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RESEARCH ARTICLE

Synthesis, Characterization and Biological Evaluation of Some 2-Azetidinones Derivative from Benzimidazole and Used as Anti-Fungicide Agents

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Abstract

Reaction of benzimidazole with ethyl chloro acetate to give ethyl –N–benzimidazole acetate (M_1) which was used the reaction with hydrazine hydrate to yield N- benzimid azole aceto hydrazide (M_2). The reaction of compound (M_2) with different aromatic aldehydes gave compounds hydrazone (M_3, M4). (P-Hydroxy (or Chloro) benzylidine) -N- aceto hydrazide benzimidazole. Then the compounds (M_3, M_4) reacted with chloro acetyl chloride in presence of tri ethyl amine using DMF as solvent to obtain 2-azetidinones compounds (M_5, M_6). The structures of all the newly synthesized compounds were confirmed by elemental analysis and FT-IR, 1H NMR and ^{13}C NMR as well as melting point. Some of these compounds showed good antimicrobial activity (M_1-M_6) and anti-bacterial activity (M_1-M_6) Antibacterial activity of some prepared compounds against two types of bacteria: *Staphylococcus aureus* (Gram positive) and E.coli (Gram negative). Some the compounds showed high inhibition activity against bacteria and two fungi (Candida albicans).

Keywords: *Benzimidazole, Schiff bases, Ester, Heterocyclic compounds.*

Introduction

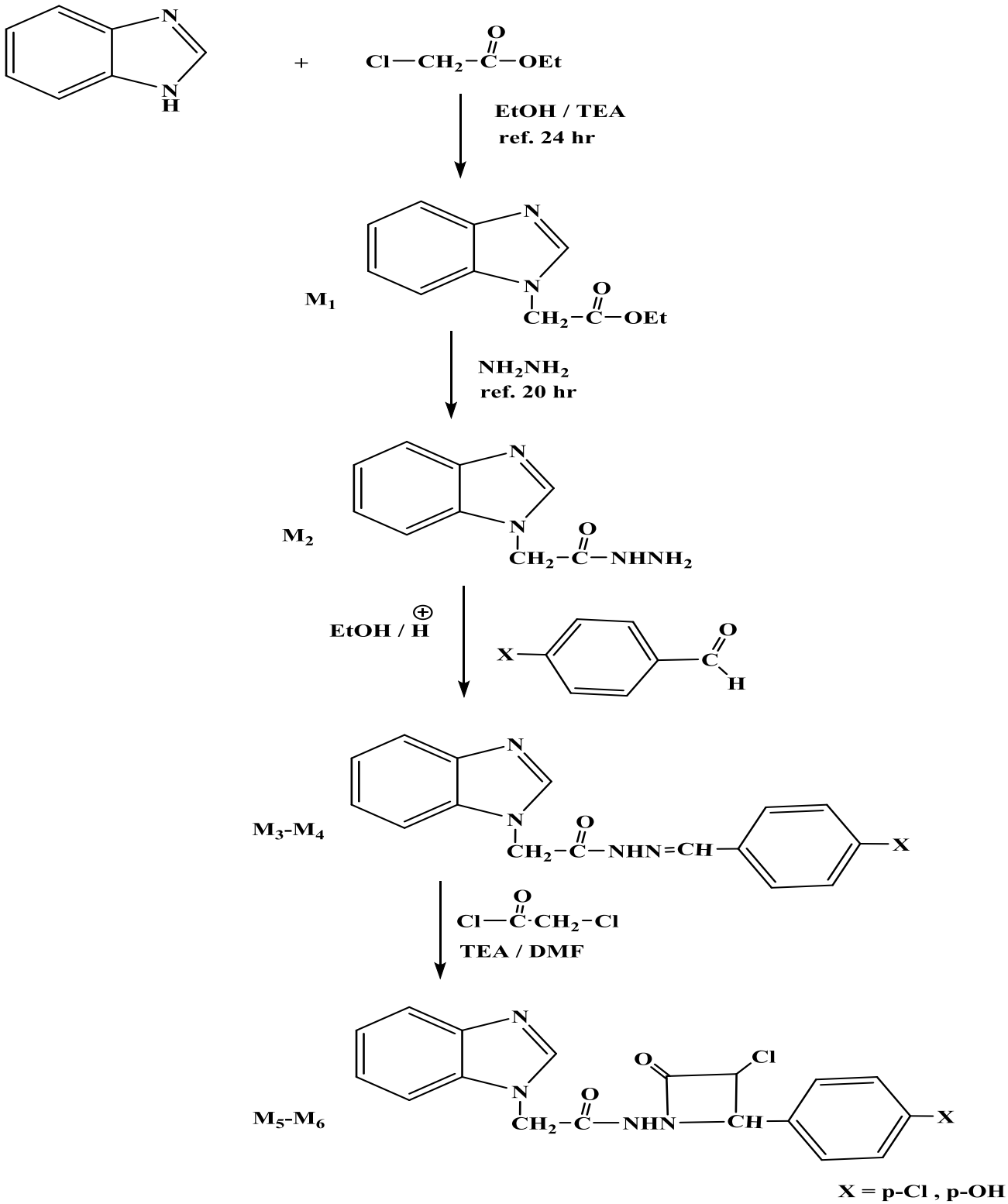
Benzimidazole derivatives are well-known biologically active N-containing heterocyclic [1], widely used as drugs such as proton pump inhibitor [2], antihelmenthetic, antidopaminergic [3], and antipsychotic agent. Specially, the 2-substituted analogs of benzimidazoles are known to be potent biologically active compounds [4]. Moreover, benzimidazole derivatives are structural isosteres of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living systems.

The benzimidazole nucleus is an important heterocyclic ring because of its synthetic utility and broad range of pharmacological activities [1].Some benzimidazole derivatives with different pharmacological activities Benzimidazole is an important pharmacophore as this moiety is an integral part of the structure of vitamin B12 antifungal [5], anti-helmintic [6], anti-HIV, antihistaminic [7], antiul8cer [9], cardio tonic [10], antihypertensive [11] and neuroleptic [12] are in clinical use.

Materials and Methods

All of the melting points were determined by open capillary using Sturat Melting point apparatus and are uncorrected. IR spectra were recorded in KBr on FTIR spectrophotometer, Shimadzu FT-IR-8300 as KBr disk in Tikrit University.

The 1H-NMR spectra were measured with a Brucker (400 MHz) spectrometer in Yildiz Teknik University Bruker using $CDCl_3$/ DMSO-d6 .Chemical reagents used in the synthesis were purchased from Riedel-Dehaen AG, Scharlau and Fluka Company. ^{1}H NMR spectra were recorded on 500 MHz in Yildiz Teknik University Bruker using $CDCl_3$/DMSO.



Scheme; preparation of Schiff bases (M_3, M_4) and 2 -azetidinones derivatives (M_5, M_6)

Preparation of Ethyl- N- acetate benzimidazole (M_1)

In a 250 ml round bottomed flask equipped with a magnetic bar stirrer and dropping funnel, a stirred solution of (2.44g,0.02mole) of benzimidazole,40ml of ethanol and (1.13g ,0.02mole) potassium hydroxide was added and heated at 78-80°C for 10 min. Then ethyl Chloro acetate (2.44ml, 0.02mole) was added in one portion, an exothermic reaction set in causing a temperature rise from 30-40°C.After stirring at 25°C for 18-hours, the reaction mixture was added to 100g of ice-water and stirred for 25 min. at 0-10 °C. The precipitate that was formed were collected by filtration, washed with water until free of chloride and air dried and recrystallized by water the yield (85%). melting point is 62-64°C.

Preparation of N-(benzimidazole) acetohydrazide (M2)

The mixture of ethyl- N- acetate benzimidazole (2.04g, 0.01mole) and hydrazine hydrate 6ml (0.01mole) are mixed well in a RBF and heated on water bath for 10 min. then dissolved in 30 ml ethanol; the reaction mixture is heated with reflux condenser for 6 hrs. After the completion of reaction .The reaction mixture was added to 100g of ice, and crystallized from ethanol. The yield (70%).Melting point is 89-92^{o}C.

General Procedure for the Preparation of Schiff bases (M3-M4)

An equimolar solution of acetohydrazide (0.567g, 0.003 mol) is dissolved in10ml of ethanol and to this solution substituted aldehydes in equimolar (0.42g ,0.003mol) is added with 4-6 drops of glacial acetic acid was added, this reaction mixture is kept under reflux for 4hours. After cooling to room temperature added to ice cold water. Compound gets separated as solid filtered, dried and recrystallized with chloroform. Melting point is noted in 239 d ^{o}C, the yield is mentioned in 75 %. The physical data of these benzimidazole derivatives were given in Table (1).

Table 1: Physical properties of the synthesized compounds (M3-M4)

Comp. No.	R	Molecular formula	Color	M.P(^{o}C)	Yield (%)
M3	p-OH	$C_{16}H_{14}N_4O_2$	yellow	239 dec	75
M4	p-Cl	$C_{16}H_{13}N_4OCl$	yellow bright	250-253	70

Synthesis of 2 -Azetidinones (M_5-M_6) [13]

To a mixture of hydrozones (M3-M4) (g, 0.005mol) and triehyl amine (TEA) as catalyst are dissolve in (10 ml) of DMF and stirred at room temperature about 30 minutes and then add Chloro acetyl chloride (0.005 mole) as drop wise. After the completion of addition, the mixture was refluxed for 8 hours and then allowed to cool to room temperature. Then the reaction poured onto crashed ice and was kept over-night at room temperature. The precipitate dried, recrystallized from ethanol to give Yellow crystals, Yield, melting points and other characterization of the synthesized compounds are given in the Table (2).

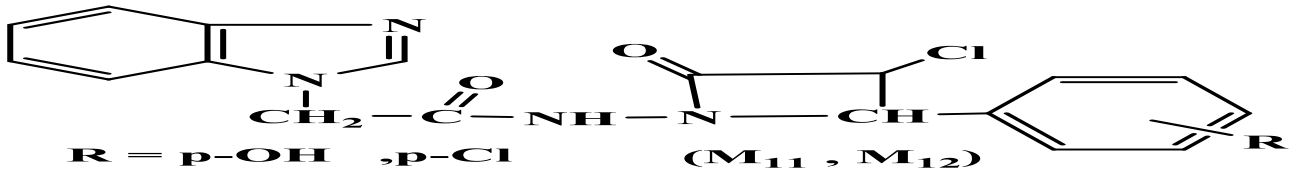


Table 2: Physical properties of the synthesized compounds (M_5-M_6)

Comp. No.	R	Molecular formula	Color	M.P(^{o}C)	Yield (%)
M_5	p-OH	$C_{18}H_{15}N_4O_3Cl$	brown	195-198	65
M_6	p-Cl	$C_{18}H_{12}N_4O_2Cl_2$	begi	182-184	60

Biological Activity of Some of the Synthesized Compounds

The synthesized compounds in this work were expected to show biological activity since they have active groups in their molecules, all of the tested compounds studies at concentration of using DMSO as a solvent (0.05 mg/ml). Thus a preliminary evaluation of antibacterial and ant-fungus activity for some of the new benzimidazole compounds were tested against types of bacteria like Escherichia coli, (Gram-negative) and Staphylococcus a urea (Gram-positive) and against *Candida tropicalis* and Candida albicans fungus. The results showed that most of the tested compounds have good antibacterial and antifungal activity. Those kinds of Bacteria and fungus have been choosing because of their wide importance in the clinical field so they cause many diseases additional to their various resistances of the

antibiotic and chemical drugs. So their biological activities were illustrated in Table (8) showed antifungal activity and antibacterial activity. The result in table (8) that the synthesized compounds have Biological activity against the chosen Fungus and Bacteria because it has ability of inhibition the chosen Bacteria and Fungus by choosing concentration of the compounds, the inhibition zone is from (16 mm the lowest inhibition zone to 36 mm the highest inhibition zone of Fungus), as for Bacteria it's about (10 mm the lowest inhibition zone to 32 mm the highest inhibition zone of Bacteria).

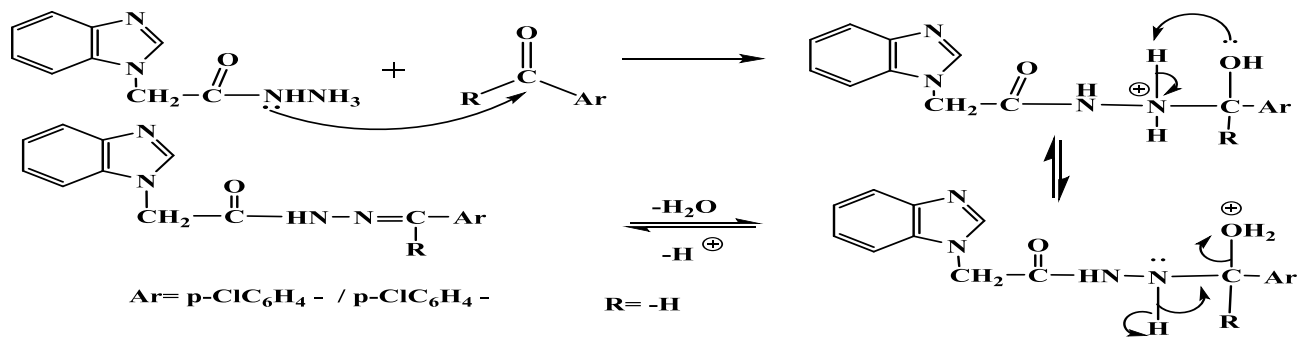
Results and Discussion

From the literature survey it reveals that the substituted benzimidazole moieties are already known for different biological activities. Here we have synthesized some new benzimidazole analogues combining with different substituted aromatic and hetero cyclic aldehydes ring system with view to get a good antimicrobial and antifungal agents with less toxic and side effects. Physiochemical properties of benzimidazole derivatives are presented in Table (8). Melting point is 62-64°C., the yield is yield 85%.

The chemical structure was confirmed by infra-red absorption spectra of all the compounds synthesized. Ethyl- N-benzimidazole acetate condensation of benzimidazole and ethyl Chloro acetate in presence of anhydrous K_2CO_3 in dry acetone to get ester (M_1).The FT-IR spectra [13] of the synthesized compound (M_1, Fig1) show disappearance of the absorption band of -NH 3253 cm^{-1} for the starting benzimidazole and appearance new clear absorption band at1747 cm^{-1} due to(C=O) of ester which proves the formation of the compound (M_1). And the appearance of characteristics bands at 2983, 2949for compound (M_1) asymmetric and symmetric aliphatic C-H stretching vibration of ester. Of aromatic ring.1450 cm^{-1}, 1350 cm^{-1} of CH_2, CH_3 bend 1226 cm^{-1}(C-O str).^{1}H-NMR spectrum of this compound (M_2), Fig (2).Showed resonating a signal at δ= 1.48 ppm that belongs to CH_3, and, at δ= 2.95 ppm for the CH_2 group protons, in -CH_2CH_3 respectively, also showed a signal at 4.2 ppm (2H, singlet) was attributed to (-CH_2) proton, a signal, while signals appeared at δ= (7.2 - 7.8) ppm were attributed to aromatic hydrogen. Synthesis of N-(benzimidazole) acetohydrazide (M_2) in 70% yield, was achieved by reaction of ethyl-N-acetate benzimidazole (M1) with hydrazine hydrate (99%).

The reaction was confirmed by the changes of the physical properties. The constitution of this compound (M_2) was supported by its melting point is 89-92°C. And IR–spectroscopy and 1H-NMR- spectroscopy .The structure was conformed via FT-IR, Fig. (3). Disappearance of 1749cm^{-1} due to (C=O) of ester and appears two strong bands at 3314, 3174 cm^{-1} asymmetric and symmetric stretching of (NH_2) group. This is a proof of the formation of Acid hydrazine.

On the other hand other great bands showed at3053cm^{-1} of N-H str.;3061cm^{-1} (C-H Ar) str.;1622 cm^{-1} (C=O) str. of amide. 1616 cm^{-1} (C=N) str.; 1542, (1498) cm^{-1} (C=C str. Ar) stretching. 1H-NMR spectrum of this compound (M_2), fig.(4) the formation of compound (M_2) was evidenced by appearance of a signal at δ= 3.5ppm due to (NH_2), also showed a signal at 8.5 ppm (H, singlet) was attributed to (-NH) proton, a signals ,while, it was between 7.3 and 7.4 ppm (m) 3H for benzene ring protons, benzimidazol acetohydrazid (M_2). Additionally a signal at δ= 2.95 ppm that belongs to CH_2N.The mechanism of this nucleophilic substitution of amino group of hydrazine hydrate on carbonyl group was preformed, Followed by elimination of ethoxy group .As it is shown in the following mechanism.



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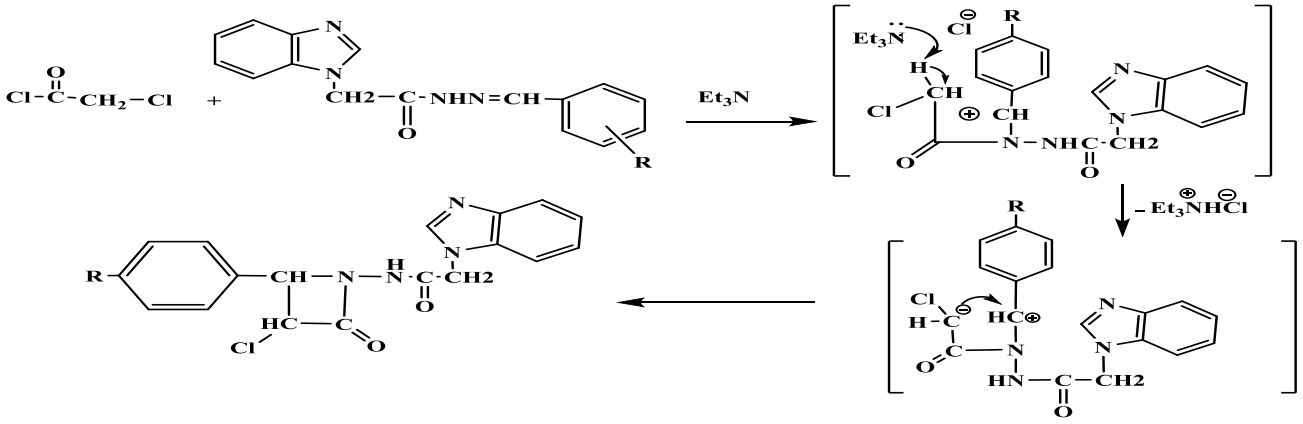
The structure of synthesis compounds Schiff's bases (M_3,M_4).were confirmed by melting-point, and FT- IR, the spectral characterization data are given in Table,(3) .Moreover**,** the condensation reaction of the (p-Hydroxy (or p-chloro) benzylidine)-N-acetohydrazide benzimidazole (M_3,M_4) were synthesized from the reaction of compound (M_2) with different aromatic aldehydes, namely, 4-hydroxy-benzaldehyde, 4-chloro Benzaldehyde, in absolute ethanol. FTIR absorption-spectra as of compounds (M_3, M_4), Fig. (3) showed disappearance of the two characteristic absorption bands at 3319 cm^{-1} and 3265 cm^{-1} due to (-NH-NH_2) group stretching vibration in compound (M_2) and appearance of new clear absorption band at1617-1620 cm^{-1} due to υ(C=N) imine. These two points are excellent proofs for the success of Schiff base formation (M_3, M_4) and showed clear absorption bands at 1735 cm^{-1} and 1706 cm^{-1} due to asymmetric and symmetric υ(C=O) imide [14].The 1H NMR spectra showed, in each case, the signals as multiplet at d 7.18-7.62 ppm attributed to Ar-H in addition to the singlet of the NH group in the region 11.85 ppm. The singlet also appeared at d 8.23 ppm attributed to one proton of N=CH. Thus, it confirmed the formation of Schiff bases.

Also showed signal at δ= 5.12 ppm belong to (CH_2) protons. All details of FT-IR spectral data of compounds (M_3, M_4) are listed in. Table (4).Condensation substituted Schiff's bases (M3, M4) reacted with chloro acetyl chloride in presence of tri ethyl amine using DMF as solvent to obtain N- (benzimidazol)-3- chloro- 4- aryl- 2 azetidinone (M_5, M_6). (Scheme1).The mechanism of this nucleophilic substitution of amino group of Schiff's bases on carbonyl group was preformed, Followed by elimination of halide hydrogen group .As it is shown in the following mechanism.



N-(benzimidazol)-3-chloro-4-aryl-2 azetidinone (M_5, M_6) were synthesized by the reaction of compounds (M_3, M_4) with Chloro acetyl Chloride. The structure of synthesized compounds were confirmed by measuring there melting-point, C.H.N, IR, ^{1}H-NMR and^{13}C-NMR data ,spectroscopic data were shown in Table (5) and Figer (7,8). The synthetic rout of these compounds is presented in Scheme (I)**.**

The FT-IR characterization compound (M5), showed appeared 1626cm^{-1} (C=O), Additionally, it showed the appearance of new absorption band between 3325 cm^{-1} that represents NH-C=O stretching vibration of amide, and appearance of new characteristic band between1693 to1647cm^{-1} that represents C=O stretching vibration of amide. Overlapped with (C-H) Ar. 1658 cm^{-1} (C=C) Ar str. 1485 cm^{-1} (C=N) str. Finally compounds M_7, M_8 showed strong absorption band at 1091 cm-1 due to υ(C-Cl). All details of FT-IR Spectral data of compounds (M_5, M_6) is shown in Table (5).H-NMR spectrum of compounds (M_5, M_6), Fig (8).Showed a signal at 9.76 ppm (1H ,singlet) which was attributed to (-NH) proton and a signal between (6.3-7.4)ppm for aromatic hydrogens, while the signal at 3.92 and 4.9 ppm (CH_2 , CH) protons, also appeared at d 9.83 ppm attributed to one proton of υ(CH-Cl) [14].Is shown in Table (6).

Conclusions

This research included new methodology for synthesis of 2 -azetidinones, Also, new derivatives of benzimidazole ring were synthesized. All the derivatives prepared by this method are analyzed by^{1}HNMR and IR.

The compounds were characterized by spectroscopic methods such as ^{1}HNMR and IR. The informational data, available in literature so far, rendered 2-azetidinones has become one of the most important heterocyclic in current chemistry research, due to its important pharmaceutical applications, especially in biological science, and medicinal chemistry. All the 2-azetidinones derivatives exhibited varied activity against their antibacterial, antifungal activities. As in Table (8).

Table 3: Physical properties of the synthesized compounds (M_3-M_4)

Comp. No.	Characteristic bands of IR. spectra (cm^{-1} , KBr disc)						
	R	υ(C=N) cm^{-1}	υ(C=C) cm^{-1}	υ(C-H) cm^{-1}arom.	υ(C-N) cm^{-1}	υ(N-H) cm^{-1}	υ others cm^{-1}
M3	p-OH	1627	1447	3063	1354	3234	(OH) 3423
M4	p-Cl	1620	1433 1591	3076	1261	3144	(Cl) 740

Table 4: ^{1}HNMR Data of compounds (M_5, M_6)

Comp. No.	R	^{1}HNMR chemical shift in ppm
M_5	p-OH	4.01 H,NH, 6.1 CH-Cl,5.1 CH-Ar , (7.05 –8.2) Ar-H, 2.35 CH_3
M_6	p-Cl	4.01(H,NH) 6.3 CH-Cl,5.4 CH-Ar , (7.05 –8.1) Ar-H, 2.35-2.85 CH_3

Table 5: Physical properties of the synthesized compounds (M_5-M_6)

Comp. No.	Characteristic bands of IR. spectra (cm^{-1} , KBr disc)						
	R	υ(C=O) cm^{-1}	υ(C=C) cm^{-1}	υ(C-H) cm^{-1} arom.	υ(C-N) cm^{-1}	υ(N-H) cm^{-1}	υ others cm^{-1}
M_5	p-OH	1634	1465	3059	1365	3255	(OH) 3445
M_6	p-Cl	1645	1598 , 1453	3085	1278	3231	(Cl) 237

Table 6: ^{13} CNMR Data of compounds (M_5, M_6)

Comp. No.	R	^{13}CNMR chemical shift in ppm
M_5	p-OH	64 C-Cl, 157 C=O, 62 C-Ar , 23 CH_3
M_6	p-Cl	23 CH_3, 163 C=O, 59 C-Ar , 140C – N

Table 7: Elemental analysis of compounds (M_5-M_6)

No. of Comp.	Molecular Weight	Molecular formula	Elemental Analysis (%)					
			Calculated			found		
			C	H	N	C	H	N
M_1	190.2	$C_9H_{10}N_4O$	55.71	5.36	29.10	55.04	5.46	29.33
M_2	204.23	$C_{11}H_{12}N_2O_2$	62.68	5.38	13.36	61.98	5.30	13.23
M_3	277.3	$C_{16}H_{14}N_4O_2$	62.27	12.85	20.07	62.35	12.57	20.27
M_4	313.76	$C_{16}H_{13}N_4OCl$	60.04	4.06	17.92	59.88	4.25	17.15
M_5	370.8	$C_{18}H_{15}N_4O_3Cl$	56.32	14.31	14.76	55.95	15.01	14.26
M_6	387.2	$C_{18}H_{12}N_4O_2Cl_2$	55.09	12.06	14.20	55.46	11.93	14.11

Table 8: Antimicrobial activities of synthesized compounds (M1-M6)

Code of Comp.	Antibacterial activity (zone of inhibition in mm)		Antifungal activity (zone of inhibition in mm)	
	Gram negative bacteria **Escherichia coli**	Gram positive bacteria **Staphylococcus aurea**	***Candida albicans***	*Candida tropicalis*
M1	**33**	**43**	**32**	**26**
M2	**12**	**22**	**20**	**17**
M3	**24**	**28**	**19**	**16**
M4	**32**	**46**	**27**	**36**
M5	**9**	**13**	**28**	**19**
M6	**21**	**28**	**25**	**21**
Ampicillin	**35**	**40**	-	-
Streptomycin	-	-	**35**	**33**

Antibacterial Activity

To determine the antibacterial activity of these agents, the cup plate method was used, with Ampicillin and Streptomycin as the reference antibiotics [15]. The prepared compounds were examined against two strains each of gram positive and gram negative bacteria. The test results, presented in Table (8), suggest that compounds M1and M4 are highly active against Escherichia coli and Staphylococcus aurea respectively .Compounds M1 and M4 are also highly active against Staphylococcus aurea.

The rest of the compounds were found to be either moderately active, slightly active against the tested microorganisms.

Antifungal Activity [15]

The antifungal activities of the prepared compounds were tested against two different fungi such as Candida tropicalis and Candida albicans. The test results, presented in Table (8), suggest that compounds M1and M5 are highly active against Candida tropical is and Candida albicans respectively Compounds M1 and M4 are also highly active against Candida albicans .by filter paper disc technique [15]. All compounds exhibited half efficiency with standard drugs against Ampicillin and Ketoconazole as shown in Tables (8) Ampicillin was used against bacteria and Ketoconazole was used against fungus as reference drugs.

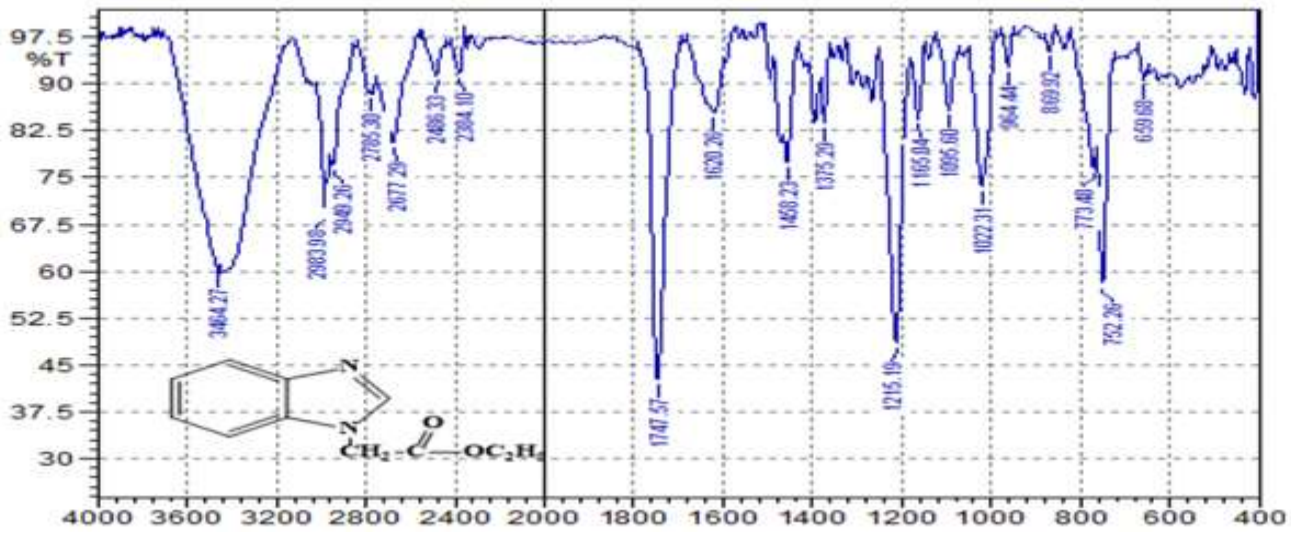


Fig.1: FT-IR spectrum for compound (M1)

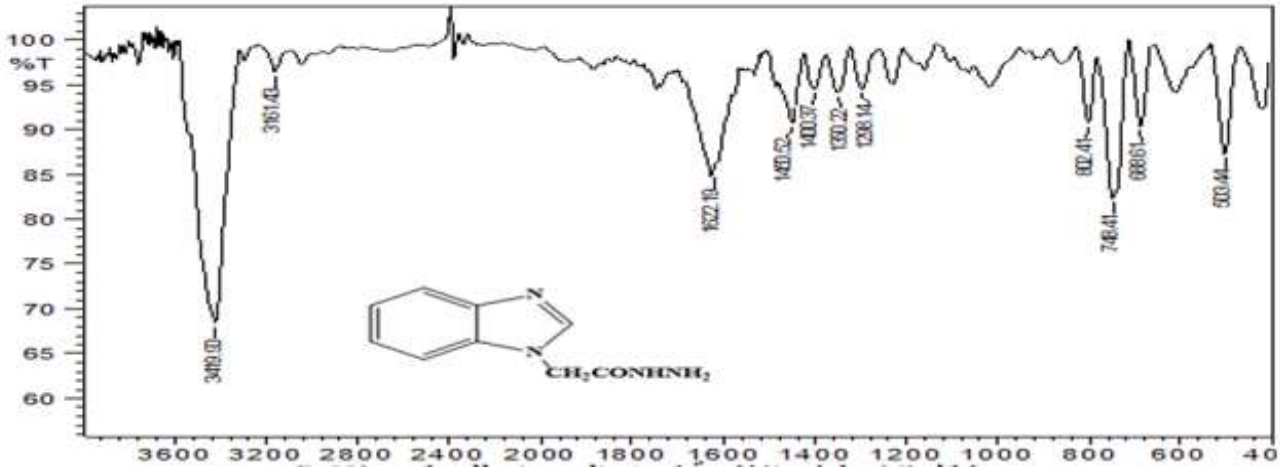


Fig.2: FT-IR spectrum for compound (M2)

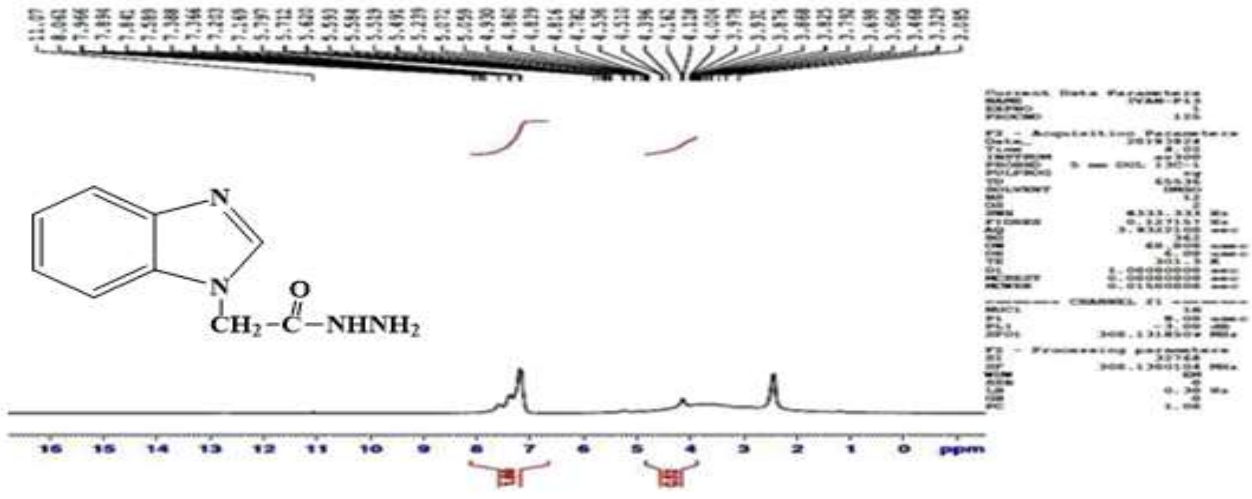


Fig.3: 1HNMR for compound (M2)

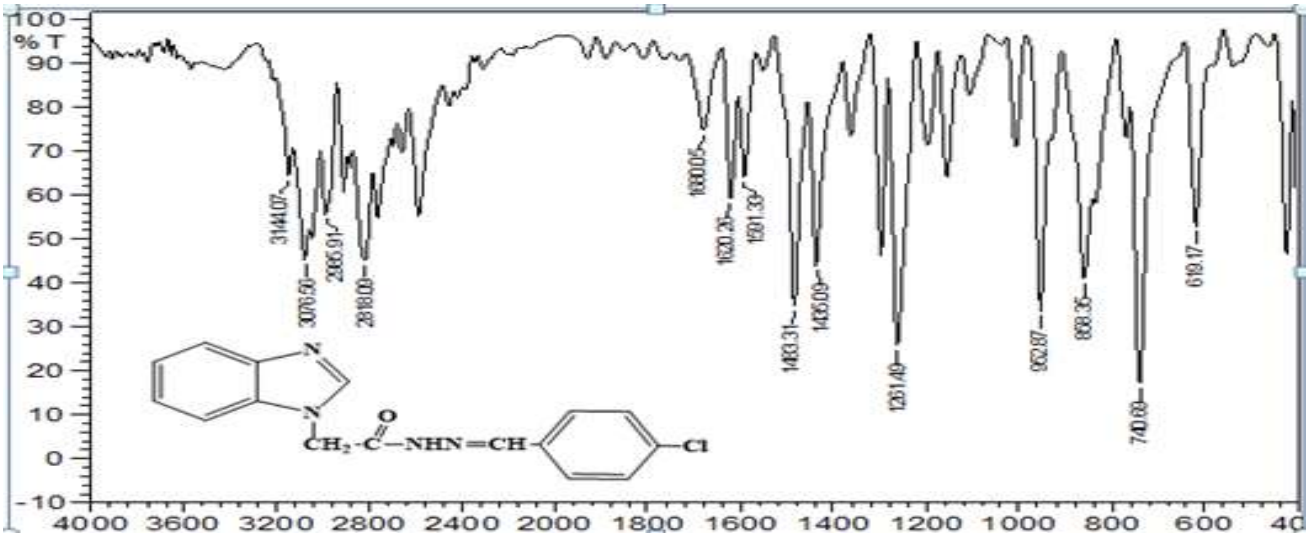


Fig.4: FT-IR spectrum for compound (M3)

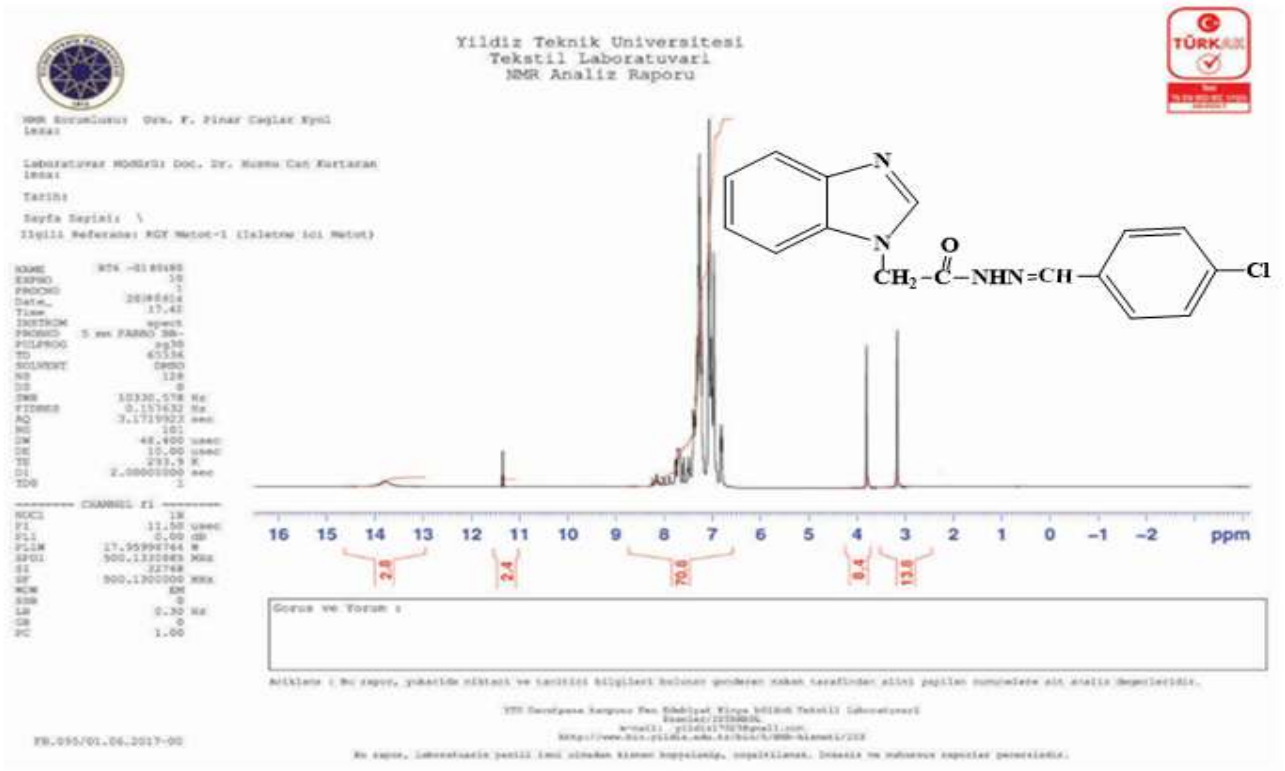


Fig.5: 1HNMR for compound (M3)

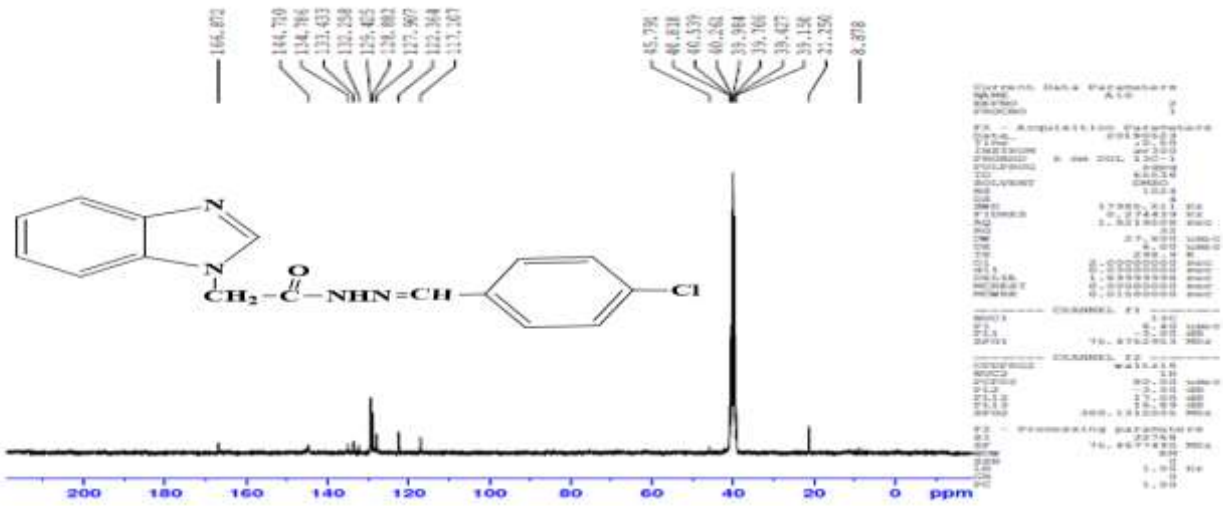


Fig.6: [13]CNMR for compound (M5)

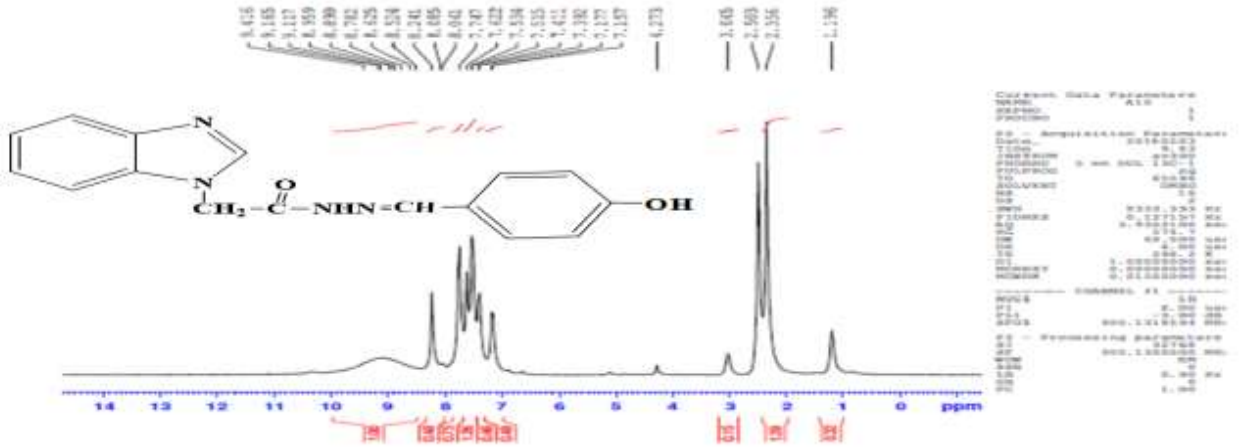


Fig.7: ^{1}HNMR for compound (M4)

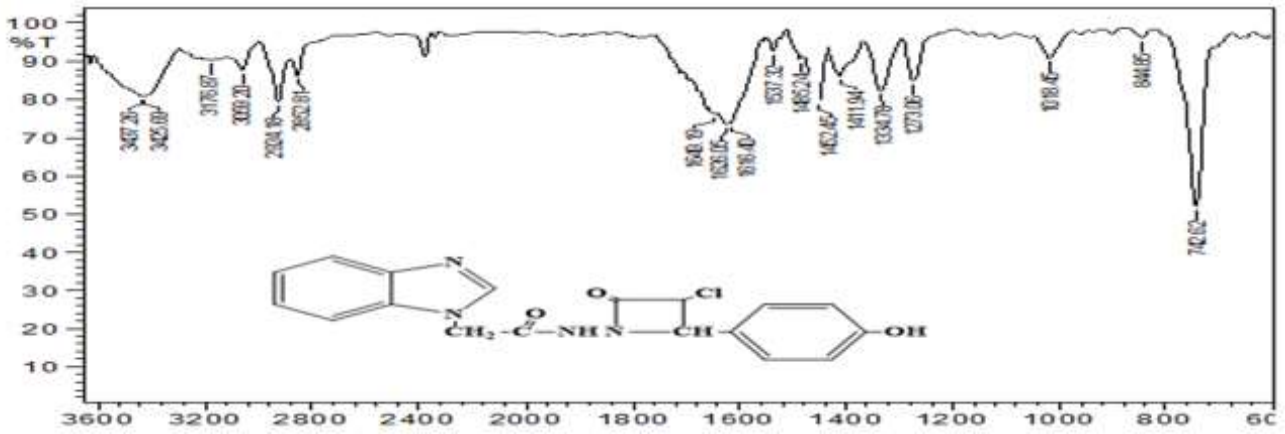


Fig.8: FT-IR spectrum for compound (M6)

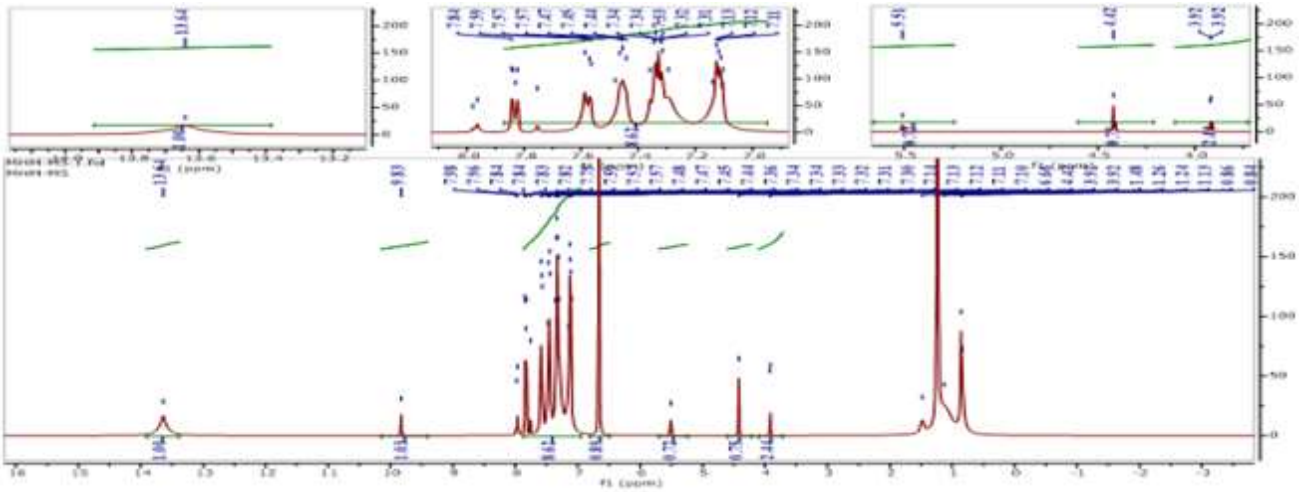


Fig.9: ^{1}HNMR for compound (M6)

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