Ten (10) compounds out of fourteen showed actions with MICs ranging from 1.25 to 25 g/ml in Mycobacterium TB H37Rv strains, while nine (09) compounds showed activity with MICs ranging from 2.50 to 25 g/ml in Clinical isolate: M. tuberculosis resistant to S, H, R, and E (MDR).

Table 4: Minimum Inhibition Concentration (MIC) of synthesized compounds

Compound	Minimum Inhibition Concentration (MIC)	
	***Mycobacterium tuberculosis* $H_{37}Rv$**	**Clinical isolate: S, H, R & E resistant *M. tuberculosis* (MDR)**
CH-1	2.25 µg/ml	2.50 µg/ml
CH-2	25.0 µg/ml	25.0 µg/ml
CH-3	1.75 µg/ml	2.25 µg/ml
CH-4	12.5 µg/ml	12.5 µg/ml
CH-5	25.0 µg/ml	50.0 µg/ml
CH-6	50.0 µg/ml	100 µg/ml
CH-7	60.0 µg/ml	100 µg/ml
CH-8	1.75 µg/ml	2.50 µg/ml
CH-9	2.50 µg/ml	5.00 µg/ml
CH-10	2.25 µg/ml	4.00 µg/ml

CH-11	12.5 µg/ml	25.0 µg/ml
CH-12	20.0 µg/ml	22.5 µg/ml
CH-13	100 µg/ml	100 µg/ml
CH-14	100 µg/ml	100 µg/ml
Isoniazid	1.25 µg/ml	2.25 µg/ml
Rifampicin	6.25 µg/ml	8.25 µg/ml
Ethambutol	3.12 µg/ml	4.12 µg/ml
Pyrazinamide	50.0 µg/ml	65.0 µg/ml

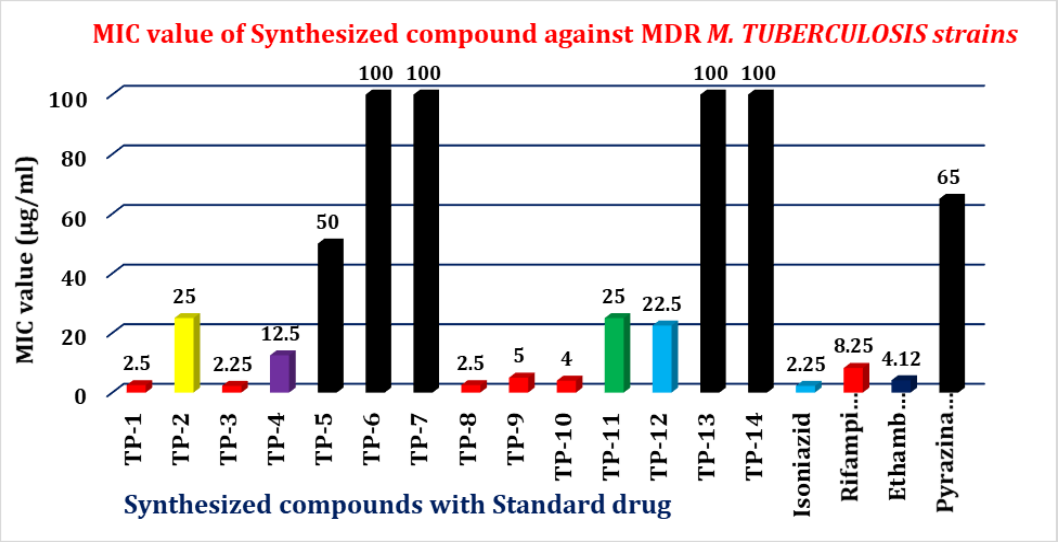


Figure 3: MIC value of Synthesized compound against MDR *M. Tuberculosis* strains.

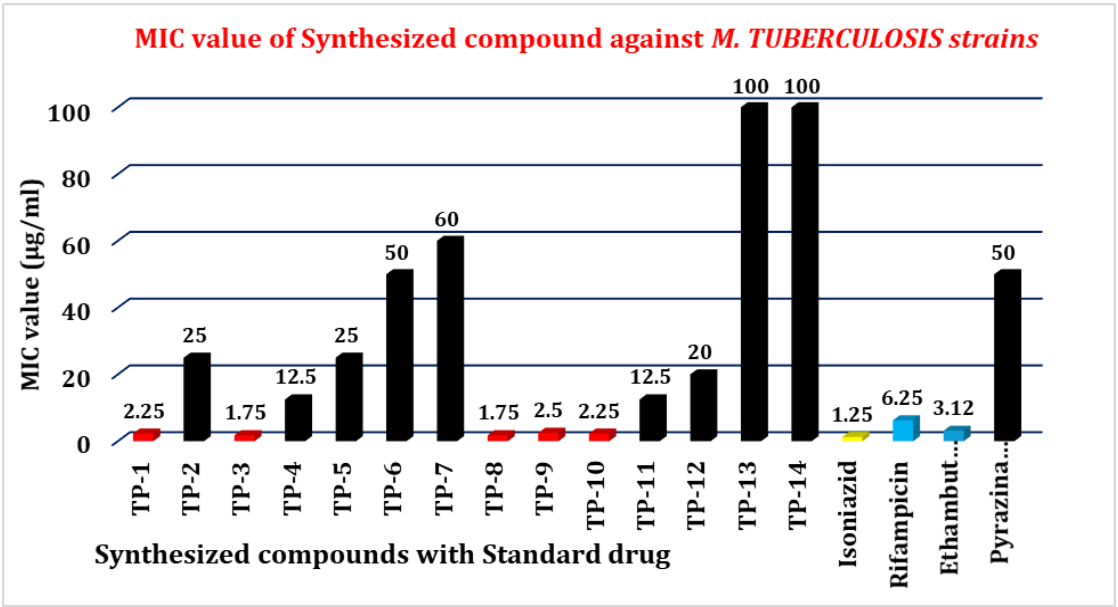


Figure 4: MIC value of Synthesized compound against *M. Tuberculosis* strains.

Luciferase Reporter Phase (LRP) assay

A total of 14 substances were investigated for antitubercular activity using the Luciferase reporter phase assay technique in this study. The synthesised compounds CH-1, CH-3, CH-8,

CH-10, and CH-11 showed more than a 50% decrease in Relative Light Units at 50 g/ml concentrations against M. tuberculosis H37RV and Clinical isolate MDR M. tuberculosis, however compounds CH-2, CH-4, CH-5, CH-6, CH-7, CH-9, CH-12, CH-13, and CH-14 At 50 g/ml concentration, 5 (five) compounds are active antitubercular drugs, whereas 09 (nine) compounds are inert (less than 50% decrease in RLU).

Table 5: Percent reduction in relative light units. (RLU)

Compound	**Percent reduction in RLU**					
	***Mycobacterium tuberculosis* $H_{37}Rv$**			**Clinical isolate: S, H, R & E resistant *M. tuberculosis* (MDR)**		
	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
CH-1	65	75	99	62	72	95
CH-2	40	65	78	38	62	75
CH-3	55	68	91	52	62	88
CH-4	42	52	78	38	59	75
CH-5	38	53	75	32	65	72
CH-6	22	45	49	20	42	60
CH-7	22	40	46	20	45	58
CH-8	72	82	98	65	72	95
CH-9	45	65	71	33	42	68
CH-10	75	88	96	74	82	92
CH-11	60	65	75	55	68	72
CH-12	33	54	72	38	48	68
CH-13	24	36	46	24	42	49
CH-14	22	38	45	25	44	48
Rifampicin	75.00			65.44		
Isoniazid	65.25			45.22		
Ethambutol	39.88			18.50		

*Where S- StreCHomycin, H- Isoniazid, R- Rifampicin, E- Ethambutol and MDR- Multi Drug Resistant.

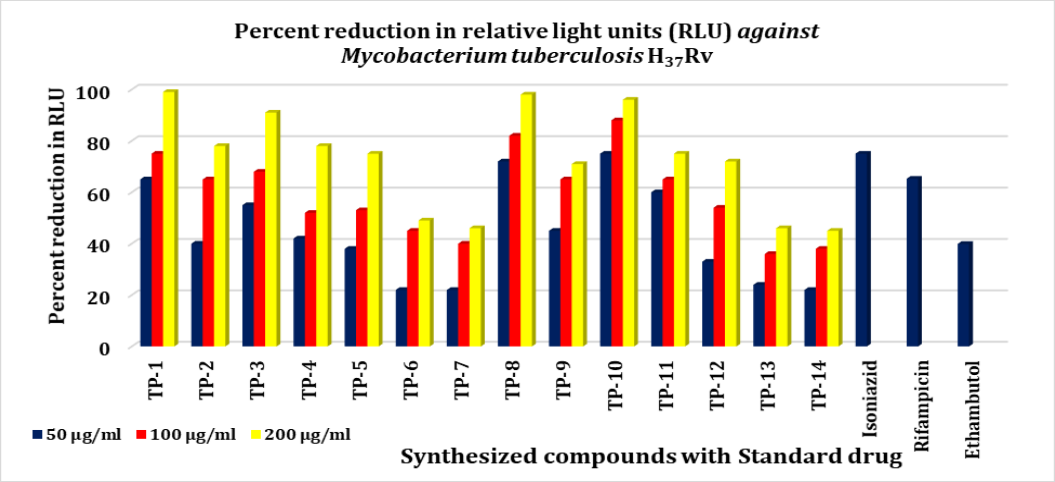


Figure 5: Percent reduction in relative light units (RLU) *against Mycobacterium tuberculosis* $H_{37}Rv$

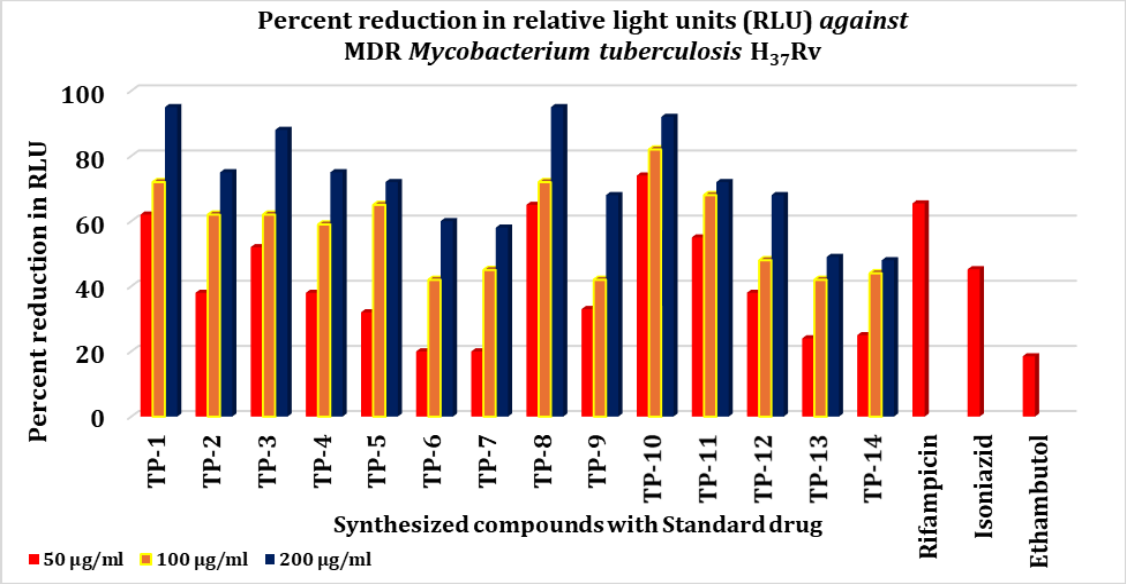


Figure 6: Percent reduction in relative light units (RLU) *against* MDR *Mycobacterium tuberculosis* $H_{37}Rv$

4. CONCLUSION

A novel series of fourteen pyrazole-thiazole-chromenone derivatives was successfully synthesized and structurally characterized using IR, 1H NMR, FAB-mass spectroscopy, and elemental analysis. The synthesized compounds were evaluated for their antitubercular activity against *Mycobacterium tuberculosis* H37Rv and multidrug-resistant (MDR) clinical isolates using MABA and Luciferase Reporter Phage assays. Several compounds exhibited promising antitubercular activity, particularly CH-1, CH-3, CH-8, CH-10, and CH-11. Compounds CH-3 and CH-8 demonstrated excellent inhibitory activity with MIC values of 1.75 µg/mL against H37Rv, while CH-10 showed the highest reduction in bacterial viability in the LRP assay. The presence of electron-withdrawing substituents, especially nitro and halogen groups, appeared to enhance antitubercular potency. Overall, the results indicate that these pyrazole-based hybrids possess significant potential as lead candidates for the development of new antitubercular agents. Further studies involving molecular docking, toxicity evaluation, pharmacokinetic profiling, and *in vivo* biological investigations are warranted to establish their therapeutic potential.

5. REFERENCES

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