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Synthesis and Study of the Biological Activity of Some Cyclohexenone Derivatives

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Abstract: ***In this work, pyrazoline (A1) was prepared from reaction ethyl acetoacetate and 2-hydrazeno benzothiazol that was prepared from (reaction 2-amino benzothiazole with hydrazine hydrate and HCl). Chalcone (α, β-unsaturated compounds) (A_{2-10}) have been prepared by two methods, the first of the reaction of appropriate aromatic aldehyde derivatives with pyrazoline (ketone) and sodium hydroxide in ethanol, and the second method using aromatic aldehyde derivatives with pyrazoline and Sodium acetate inglacial acetic acid. The Cyclohexenon derivatives (A_{11-19}) were obtained from reaction Chalcones (A_{2-10}) with ethyl acetoacetate in presence of 10%NaOH.All these synthesized compounds were characterized by some physical properties such as the color and melting point and spectrophmatic methods using (IR, ^{13}C-NMR and ^{1}H-NMR). The study has shown biological activity for chemical compounds, at three concentrations 0.01, 0.001, 0.0001) mg/ml The minimum inhibitory concentration [MIC] have been determined by the reference of stander drugs the results showed that the cyclohexenone derivatives are better than growth of both types of bacteria (gram- positive and gram–negative) compared to drugs.***

Keywords: cyclohexenone, Pyrazoline, biological activity, chalcones, α, β-unsaturated compounds

1. Introduction

Chalcones are natural products that can also be obtained synthetically using a relatively simple synthesis procedure. The general method applied to synthesize chalcones is the Claisen-Schmidt reaction. An important feature of chalcones is their ability to act as an intermediate for the synthesis of biologically active heterocyclic compounds such as, pyridine, pyrimidine, cyclohexenone, pyrazoline and isoxazoline derivatives [1, 2].The Michael reaction of chalcones with active methylene compounds such as 1, 3-dicarbonyls have been the subject of many investigations.[3–6] It is known that a weak base or acid such as piperidine [7] or phosphorus trichloride [8] often affords open chain adducts, while cyclic products have been obtained in the presence of sodium methoxide [9] or sodium hydroxide. [10]

Chalcones represent an essential group of natural as well as synthetic products and some of them have wide range of pharmacological activity such as anti-inflammatory, anti-fungal, antibacterial and anti-oxidant agents [11-13]. The indazole moiety has a great interest in the biological field, including antiviral [14], anticancer [15] and antihypertensive [16]. Al-Bogami [17] reported the synthesis and antibacterial activity of new indazole derivatives. The aim of the work is a synthesis of chalcone derivatives fused with cyclohexenone via pyrazoline as an intermediate.

2. Materials and Methods

Chemicals and reagent

Pyrazoline**,** Various aldehyde, Ethanol, NaOH, Ethyl acetoacetate, Sodium acetate glacial acetic acid.

Experimental

All the chemicals and solvents used were of Aldrich and Fluka products and were used without further purification**.** Melting points are uncorrected and were recorded in an open capillary tube on Stuart melting point apparatus. Infrared spectra have been recorded on a Shimadzo FTIR-8100 spectrophotometer using KBr discs–, ^{1}H-NMR Spectra have been measured on a MH_Z spectrometer using (DMSO-d_6) as solvent and ^{13} C-NMR Spectra have been measured on a MH_Z spectrometer using (DMSO-d_6) as solvent. Reaction monitoring and verification of the purity of the compounds was done by TLC on silica gel-percolated alumni sheets (type 60 F254 Merck, Darmstadt, Germany).

1-Preparation of 2- (benzothiazol-2-yl) -5-methyl-2, 4-dihydro-3H-pyrazol-3-one (A_1) [18]

In a conical flask puts (0.01mol) from ethyl acetoacetate and added to conical gradually (0.01mol) from 2-hydrazino benzothiazole dissolved in (40mL) from ethanol with stirring at a temperature of less than (60 °C), leaves the mixture for (80 min) at temperature of no exceeding (60 °C), The mixture cools or leaves in the freezer to give compound.

2-Preparation of: E) -2- (benzo[d]thiazol-2-yl) -4-(sub.benzylidene) -5-methyl-2, 4-dihydro-3H-pyrazol-3-one (A_{2-10}) Method (A) [19]

A mixture of appropriate pyrazoline (0.01mol) and aromatic benzaldehyde derivatives (0.01mol) have been added to a solution of (10%) sodium hydroxide (5mL), and (3mL) of ethanol. The mixture was stirred for (2-3) hr. at (20-40) $^{\circ}$ C and kept in a refrigerator for (12) hr. Then it was diluted with ice-cold distilled water (30mL), filtered washed with cold water, dried and recrystallized from ethanol. The physical properties are shown in Table (1)

Method

A mixture of pyrazoline (0.01 mol) and substituted aromaticaldehydes (0.01 mol) and Sodium acetate (0.02 mol) was stirred in glacial acetic acid (15 mL). The mixture was poured into crushed ice. The solid separated was filtered. The physical properties are shown in Table (1)

3-Preparation of: ethyl 1- (benzo[d]thiazol-2-yl) -4-(Sub.phenyl) -3-methyl-6-oxo-3a, 4, 5, 6-tetrahydro-1H-indazole-5-carboxylate (A_{11-19}) [20]

A mixture of chalcone (3 mmol) and ethyl acetoacetate (0.40 mL, 3 mmol) was refluxed for 2 hrs in 10-15 mL ethanol in presence of 0.5 mL 10% NaOH. The reaction mixture was

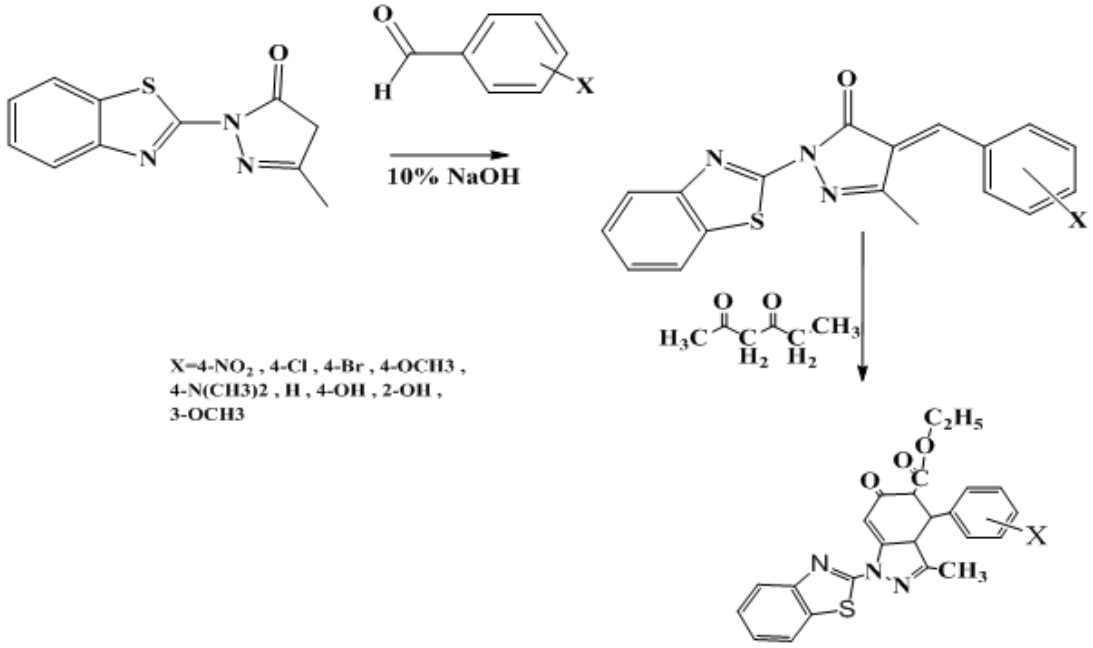
then poured with good stirring into 200 mL ice-cold water and kept at room temperature until the reaction product separated as a solid, which was filtered off and recrystallized from ethanol. In synthesis twin compounds, a twice mole from ethyl acetoacetate and twice amount of 10% NaOH was used**.** The physical properties are shown in Table (2)

3. Result and Discussion

The synthesis of chalcones and cyclohexenone derivatives were performed as Shown in scheme (1). Pyrazoline (A_1) was prepare from reaction of 2-hydrazino benzothiazole with ethyl acetoacetate, the IR spectra of compound (A_1) showed characteristic (C=O) stretching at (1724cm^{-1}) and (C=C) stretching frequencies at (1589 cm^{-1}), and band at (1554cm^{-1}) for **(C C)** group and band at (3062cm^{-1}) for (Ar-H) group and a band at (1604cm^{-1}) for (C=N) group. ^{1}HNMR Spectrum of compound (A_1)**,** (Figure 1), Showed the following signals: A singlet signal at (δ=2.01ppm) due to a protons of (3H-CH_3) group, multi signal at (δ=7.02-8.06ppm) due to a protons of phenyl, and signal at (3.57ppm) for (2H, CH_2). The reaction of pyrazoline with aromatic aldehydes yielded the compounds chalcone ($A_{2\text{-}10}$), the IR spectra of compounds ($A_{2\text{-}10}$) showed characteristic (C=O) stretching at (1639-1705cm^{-1}), (**C C**) stretching frequencies at (1514-1525cm^{-1}), band at (3057-3068cm^{-1}) for (Ar-H) group and a band at (1589-1620cm^{-1}) for (C=N) group the IR data showed in the (table3).^{1}HNMR Spectrum of compound (A_5)**,** (Figure 2), Showed the following signals: a singlet signal at (δ 2.23 ppm) due to a proton of (3H-CH_3) group, and multi signal at (δ 7.28-7.96) ppm due to a proton of phenyl, signal at (4.65 ppm) for (C=CH)) figure (2). ^{13}C-NMRSpectrum of compound (A_5)**,** (Figure 3), Showed the following signals : single at (28.23ppm) for atom of C in (CH_3), and single at (119.01-145.04 ppm) for atoms in ring benzene, and single at (192.84ppm) for atom of C2 in ring benzothiazole and single at (158.11ppm) for atom C in (C=O), and single at (151.55ppm) for atom C in (C=N), and single at (139ppm) for atom CH in (CH=C), and single at (129.86ppm) for atom C in (CH=C).

The reaction of chalcone ($A_{2\text{-}10}$) with ethyl acetoacetate yielded the compound scyclohexenone derivatives ($A_{11\text{-}19}$). The IR spectral data of these compounds ($A_{11\text{-}19}$) showed band at (1525-1514cm^{-1}) due to stretching (**(C C)**) group, and a band at (1658-1583 cm^{-1}) for (C=N) group, and (C=O) stretching at (1641-1781). A band at (3074-3055) for (Ar-H) group, and other bands. The IR data showed in the Table (4). H^1 NMR spectrum of compound (A_{14}), (Figure 4), showed the following signals : signal at (δ1.23PPM) could be attributed to three protons of (3H, CH3) in CH_3CH_2O group, signals at (δ 2.17 ppm) could be attributed to three protons of (3H, CH_3) group-pyrazoline, signal at (δ2.51 PPM) for (1H, CH) in ring pyrazoline, signal at ((δ3.34 PPM) for (1H, CH) (in C5, Hexenon), signal at (δ3.91PPM) for (1H, CH) (in C4, Hexenon), signal at (δ4.05PPM) for (1H, CH) (in C3, Hexenon), signal at (((δ5.17PPM) for (1H, CH) (in C1, Hexenon), signal at (δ4.50PPM) for (2H, CH_2), many signals (aromatic protons) appeared in the region (7.35to 8.18ppm).

The^{13}C-NMRSpectrum of compound (A_{14} **),** (Figure 5), Showed the following signals : single at (11.23ppm) for atom of C in (CH_3) (CH_3CH_2O), single at (14.10ppm) for atom of C in (CH_3) (pyrazoline), single at (121.20-152.69ppm) for atoms in ring benzene, single at (104.93ppm, C1in ring Hexenon), single at (60.61ppm, C3in cycle Hexenon), single at (61.66ppm, C5in cycle Hexenon), single at (201ppm, C=O in cycle Hexenon), single at (78.99ppm, CH_2 in OCH_2CH_3), single at (177ppm, C2inBenzothizole), single at (167.86ppm, C=O in ester), (167.57ppm, C-Nin pyrazoline), and single at (161.25ppm, C=Nin pyrazoline).



Scheme 1: synthesis of chalcone derivatives ($A_{2\text{-}10}$) and cyclohexenone derivatives ($A_{11\text{-}19}$)

Evaluation of biological activity

All the synthesized compounds were tested for their antimicrobial activity against Gram negative bacteria (Pseudomonas aeruginosa) and Gram positive bacteria (*Proteusspp*) using the agar diffusion method [21]. Each compound was dissolved in DMSO to give concentration 1ppm. The plates were then incubated at 37 0C and examined after 24 hrs. The inhibition zone diameter in mm

(IZD) was used as a criterion for the antimicrobial activity. The lowest concentration required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC, µg/mL), was determined for all the compounds and compared with the control. The investigation of antibacterial screening data revealed that cyclohexenone derivatives (A12, A13, A14, A15, A16, A17) Compounds. The maximum activity (MIC = 12.5 µg/mL) was indicated for compounds. he results are summarized in Table (5)

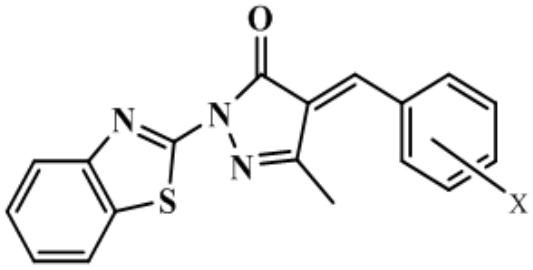


Table 1: The physical properties of compounds (A_2-A_{10})

Comp. No.	X	Molecular formula	MP (C) ^{0}	Yield	Color
A_2	H	$C_{18}H_{13}N_3OS$	206 – 208	%72	Yellow
A_3	4-OH	$C_{18}H_{13}N_3O_2S$	218 – 220	%54	Yellow
A_4	4-Cl	$C_{18}H_{12}N_3OSCl$	197 – 200	%66	Orang
A_5	4-Br	$C_{18}H_{12}N_3OSBr$	210 – 213	%67	Pink
A_6	4-OH, 3-OCH_3	$C_{19}H_{15}N_3O_3S$	215-219	%57	Yellow
A_7	4-NO_2	$C_{18}H_{12}N_4O_3S$	212 dss	%56	Orang
A_8	2-OH	$C_{18}H_{13}N_3O_2S$	216 – 218	%71	Yellow
A_9	4-N $(CH_3)_2$	$C_{20}H_{18}N_4OS$	177-180	%38	Orang
A_{10}	4-OCH_3	$C_{19}H_{15}N_3O_2S$	215 – 220	%75	Yellow

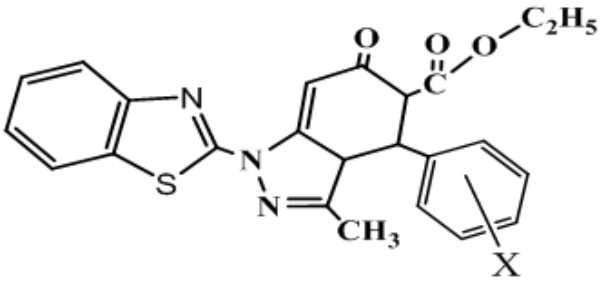


Table 2: The physical properties of compounds (A_{11}-A_{19})

Comp NO	X	Molecular Formula	M.P (°C)	Yield	Color
A_{11}	4-Br	$C_{24}H_{20}N_3O_3SBr$	147-150	%78	Pink
A_{12}	4-Cl	$C_{24}H_{20}N_3O_3SCl$	136-140	%66	Pink
A_{13}	4-N $(CH_3)_2$	$C_{26}H_{26}N_4O_3S$	225-230	%66	Orang
A_{14}	4-NO_2	$C_{24}H_{20}N_4O_5S$	132-135	%77	Red
A_{15}	4-OCH_3	$C_{25}H_{23}N_3O_4S$	127-130	%65	Yellow
A_{16}	4-OH	$C_{24}H_{21}N_3O_4S$	240-245	%52	Yellow
A_{17}	H	$C_{24}H_{21}N_3O_3S$	196-200	%22	Pink
A_{18}	4-OH, 3-OCH_3	$C_{25}H_{23}N_3O_5S$	187-190	%40	White
A_{19}	2-OH	$C_{24}H_{21}N_3O_4S$	136-140	%54	Orang

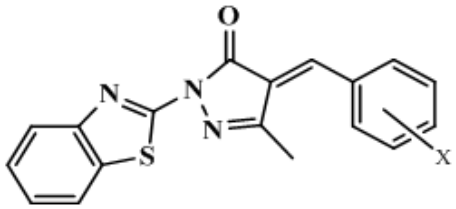


Table 3: IR –spectral data of compounds (A_2-A_{10})

Comp .No	X	FT.IR cm^{-1} (KBr)				
		υ (C=N)	υ (C=O)	υ (Ar-H)	υ (C C)	Others
A_2	H	1614	1703	3057	1523	2918sy υ (CH_3)
A_3	4-OH	1612	1658	3057	1523	2918sy υ (CH_3), 3407 υ (OH)
A_4	4-Cl	1620	1660	3057	1525	789 υ (C-Cl), 2918asy, 2970sy υ (CH_3)
A_5	4-Br	1612	1695	3057	1521	858 υ (C-Br), 2918asy, 2970sy υ (CH_3)
A_6	4-OH, 3-OCH_3	1610	1657	3068	1516	2950sy υ (CH_3)
A_7	4-NO_2	1589	1705	3056	1521	2850sy υ (CH_3)
A_8	2-OH	1603	1639	3059	1522	2846asy, 2922sy υ (CH_3)
A_9	4-N $(CH_3)_2$	1595	1677	3059	1515	2912sy υ (CH_3)
A_{10}	4-OCH_3	1612	1680	3059	1514	2835asy, 2924sy υ (CH_3)

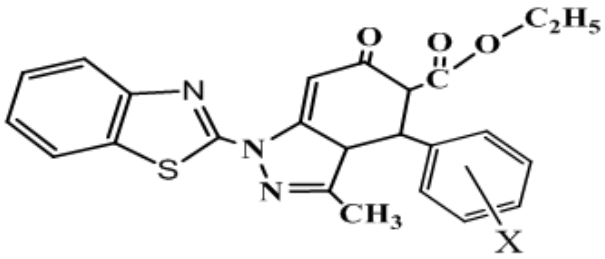


Table 4: IR –spectral data of compounds (A_{11}-A_{19})

Comp. No	X	FT.IR cm^{-1} (KBr)				
		υ (C=N)	υ (C=O)	υ (Ar-H)	υ (C C)	Others
A_{11}	4-Br	1643	1712, 1739	3064	1523	υ (CH_3) 2927
A_{12}	4-Cl	1658	1714, 1739	3074	1525	υ (CH_3) 2920
A_{13}	4-N $(CH_3)_2$	1622	1722, 1781	3058	1515	υ (CH_3) 2923
A_{14}	4-NO_2	1633	1658, 1739	3074	1521	υ (CH_3) 2918
A_{15}	4-OCH_3	1612	1710, 1739	3056	1514	υ (CH_3) 2927
A_{16}	4-OH	1612	1660, 1708	3058	1515	υ (CH_3) 2921 υ (OH) 3427
A_{17}	H	1614	1654, 1735	3058	1523	υ (CH_3) 2925
A_{18}	4-OH, 3-OCH_3	1627	1714, 1737	3055	1515	υ (CH_3) 2933 υ (OH) 3373
A_{19}	2-OH	1583	1641, 1730	3058	1525	υ (CH_3) 2927 υ (OH) 3369

Table 5: Antibacterial activity of the synthesized compounds (A12-A17)

Comp .No	antibacterial activity (zone of inhibition in mm)		
	Conc. (mg/m)	Pseudomonas aeruginosa	*Proteus spp*
A12	0.01	15	14
	0.001	30	16
	0.0001	18	21
A13	0.01	24	19
	0.001	22	30
	0.0001	15	30
A14	0.01	27	16
	0.001	24	19
	0.0001	21	14
A15	0.01	18	21
	0.001	20	17
	0.0001	25	28
A16	0.01	13	15
	0.001	20	25
	0.0001	25	14
A17	0.01	12	13
	0.001	17	19
	0.0001	21	11
Ciprofloxacin	MIC	12.5	12.5

Slight activity 15-18 mm, moderate activity 18-20 mm and high activity 21-25 mm; MIC: minimum inhibition concentration (μ g / mL)

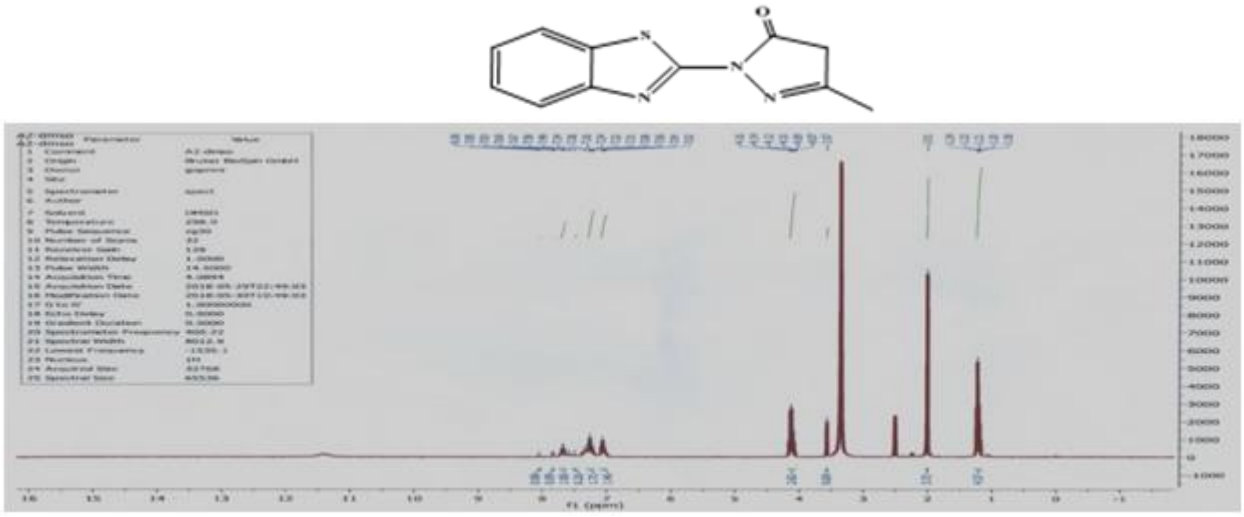


Figure 1: ^{1} H-NMR spectrum of A_1

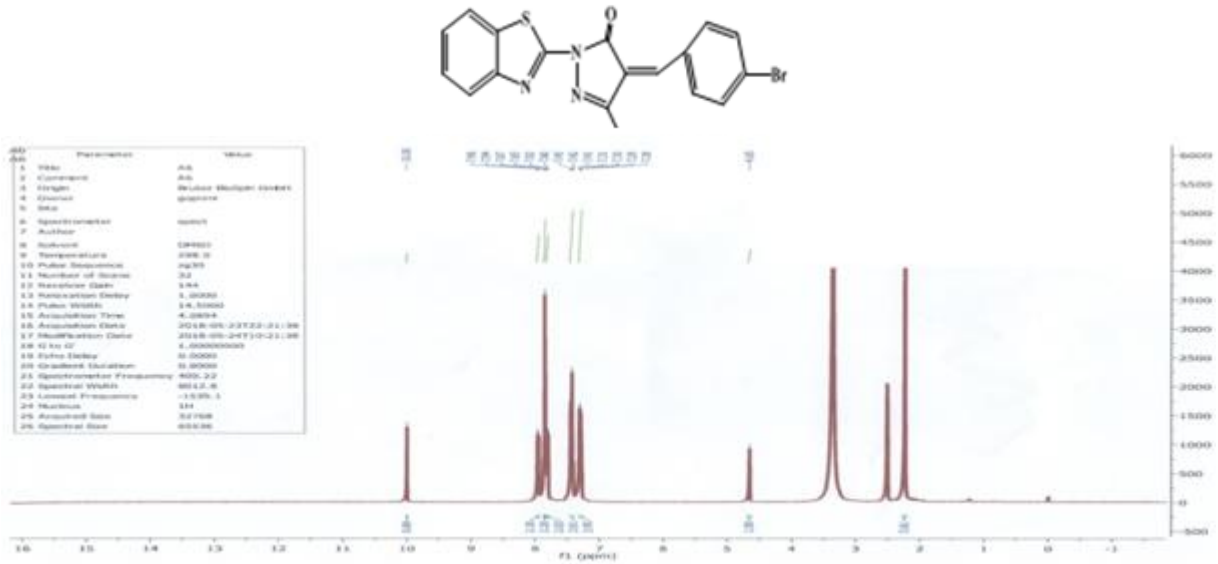


Figure 2: ^{1} H-NMR spectrum of A_5

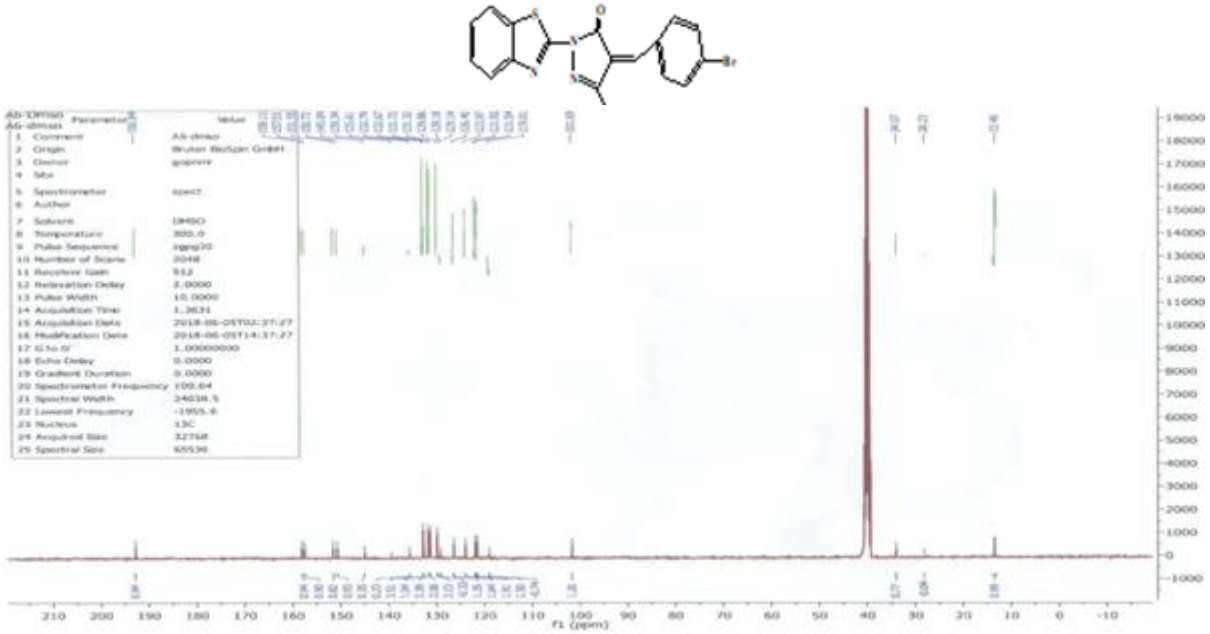


Figure 3: ^{13} C-NMR spectrum of A_5

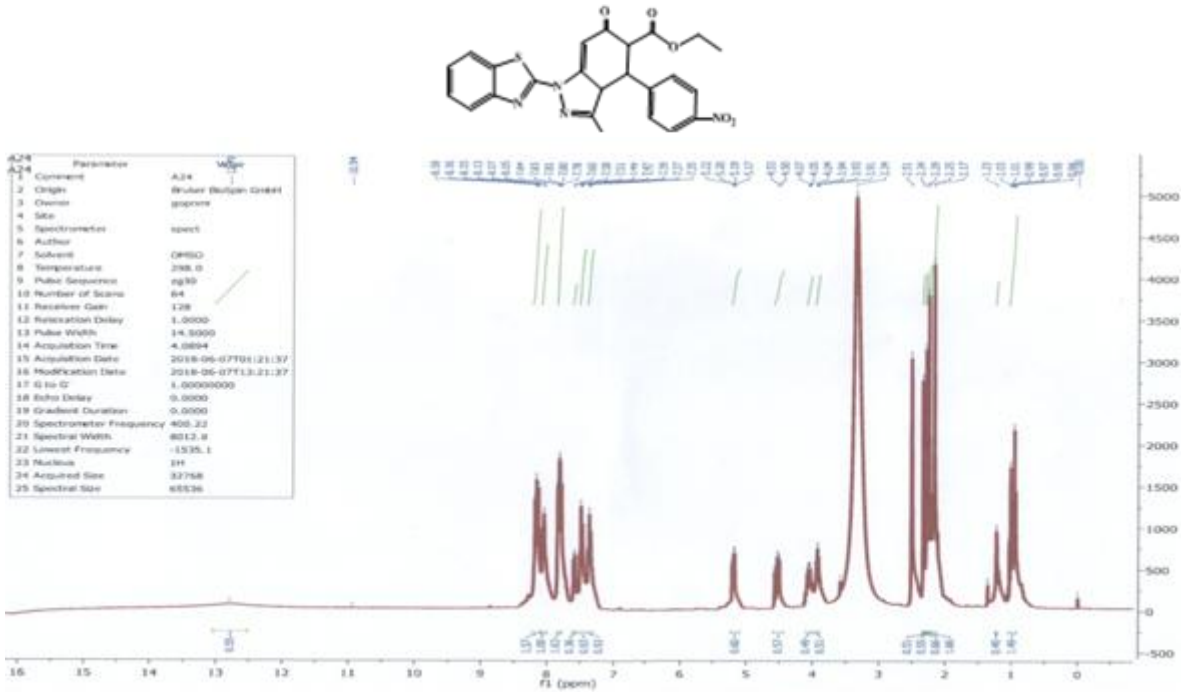
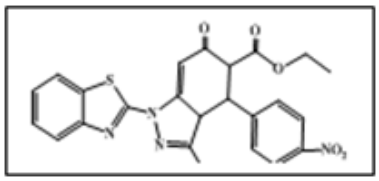


Figure 4: ^{1} H-NMR spectrum of A_{14}



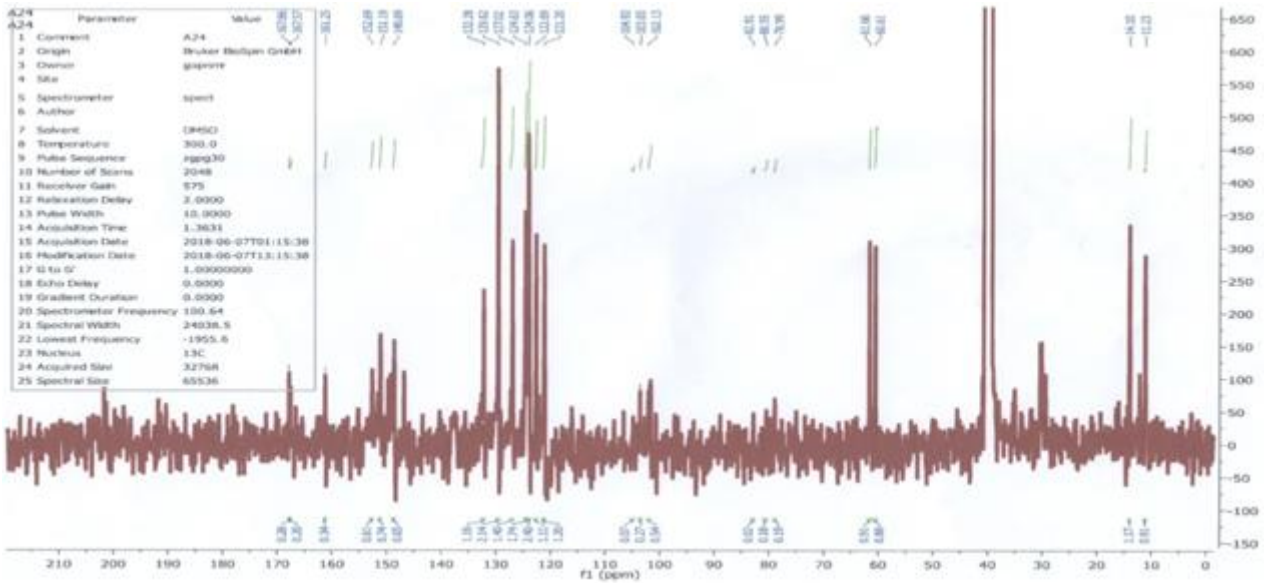


Figure 5: ^{13} C-NMR spectrum of A_{14}

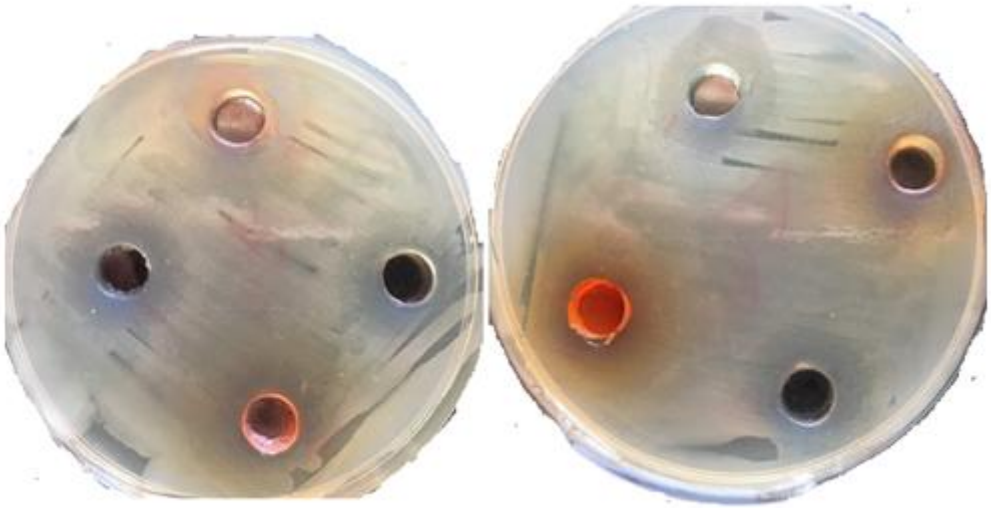


Figure 6: Compound (A_{12}) inhibits growth of bacteria Proteus Mirabil

Figure 7: Compound (A_{15}) inhibits growth bacteria Pseudomonas aeruginosa Mirabilis

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