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Research Article
Hepatoprotective Potentials of Promising Newly Synthesized 3-substituted-2-biphenyl Imidazo (1,2-a) Pyrimidine Derivatives on CCl_4 Induced Albino Male Mice

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Abstract

Background and Objective: Synthesis of new compound with appropriate therapeutic importance is a major challenge in medicinal chemistry. Recently fused rings compounds of pyridine and pyrimidine have significant importance in the pharmaceutical industry due to their various interesting biological activities displayed over a broad range of therapeutic classes, therefore development of some novel fused heterocycles is the main goal of the present study. **Materials and Methods:** Thus, a simple and cost effective procedure, novel fused heterocycles compounds of 3-substituted heterocyclic compounds containing bridge head nitrogen were synthesized through multi step reactions to give new compound of 2-biphenyl-3-oxypyrimidine imidazo (1,2-a) pyrimidine. In addition, all prepared compounds were characterized via Fourier Transform Infrared (FT-IR) spectroscopy, some of them were characterized by Hydrogen-1-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy. These new 3-substituted derivatives of imidazo/pyrimidine rings were tested *in vivo* by determining these activities on liver function enzymes Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Alanine Aminotransferase (ALT) in addition to evaluating the hepatoprotective activity on liver tissue after treatments with these compounds alone or after interaction with the toxic compound carbon tetrachloride CCl_4. **Results:** These compounds showed promising antitumor activity by reducing the level of liver function enzyme to or near the normal level when given alone or after interactions with CCl_4 for GOT, GPT and ALP, respectively. Also, all synthesized compounds had the ability to return liver tissue to normal state after damaged by CCl_4. **Conclusion:** The newly synthesized compounds 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde, (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one and 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2 (1H)-one expressed hepatoprotective activity against damaged caused by CCl_4 at enzyme level or liver tissue.

Key words: Imidazo, pyrimidine, chalcone, oxypyrimidine, hepatoprotective, antitumor

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the most important imidazole compounds are aza-indolizidine, which contains a phenyl ring fused to a imidazole ring, which also known as imidazo (1,2-a) pyridine[1,2].

Imidazo [1,2-a] pyridine are bridge head nitrogen heterocycles and compounds containing this heterocycles have been reported for various biological activities and received considerable interest from the pharmaceutical industry like antifungal and antimicrobial agents[3-11]. In order to prepare parent compound of 2-substituted imidazo (1,2-a) pyridine, a known procedure will be used by condensation of suitable 2-amino pyridine with different α-halo ketones in refluxing ethanol to give 2-substituted imidazo (1,2-a) pyridine and introduce it in different reactions[4].

The easy electrophilic attack on position-3 in this fused system will be permitted in the preparation of a variety of 3-substituted fused rings of pyridine. Therefore, the second step will be introduced in aldehyde group at position-3 by Vilsmeier-Haack reaction with using mixture of Phosphoryl Chloride ($POCl_3$) and Dimethylformamide (DMF) in the presence[5] of Chloroform ($CHCl_3$). Moreover, hydrazone derivatives of fused rings of imidazo/pyridine have been explored to have interesting bioactivity such as anti bacterial and anti fungal[5-9] here this research has designed and synthesized hydrazones, semicarbazones and oximes derivatives of imidazo [1,2-a] pyridine[5]. In addition, new chalcones derivatives of imidazo (1,2-a) were synthesized. Chalcones have been proved to be an important intermediate for the synthesis of many heterocyclic compounds in organic chemistry[6-8]. These facts encouraged us to synthesize some new chalcone derivatives bearing imidazo (1,2-a) pyridine nucleus, which were reported to possess various biological activities such as antibacterial, antimicrobial, antiviral, anti HIV, antitumor and anticancer[4,7,8]. The chalcones have been discovered to be useful for the synthesis of variety of heterocyclic compounds such thiopyrimidines and oxopyrimidines. It is worth to mention oxypyrimidine and thiopyrimidines derivatives represent one of the most important class of compounds having a wide range of biological activities such as anti HIV, antiviral and herbicidal[10]. These active compounds have been synthesized by cyclocondensation of chalcones with urea and thiourea[2-9]. The aim of the research was to synthesis new compound of imidazo (1,2-a) pyridine and study their bioactive entities, especially with pharmacological activities bearing heterocyclic ring system namely imidazo [1,2-a] pyridine.

MATERIALS AND METHODS

The study was conducted in 2017 at synthesis lab in Department of chemistry, College of Sciences, University of Baghdad, Iraq from October-April, 2017 and Biology work carried out at Biotechnology lab from May-July, 2017.

Melting points recorder using electro thermal melting point apparatus. All the (^1H and ^{13}C-NMR) spectra were recorded on Bruker UltraShield 400 MHz spectrometer using DMSO-d6 as solvent as an internal standard chemical shift values are listed in δ scale. The IR spectra were recorded on Shimadzu FTIR spectrophotometer by using potassium bromide discs.

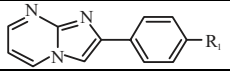
Experimental section of compounds 1, 2, 3 and 4

Synthesis of 2-(biphenyl) imidazo (1,2-a) pyrimidine 1: A mixture of 2-amino pyrimidine (0.95 g, 0.01 mol), 4-phenyl phenacyl bromide (2.74 g, 0.01 mol) are dissolved in 20 mL of ethanol. The mixture was heated under reflux in water bath for 6 h. Then, the solution was cooled and basified with 5% NaOH until pH 10. The resulting solid washed with water filtered and recrystallized with ethanol. Physical properties of compound 1 shown in the Table 1.

Synthesis of 2-(biphenyl) imidazo (1,2-a) pyrimidine-3-carbaldehyde derivatives 2: To an ice cold solution of DMF (1 mL, 0.012 mol) in $CHCl_3$ (5 mL , 0.060 mol), was added $POCl_3$ (2 mL, 0.021 mol) drop wise and the temperature was maintained below 10°C since an exothermic reaction took place. To the reaction mixture, an ice-cold solution of compounds 1 (1 g, 0.0036 mol) in chloroform was added slowly. After completion of addition, the reaction mixture was refluxed in water bath for about 2 h. The reaction mixture was cooled and washed with ice water and filtered. The product solid obtained was purified by recrystallization from mixture of acetone and ethanol (1:1) to give derivatives of 2, respectively. Physical properties of compound 2 is shown in the Table 2.

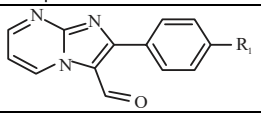
Synthesis of (2E)-3-[2- (bi phenyl) imidazo (1,2-a) pyrimidine-3-yl]-1- (4-nitro phenyl)-prop-2-en-1-one. 3: To a solution of substituted acetophenone 0.5 g, 0.003 mol in ethanol (15 mL), a solution of 40% NaOH (1 mL) was added till the solution became basic and stirred for 20-25 min, after that, compounds of 2 (0.85 g 0.003 mol) was added. The resulting mixture was stirred for 24 h. The content poured on crushed ice and neutralized with concentrated acetic acid. The solid

Table 1: Physical properties of compound 1

Compound number	Compound structure	R_1	Molecular formula	Melting point (°C)	Color	Yield (%)
1		C_6H_6	$C_{18}H_{13}N_3$	180	Orange	88

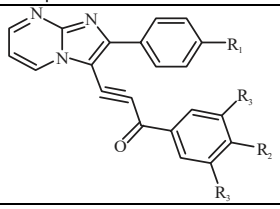
R_1: Radical group

Table 2: Physical properties of compounds 2

Compound number	Compound structure	R_1	Molecular formula	Melting point (°C)	Color	Yield (%)
2		C_6H_6	$C_{19}H_{13}N_3O$	184	Off white	93

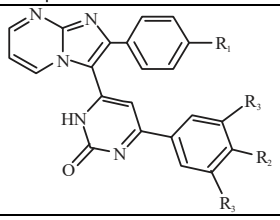
R_1: Radical group

Table 3: Physical properties of compounds 3

Compound number	Compound structure	R_1	R_2	R_3	Molecular formula	Melting point (°C)	Color	Yield (%)
3		C_6H_6	NO_2	-	$C_{21}H_{13}N_4O_3$	178	Orange	75

R_1, R_2, R_3: Radical groups

Table 4: Physical properties of compounds 4

Compound number	Compound structure	R_1	R_2	Molecular formula	Melting point (°C)	Color	Yield (%)
14e		C_6H_6	NO_2	$C_{28}H_{18}N_6O_3$	236	Off orange	71

R_1, R_2: Radical groups

was separated, filtered and crystallized from mixture of ethanol and chloroform to give derivatives 3, respectively. Physical properties of compounds are shown in the Table 3.

Synthesis of 6-[2-(biphenyl) imidazo (1,2-a) pyrimidine -3-yl]-4-nitro pyrimidine -2 (1H)-one. 4: A mixture of chalcone (3) (1.31 g, 0.003 mol) and urea (0.26 g, 0.003 mol) in ethanol (10 mL) was refluxed on water bath in presence of (40%) alcoholic KOH for 8 h. The reaction mixture cooled and neutralized with 20% HCl. The separated solid was filtered and recrystallized by using ethanol to give derivatives 4, respectively. Physical properties of compounds are shown in the Table 4.

Assessment of hepatoprotective effects: Hepatoprotective effects were assessed in albino male mice after inducing hepatic damage with carbon tetrachloride (CCl_4). The parameters of assessment in determined after treatment of mice with 3 chemical compound 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde, (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one and 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine-3-yl]-4-nitro pyrimidine-2 (1H)-one determination liver function enzymes in serum and histopathological evaluation of liver tissue.

Experimental design

Experimental design includes two stages: For determination of liver function enzymes in serum and histopathological evaluation of liver tissue the details of these stage explained below:

First stage:

Group I : Mice were administrated with a single daily dose (0.1 mL) of DMSO and distilled water for 7 days (Control I)

Group II : Mice were administered with a single dose of 0.2% CCl_4 in olive oil (0.1 mL) in day 1 and then received distilled water (0.1 mL) as a single daily dose for 7 days (Control II)

Group III : Mice were administered with a single dose (0.1 mL) of 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde as a single daily dose for 7 days

Group IV : Mice were administered with a single dose (0.1 mL) of (2E)-3-[2-(biphenyl) imidazo [1,2-a]pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one as a single daily dose for 7 days

Group V : Mice were administered with a single dose (0.1 mL) of 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2(1H)-one as a single daily dose for 7 days

Second stage: An interaction between CCl_4 and 2-(biphenyl) imidazo[1,2-a]pyrimidine-3-carbaldehyde,(2E)-3-[2-(biphenyl) imidazo[1,2-a]pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one and 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4-nitro pyrimidine-2 (1H)-one were made and 3 groups of mice were used:

Group VI : Mice were administered with a single dose of 0.2% CCl_4 in olive oil (0.1 mL) in day 1 and then received 0.1 mL of the first dose (0.0312 mg kg^{-1}) of 2-(biphenyl) imidazo[1,2-a] pyrimidine-3-carbaldehyde from 2-7 days

Group VII : Mice were administered with a single dose of 0.2% CCl_4 in olive oil (0.1 mL) in day 1 and then received 0.1 mL of the second dose (0.0312 mg kg^{-1}) of (2E)-3-[2-(biphenyl) imidazo [1,2-a]pyrimidine-3-yl]-1-(4-nitro phenyl) -prop-2-en-1-one from 2-7 days

Group VIII : Mice were administered with a single dose of 0.2% CCl_4 in olive oil (0.1 mL) in day 1 and then received 0.1 mL of the third dose (0.0312 mg kg^{-1}) of 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine-3-yl]-4-nitro pyrimidine-2(1H)-one from 2-7 days

The tested materials were IP injected and mice were sacrificed and dissected in day 8. Before sacrificing the mouse, blood was collected by heart puncture, transferred to Eppendorf tube and allowed to clot at room temperature for 15 min and then serum was separated by centrifugation at 3000 rpm for 10 min. The serum was used for the assessment of liver function enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), in addition to Alkaline Phosphatase (ALP). After blood collection, the mouse was sacrificed and dissected to obtain the liver. The liver was fixed in 10% formalin for histopathological examination.

Determination of enzyme activity of AST and ALT: The enzyme activities of AST and ALT were determined in mouse serum following the enzymatic colorimetric method using a commercial kit (Randox Company)[12].

Alkaline phosphatase (ALP): The enzyme ALP was assessed in mouse serum using a commercial kit produced by Bio Merieux Company. The reaction started by di-sodium phenyl phosphate was hydrolyzed with liberation of phenol and formation of sodium phosphate. The amount of phenol formed was estimated colorimetrically[13].

Histopathological evaluation of liver: The liver was fixed in 10% formalin[14] for 48 h. The procedure is outlined as the following:

- **Washing:** Sample was placed in 70% ethanol overnight
- **Dehydration:** Sample was dehydrated with ascending concentrations (50, 70, 90 and 99%) of ethanol (2 h for each concentration)
- **Clearing:** Sample was placed in xylene for 2 h
- **Infiltration:** Sample was first placed in paraffin-xylene (1:1) for 30 min at 57-58°C and then in paraffin alone for 2 h at 60-70°C
- **Embedding:** Sample was embedded in pure paraffin wax (melting temperature: (60-70°C) and left to solidified at room temperature
- **Sectioning:** Paraffin block was sectioned (rotary microtome) at a thickness of 5 µL and then the sections were transferred to a slide covered with Mayer's albumin. The section of tissue was placed in a water bath (35-40°C) for few sec

RESULTS

Synthesis of 2-biphenyl imidazo (1,2-a) pyrimidine derivative 1: Synthesis of compound 1 was achieved by condensation reaction of 2-amino pyrimidine with 2-4-phenyl phenacyl bromide in ethanol with using sodium bicarbonate (to get rid of the 2 molecules of HBr and H_2O) to give 2-biphenyl) imidazo [1,2-a] pyrimidine 1. The mechanism of formation these known compound 1 is shown in Fig. 1.

The FT-IR spectra of compounds 1 showed a strong absorption bands at 1633, 1616, 1614, 1595 and 1519 cm^{-1} owing to (C = N) pyridine, (C = N) pyrimidine, C = N) imidazo/pyridine and imidazo/pyrimidine, respectively (Table 5).

Disappearing bands of NH_2 group of 2-amino pyridine and pyrimidine at 3200-3300 cm^{-1} in these spectra was good

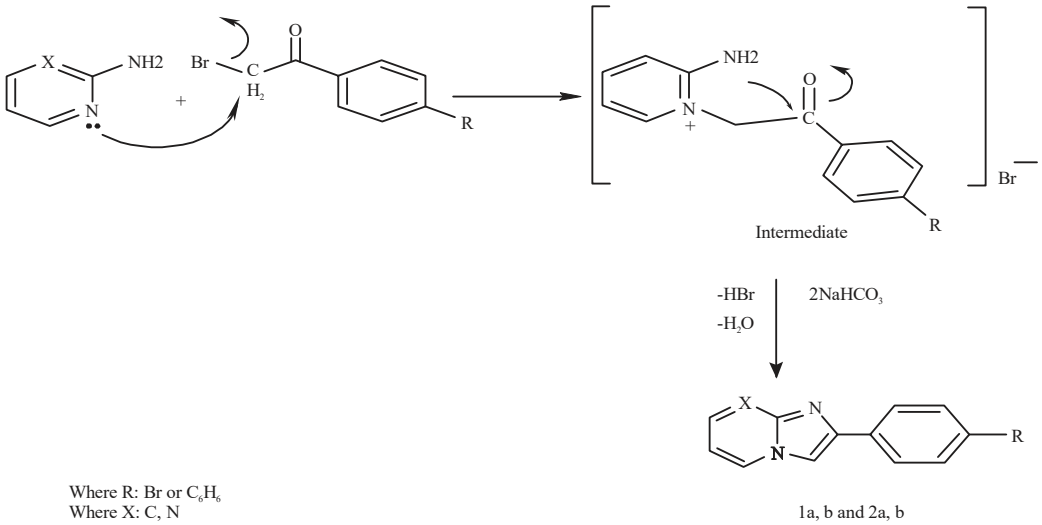


Fig. 1: Mechanism of formation of compounds 1

Table 5: FT-IR spectral data (cm^{-1}) of compounds 1

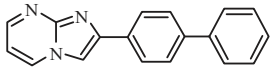
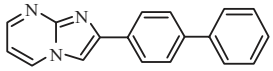
Compound number	Compound structure	Arom υ (C-H)	Pyrimidine υ (C = N)	Imidazo υ (C = N)	Arom υ (C = C)	Other bands
1		3029	1616	1519	1479	C-Ph 767

Table 6: 1H-NMR spectral data (ppm) compounds 1a

Compound number	Compound structure	Chemical shifts (ppm)
1		δ 7.15- 7.19 (d, 2H, Ar-H) δ 7.37-7.39 (d, 2H, Ar-H) δ 7.68- 7.74 (m, 4H, Ar-H) δ 8.66 (d,2H, -CH) δ 9.66- 9.69 (d,2H, Ar-H)

evidence for the formation of fused imidazo/pyridine and imidazo pyrimidine derivative 1 Fig. 2. ^{1}H-NMR spectra of these compounds 1 (Fig. 3) showed characteristic signals at 7.56-7.59 ppm (d, H, -CH) for CH at position-3 in imidazo (1,2-a) pyridine and also at 7.63-7.66 ppm (d, H, -CH) for 2-substituted imidazo (1,2-a) pyrimidine. The reason for splitting this signal to doublet was the effecting of neighboring protons in pyridine ring or pyrimidine ring (Table 6). It worth to mention that all signals of these compounds appeared in down filed region in NMR spectra due to the deshielding effect of aromaticity of these bridge head nitro fused rings.

Synthesis of 2-biphenyl imidazo (1,2-a) pyrimidine-3-carbaldehyde derivatives 2: Compound 2 was prepared by Vilsmeier-Haack reaction to introduce aldehyde group CHO at position-3 by reaction mixture of $POCl_3$ and DMF in presence of $CHCl_3$ with 2-substituted imidazo (1,2-a) pyrimidine (2) (Fig. 4).

The synthesis mechanism formation of imidazo[1,2-a]pyridine-3-carbaldehyde and imidazo [1,2-a]pyrimidine-3-carbaldehyde derivatives 2 as shown in Fig. 5. The reaction of the dimethylformamide with phosphorus oxychloride produces an electrophilic iminium cation followed by electrophilic aromatic substitution yields an iminium ion intermediate, which is hydrolyzed to afford desired aryl aldehyde.

The FT-IR spectra of compounds 2 (Fig. 6) displayed a strong absorption bands at 1653, 1635, 1693 and 1678 cm^{-1} belong to carbonyl of aldehyde group (CH = O) in fused ring of imidazo/pyridine-3-carbaldehyde derivatives and imidazo/pyrimidine-3-carbaldehyde derivatives, respectively. These bands and other bands appeared at 2850 cm^{-1} corresponding to (C-H) of aldehyde were good evidence for the formation of the aldehyde derivatives 3. All details of FT-IR spectral data of compounds 2 are listed in Table 7.

Moreover, ^{1}H-NMR spectra of these compounds 2 Fig. 7 exhibited characteristic signal at 10.1 ppm (s, 1H, -CHO) for fused rings of imidazo/pyridine-3-carbaldehyde, While this signal appeared at 10.15 (s,1H, -CHO) for imidazo/pyrimidine -3-carbaldehyde. In addition to that, disappearing signal of CH

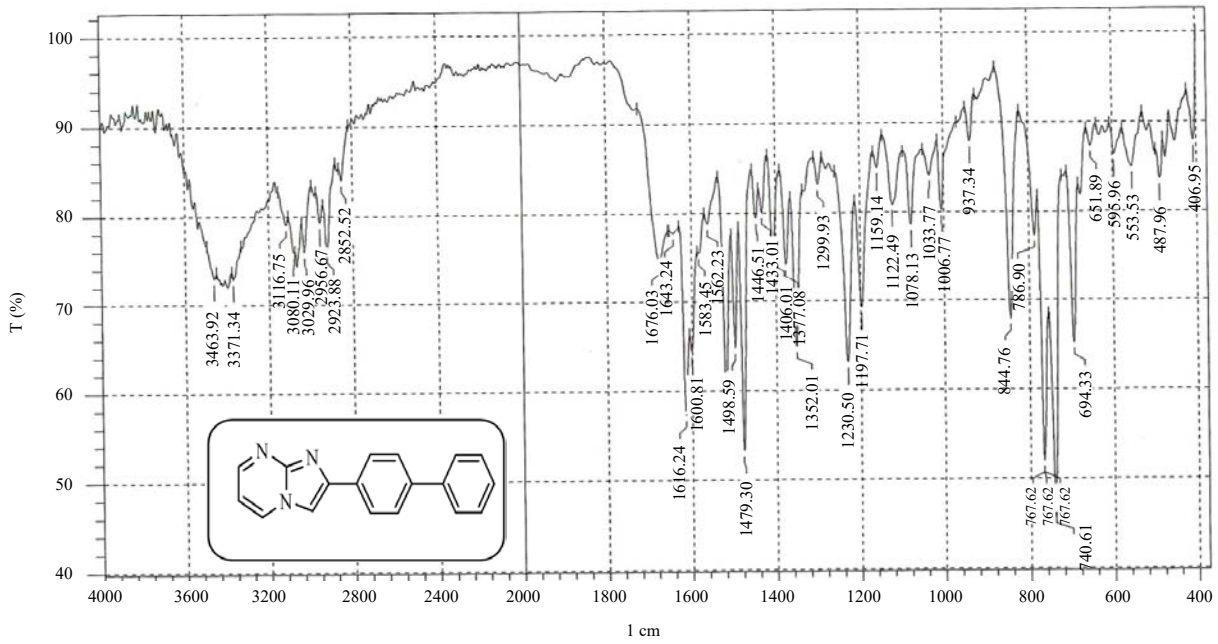


Fig. 2: FT-IR spectrum of compound 1

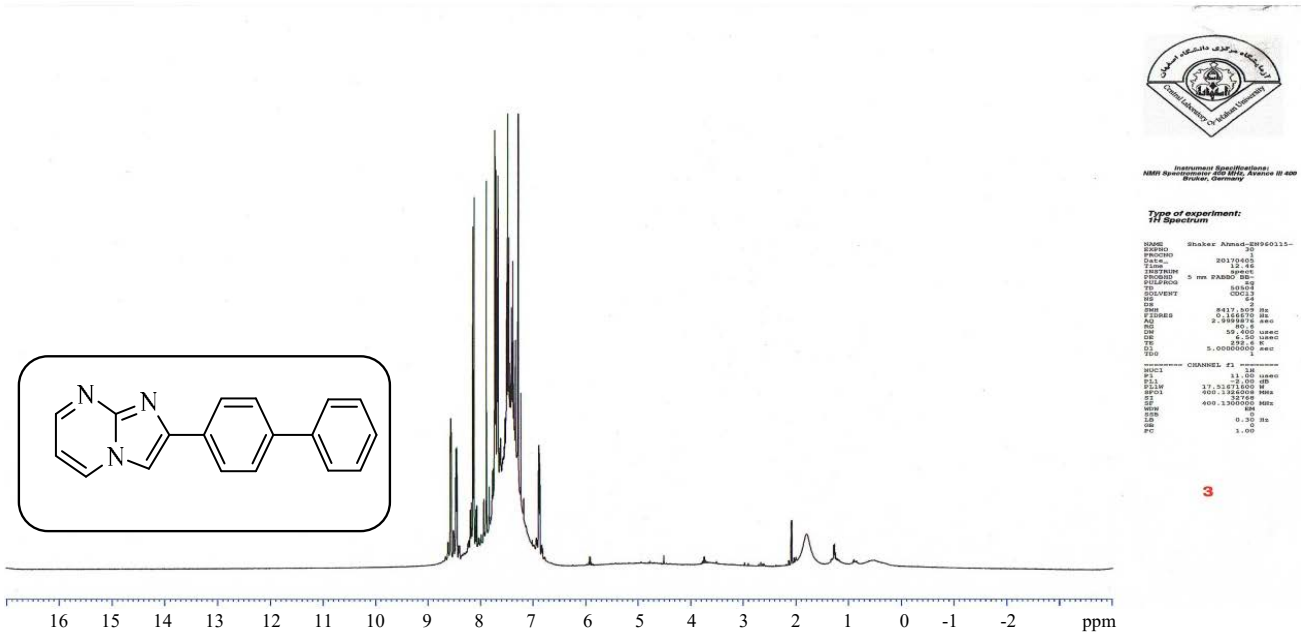
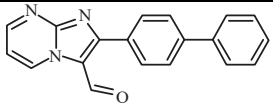


Fig. 3: ^{1}H-NMR spectrum of compound 1

Table 7: FT-IR spectral data of compound 2

Compound number	Compound structure	Aldehyde υ (C-H)	Aldehyde υ (C = O)	Pyrimidine υ(C = N)	Imidazo υ (C = N)	Arom υ(C = C)	Other bands
4b		2860	1678	1610	1560	1518	C-Ph 765

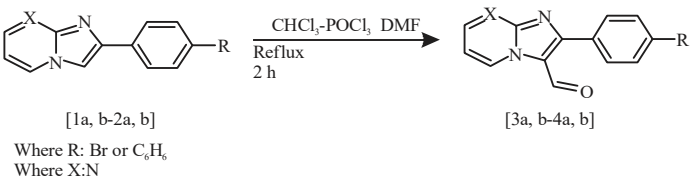


Fig. 4: Vilsmeier-Haack reaction of compounds 2

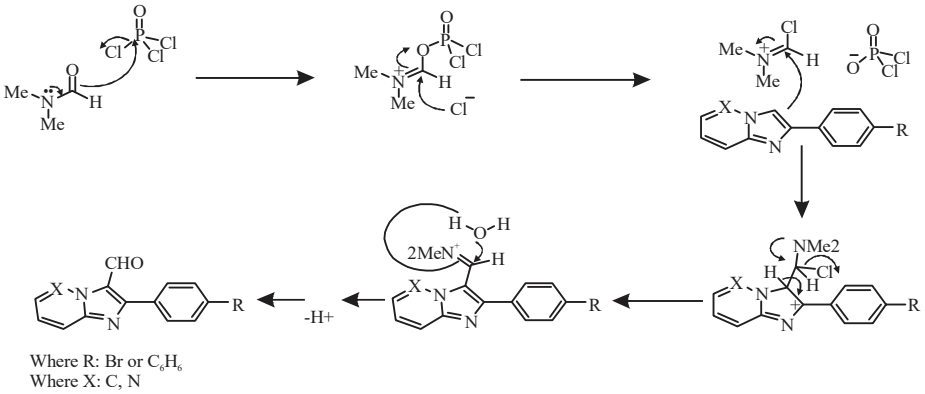


Fig. 5: Synthesis mechanism of compound 3

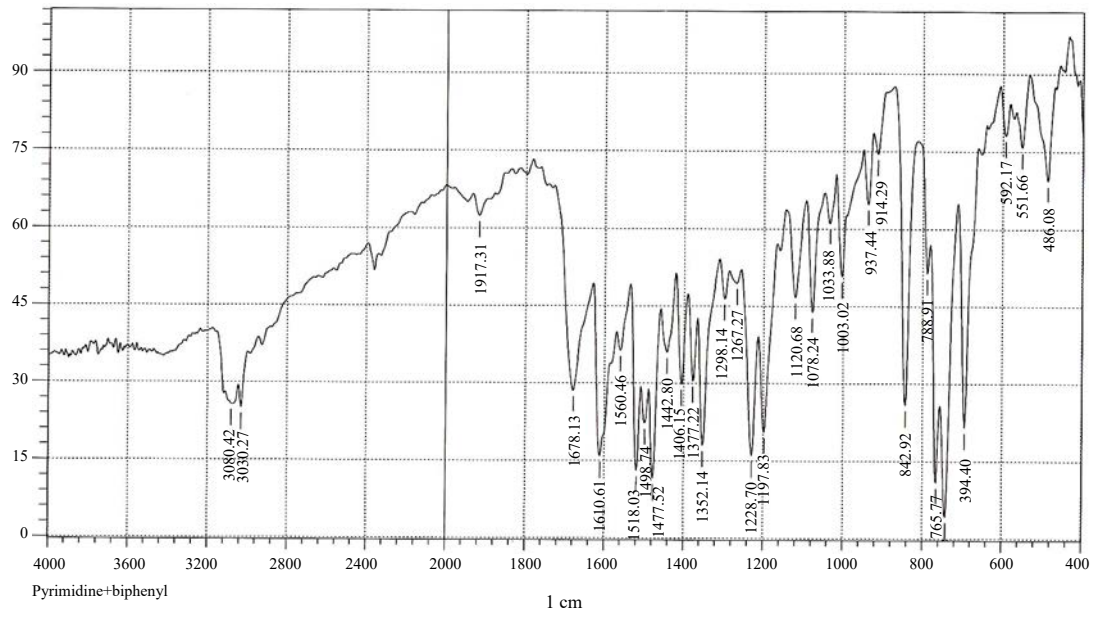
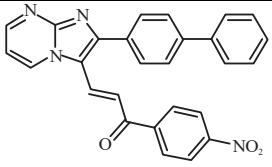


Fig. 6: FT-IR spectrum of compound 2

Table 8: FT-IR bands of compounds 3

Compound number	Compound structure	Arom υ (C-H)	Ketone υ (C = O)	Alkene υ (C = C)	Arom υ (C = C)	Other bands
3		3058	1681	1523	1485	C-NO2750

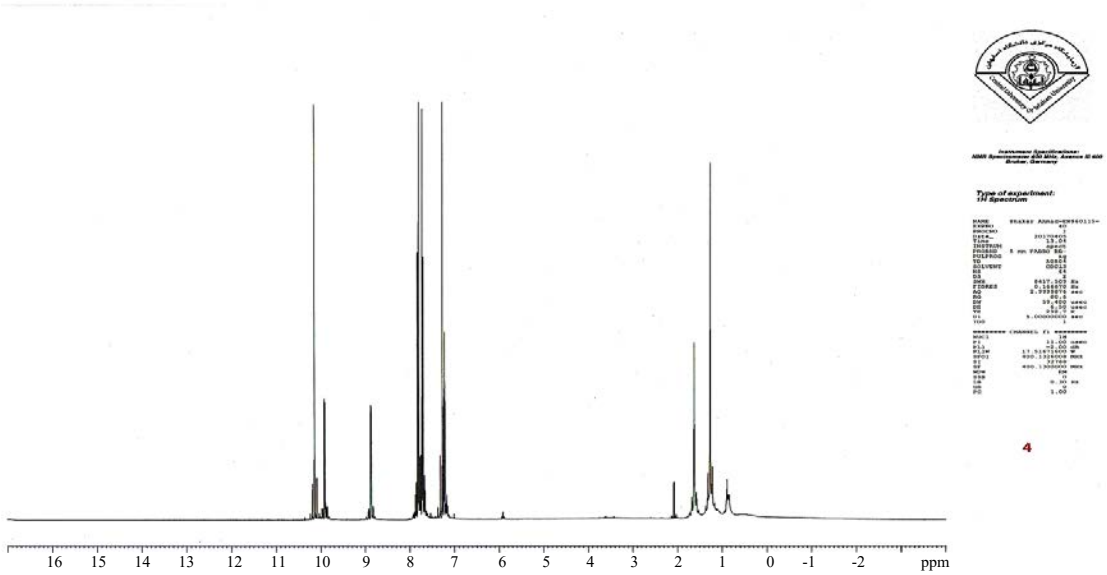


Fig. 7: [1]H-NMR spectrum of compound 2

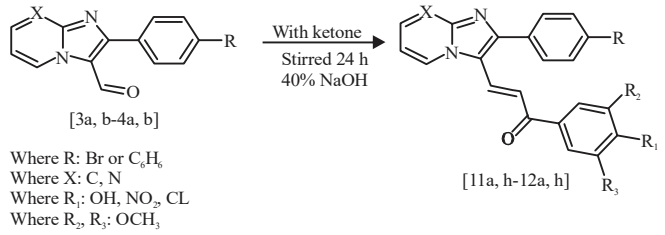


Fig. 8: Formation of chalcones derivative 3

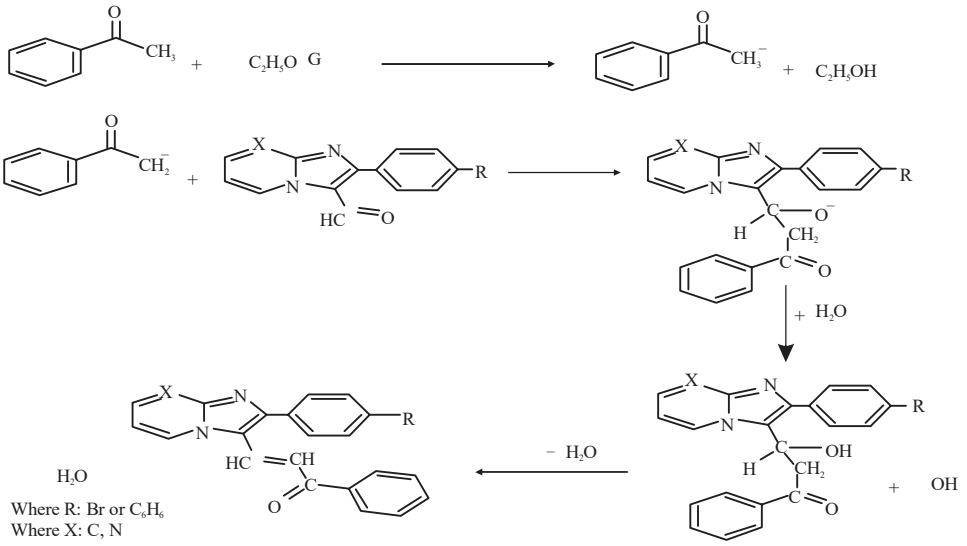


Fig. 9: Mechanism of synthesis chalcone derivatives 3

at position-3 in these spectra for occupation with aldehyde group. These results with other aldehyde tests were unambiguous evidence for introduction aldehyde group at position-3 as shown in Table 8.

Synthesis of chalcones of 2-biphenyl imidazo[1,2-a] pyrimidine 3: The α, β-unsaturated compounds 3 was prepared according to crossed aldol condensation reaction Claisen-schmidt reaction[4] (Fig. 8). A variety of methods are

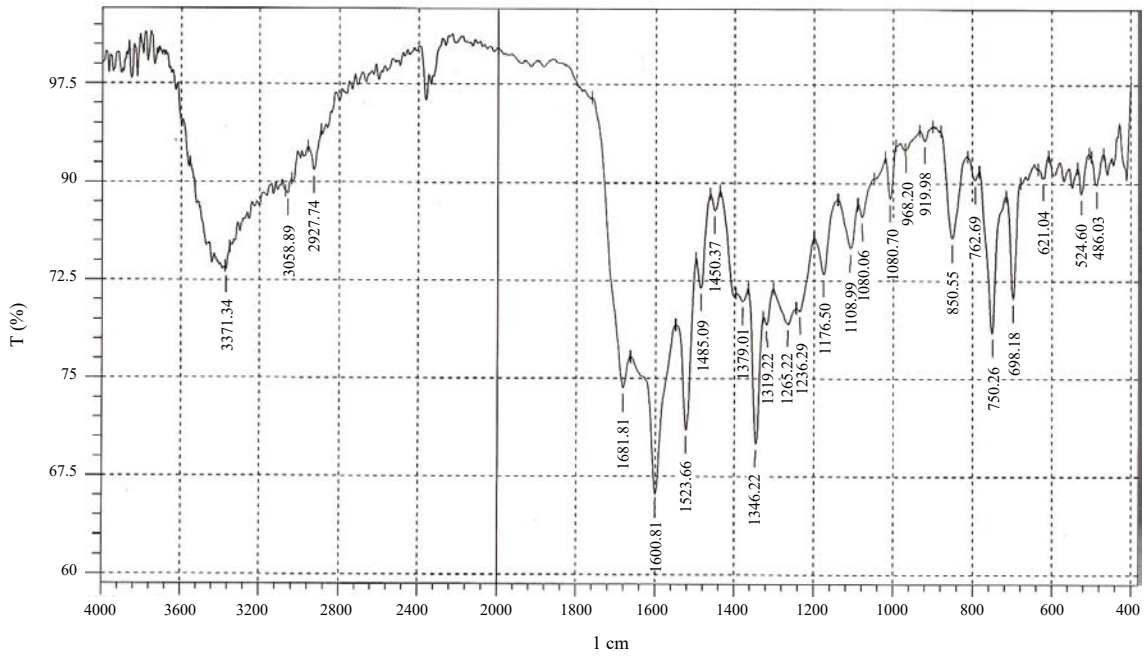


Fig. 10: FT-IR spectrum of compound 3

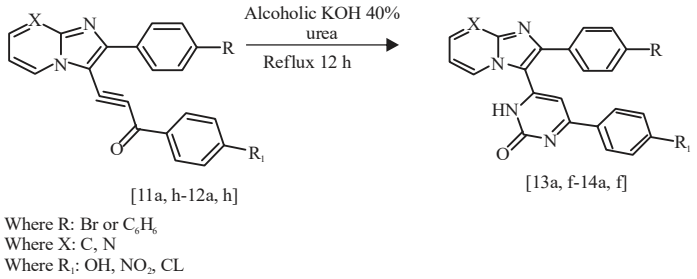


Fig. 11: Cyclization reaction of chalcones to form 3-cyclic oxypyrimidine derivatives 4

available for the synthesis of chalcones, the most convenient method is the one that involved the Claisen-Schmidt condensation of equimolar quantities of a substituted acetophenone with substituted aldehydes (2) in the presence of aqueous alcoholic alkali (Fig. 9).

The FT-IR spectra of compounds (3) (Fig. 10) showed characteristic identification bands at 1681-1631cm^{-1} corresponding to stretching of (C = O) Chalcones of fused imidazo/pyridine derivatives and (1681-1616 cm^{-1}) for Chalcones of fused imidazo/pyrimidine derivative. These bands which are less than usual stretching vibration bands for carbonyl group of acetyl group due to extend the conjugated system[5], while ^{1}H-NMR spectrum of compound (3) showed signals at 7.56_7.60 ppm (d, 2H, HC = CH) for Chalcone imidazo/pyridine derivative, while compound (3) displayed signals at δ 7.56_7.59 ppm (d, 2H, HC = CH). This signal appeared in spectrum of compound (3) at 7.48-7.51 (d, H and CH = CH). ^{13}C-NMR spectra of compound showed characteristic signals at 55,122,127-132,146 and 188 belong to OCH_3, C-Br, CH = CH, CH = N and C = O, respectively.

Synthesis of oxopyrimidines of 2-substituted imidazo [1,2-a] pyrimidine [4]: The cyclization of chalcones derivatives 3 with urea in presence of base as catalyst gave the corresponding oxopyrimidines 4 (Fig. 11).

The FTIR spectra of compounds 4 displayed characteristic identification bands at 1649-1654 cm^{-1} corresponding to stretching of carbonyl C = O) of 3-cyclic oxopyrimidines of imidazo/pyridine derivatives. These bands appeared at (1627-1683 cm^{-1}) in spectrum of 3-oxopyrimidines of imidazo/pyrimidine derivative (Fig. 12). While ^{1}H-NMR spectrum of compound showed signal at 8.10 ppm (s, H, Ar-NH) for imidazo/pyridine-3- oxopyrimidine derivatives (Fig. 13). ^{13}C-NMR spectra of compound 4 showed

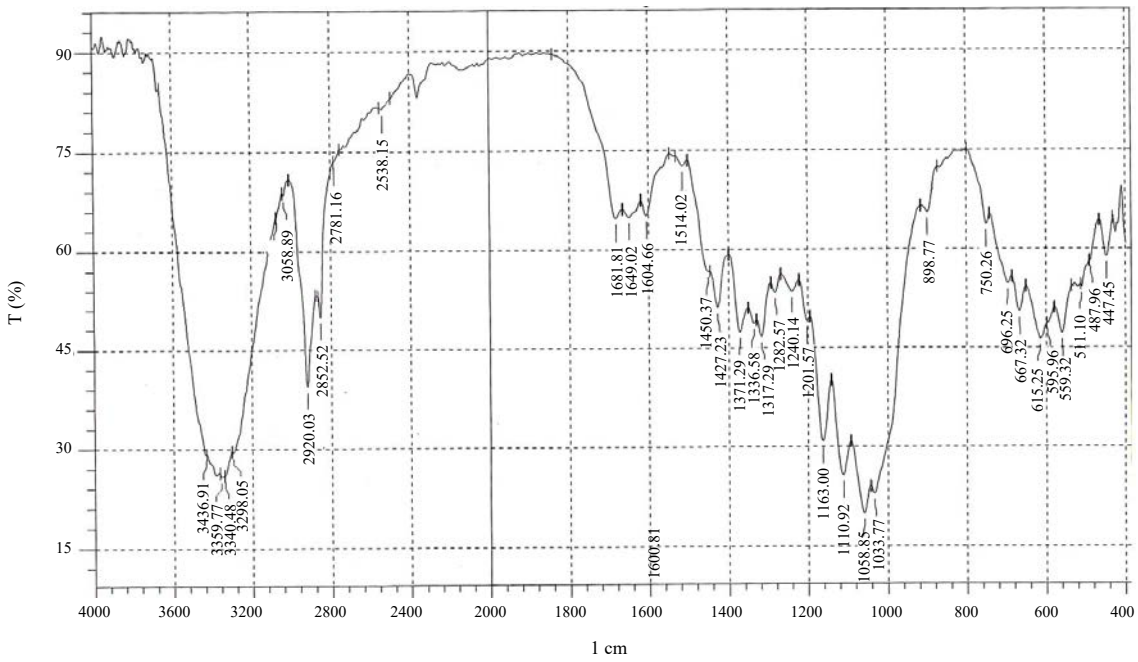


Fig. 12: FT-IR Spectrum of compound 4

Table 9: FT-IR spectral data of compound 4

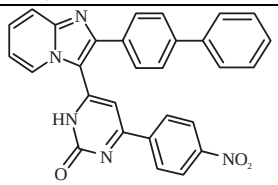
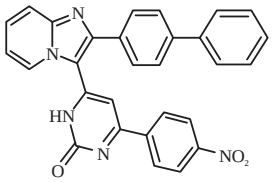
Compound number	Compound structure	Imidigon υ (N-H)	Arom υ (C-H)	Imidazo υ (C = N)	Oxopyrimidine υ (C = O)	υ (other bands)
4		340	3033	1604	1681	C-$NO_2$750

Table 10: Characteristic ^{1}H and ^{13}C-NMR spectral data (d ppm) compound 4

Compound number	Compound structure	Chemical shifts (ppm)
14e		δ 7.43-7.54 (m, 6H, Ar-H) δ 7.78-7.97(m, 4H, Ar-H) δ 7.46 (m, 4H, Ar-H) δ 7.78-7.80 (d, H, Ar-H.) δ 7.89-7.97 (d, H, Ar-H.) δ 8.10 (s, H, Ar-NH) δ 8.12-8.85 (d, 4H, Ar-H) δ 125.9-126.3 (m, Ar) δ 126.39-126.7 (m, Ar) δ 128.8-128.9 (m, Ar) δ 128.7-132.4 (m, Ar) δ 135 (C-NO_2) δ 139.6 (CH = N imidazo) δ 144 (CH = NH cyclic) δ 150.3 (C = O oxo)

characteristic signals at 139.6,144 and150.3 belong to CH = N imidazo, CH = N cyclic and C = O, respectively (Fig. 14).

Histopathological evaluation of liver: The results of treatment mice with (2-(biphenyl) imidazo [1,2-a]pyrimidine-3-carbaldehyde and (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one and 6-[2-(bi phenyl) imidazo[1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2(1H)-one) and for interaction treatment indicated that all compounds had the ability of antioxidant activity for free radical produced by CCl_4 as shown in Table 10.

The results of liver function enzymes (GOT, GPT and ALP) revealed that the compounds reduced these enzymes to (12.66 ± 1.73^E, 21.33 ± 2.40^D, 35.66 ± 2.96^D U L^{-1}) for GOT, GPT, ALP, respectively when mice treated with compound (2-(biphenyl) imidazo [1,2-a]pyrimidine-3-carbaldehyde) and when mice treated with compound ((2E)-3-[2-(biphenyl) imidazo[1,2-a]pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one) also enzymes of liver reduced to (16.66 ± 1.73^E, 26.66 ± 5.69^D, 38.66 ± 4.37^D U L^{-1}) for GOT, GPT and ALP, respectively as shown in Table 11.

The same results obtained when mice treated with compound (6-[2-(bi phenyl) imidazo [1, 2-a] pyrimidine -3-yl]-

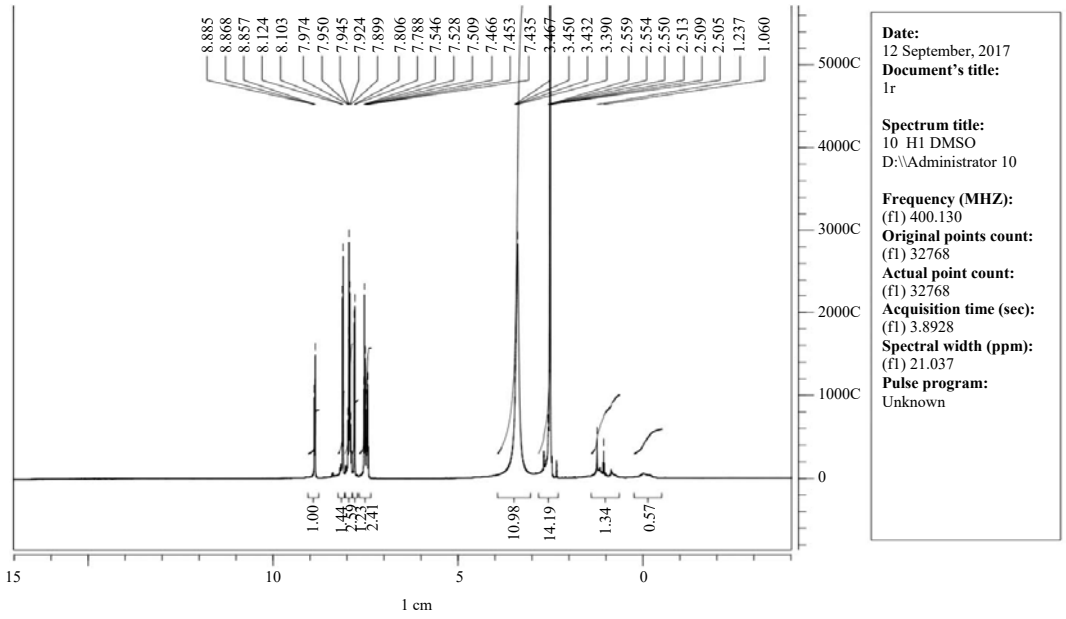


Fig. 13: [1]H-NMR Spectrum of compound 4

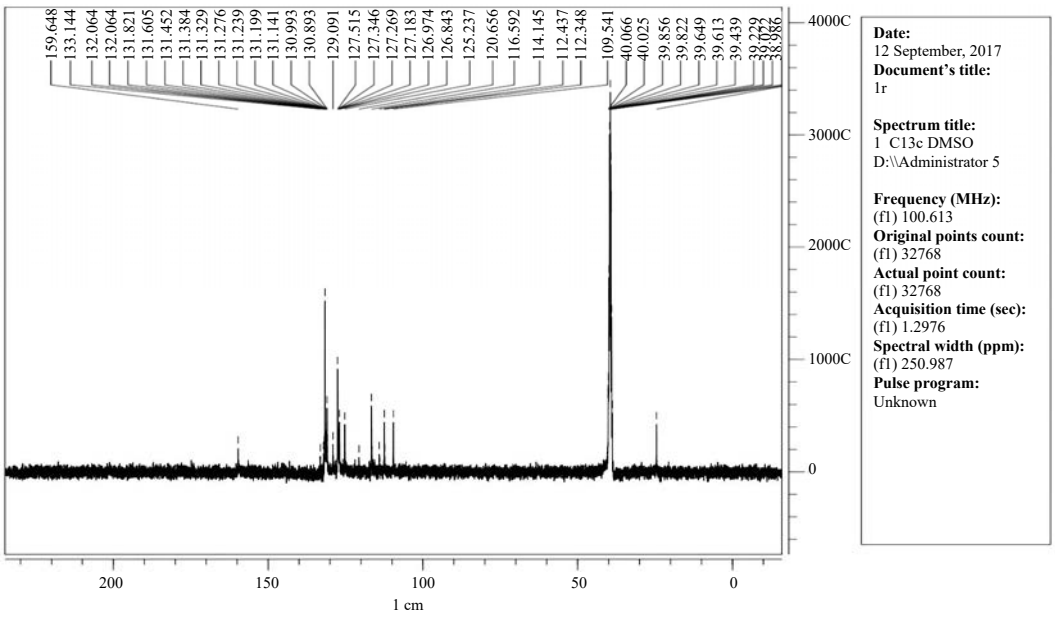


Fig. 14: [13]C-NMR Spectrum of compound 4

4-nitro pyrimidine-2(1H)-one) (24.00 ± 3.05^{D}, 27.33 ± 2.40^{D}, 38.33 ± 2.84^{D} U L^{-1}) for GOT, GPT and ALP, respectively as compared with the results of control negative (38.33 ± 2.02^{BC}, 45.01 ± 5.13^{BC}, 69.66 ± 2.027^{B} U L^{-1}) and control positive (CCl_4) (55.01 ± 1.73^{A}, 61.33 ± 6.33^{A}, 108.33 ± 10.92^{A} U L^{-1}) for all enzymes tested (GOT, GPT and ALP).

The results of interactions indicated that all compounds had ability to repair damage produced by CCl_4 for all enzymes for (2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde) the enzymes concentrations (32.66 ± 2.90^{C}, 40.66 ± 2.18^{C}, 54.03 ± 2.30^{C} U L^{-1}) for GOT, GPT and ALP, respectively.

For ((2E)-3-[2-(biphenyl) imidazo [1,2-a]pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one) the enzymes concentrations were (39.66 ± 1.20^{BC}, 45.00 ± 2.08^{BC}, 62.01 ± 2.08^{BC} U L^{-1}) for GOT, GPT and ALP, respectively. The same results obtained for (6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine-3-yl]-4-nitro pyrimidine-2(1H)-one) the enzymes concentrations were (42.66 ± 3.71^{B}, 54.66 ± 1.45^{AB}, 61.33 ± 2.40^{BC} U L^{-1}) for GOT,

Table 11: Effect of different groups on GOT, GPT and ALP enzyme in sera of carbon tetrachloride (CCl_4) treated albino male mice

Groups	Dose (mg kg^{-1})	Mean±SD (U L^{-1}) GOT	GPT	ALP
Control I: (DMSO)	0.2	38.33±2.02^{BC}	45.01±5.13^{BC}	69.66±2.027^{B}
Control II: (CCl_4)	0.2	55.01±1.73^{A}	61.33±6.33^{A}	108.33±10.92^{A}
Group III: (2-(biphenyl) imidazo [1,2-a]pyrimidine-3-carbaldehyde)	0.0312	12.66±1.73^{E}	21.33±2.40^{D}	35.66±2.96^{D}
Group IV:((2E)-3-[2-(biphenyl) imidazo [1,2-a]pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one)	0.0312	16.66±1.73^{E}	26.66±5.69^{D}	38.66±4.37^{D}
Group V: (6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2(1H)-one)	0.0312	24.00±3.05^{D}	27.33±2.40^{D}	38.33±2.84^{D}
Interactions				
CCL_4+ 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde	0.0312	32.66±2.90^{C}	40.66±2.18^{C}	54.03±2.30^{C}
CCL_4+ (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one	0.0312	39.66±1.20^{BC}	45.00±2.08^{BC}	62.01±2.08^{BC}
CCL_4+ 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2(1H)-one)	0.0312	42.66±3.71^{B}	54.66±1.45^{AB}	61.33±2.40^{BC}

AST (GOT): Aspartate aminotransferase, ALT (GPT): Alanine aminotransferase, ALP: Alkaline phosphatase CCl_4: Carbon tetrachloride

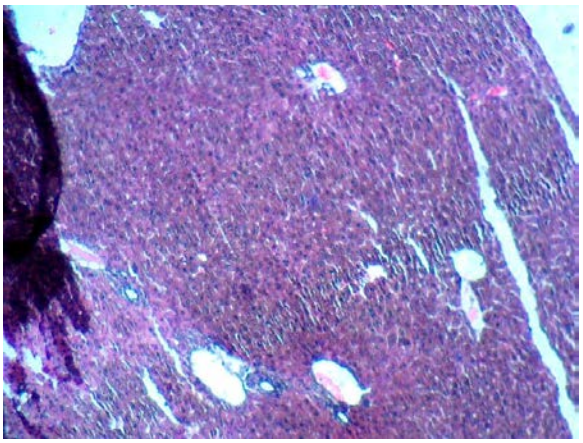


Fig. 15: Section of a liver tissue in mouse treated with carbon tetrachloride and then with distilled water (control positive)
Slight necrosis and degeneration of hepatocytes and mild inflammatory cell infiltrate (mononuclear cells), especially in portal area are observed (200X, H and E)

GPT and ALP, respectively as compared with positive and negative control all compounds (2(biphenyl) imidazo [1,2-a]pyrimidine-3-carbaldehyde and (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one and 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4-nitro pyrimidine -2(1H)-one) alone and in interactions with CCl_4 had significant differences in compared with positive and negative control. The results o f liver histology indicated the ability of all synthesized compound to repair the damaged caused by CCl_4 that resulted in slight necrosis and degeneration of hepatocytes and mild inflammatory cell infiltrate (mononuclear cells), especially in portal area in comparison to newly synthesized compounds which return the his to section of liver to be look like normal (Fig. 15-22).

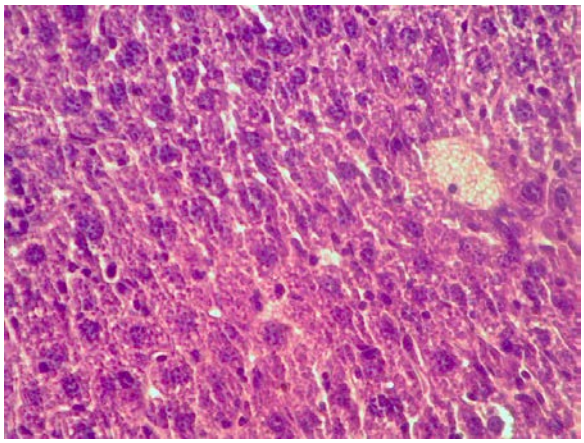


Fig. 16: Section of normal liver structure, which consists of central vein, surrounded by hepatocyte cells (H and E) 200X
Control negative (DMSO)

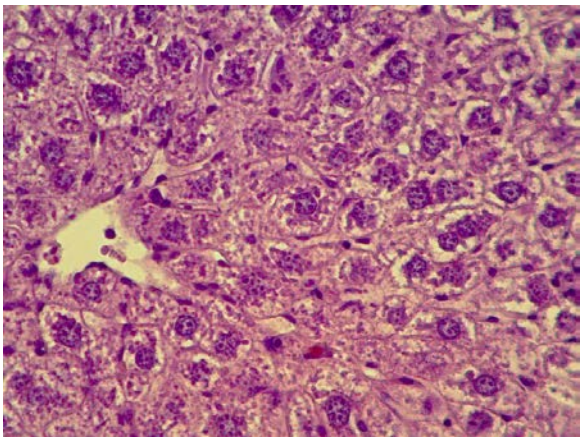


Fig. 17: Mice treated with 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde for 7 days
Liver showing look like normal architecture with accumulation of glycoproteins granules and the cell become enlarged (H and E) 200X

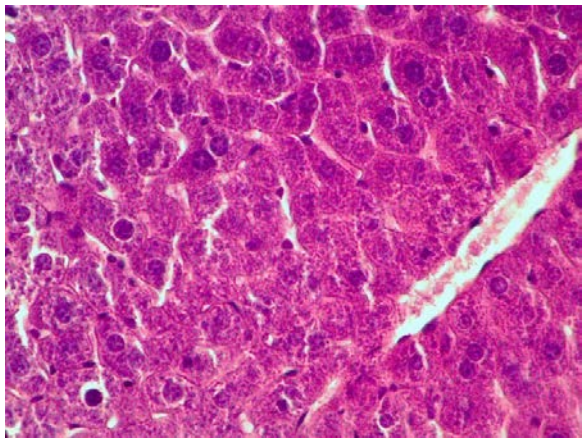


Fig. 18: Mice treated with (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitrophenyl)-prop-2 -en-1-one for 7 days
Liver showing normal appearance structure, consist of central vein and threads of hepatocyte cells (H and E) 200X

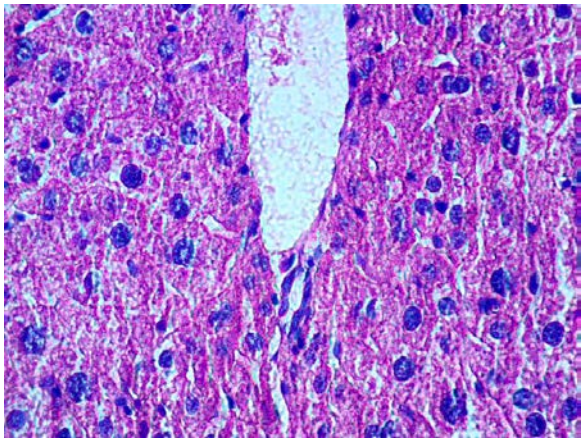


Fig. 20: Section of a liver tissue in mouse treated with carbon tetrachloride and then with 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde
Dilation of sinusoids is observed together with a presence of mild necrotic cells around portal area (200X, H and E)

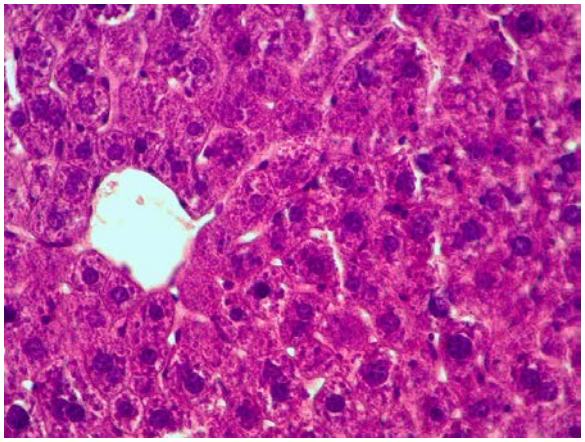


Fig. 19: Mice treated with 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4-nitro pyrimidine -2(1H)-one for 7 days
Liver showing look like normal architecture with accumulation of glycoproteins this indicate the hepatocyte cells (H and E) 200X

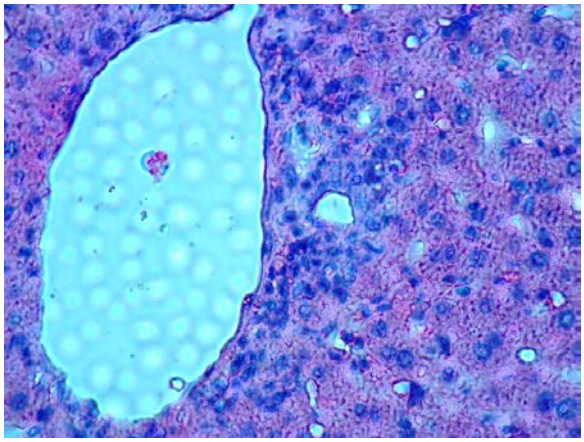


Fig. 21: Section of a liver tissue in mouse treated with carbon tetrachloride and then with (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitrophenyl) -prop-2-en-1-one
Dispersed focal areas of necrosis are still present together with inflammatory cells (mononuclear cells) around central venule (200X, H and E)

DISCUSSION

Liver is the major site of detoxification and the primary target of drug exposure in the body. High levels of drugs cause various hepatic disorders by producing pro-oxidants Reactive Oxygen Species (ROS), which are able to induce cellular damage in a variety of ways by affecting the cellular biomolecules, such as lipids, DNA and proteins[15].

The hepatotoxicity induced by CCl_4 is mainly due to its metabolite CCl_3^-, which is a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids. In the presence of oxygen, lipid peroxides are produced, leading to liver damage, which is characterized by fatty liver, cirrhosis and necrosis[16].

Oxidative stress is considered to play a prominent causative role in many diseases, including liver damage[17]. Oxidative stress is the state of imbalance between the level of antioxidant defense system and production of ROS, such as

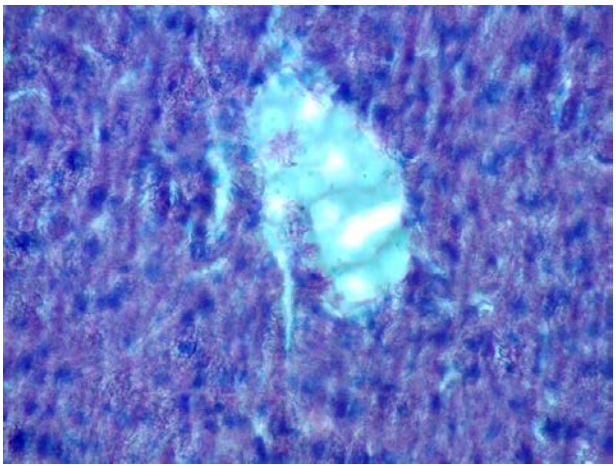


Fig. 22: Section of a liver tissue in mouse treated with carbon tetrachloride and then with 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2(1H)-one
Section is look-like with normal hepatocytes (200X, H and E)

superoxide radical (O^{2-}), hydroxyl radical (OH^{-}) and hydrogen peroxide (H_2O_2). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important for protection against CCl_4 induced hepatotoxicity[18].

In addition, in CCl_4 induced hepatotoxicity, the extent of hepatic damage is assessed by the increased level of cytoplasmatic enzymes (ALT, AST and ALP), which leads to leakage of large quantities of the enzymes into the blood circulation and could be regarded as an index of the liver parenchymal cells damage[19]. Hepatocellular necrosis and liver injury leads to elevation of these serum marker enzymes, which are released from the liver into blood[20].

The present study revealed a significant increase in the activities of ALT, AST and ALP upon exposure to CCl_4, indicating considerable hepatocellular injury. Clinically, the general strategy for prevention and treatment of the CCl_4 induced hepatotoxicity includes reducing the production of reactive metabolites[21] increasing evidence indicates that oxidative stress causes organ injury and carcinogenesis[22].

Carbon tetrachloride induced hepatotoxicity in mice caused a severe centrizonal necrosis, steatosis and damage to the structural integrity of liver and was reflected by increase in the liver hepato specific enzymes (ALP, ALT and AST) in the serum, because they are cytoplasmic in location and are released into circulation after cellular damage[23]. Excessive ROS generation triggers the process of lipid peroxidation in cell membranes and causes the destruction of cell components and cell death[24].

CONCLUSION

The newly synthesized chemical compounds 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde, (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl) -prop-2-en-1-one and 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine -3-yl]-4- nitro pyrimidine -2(1H)-one had the ability to counteract the damaged caused by CCl_4 in liver of mice by returning its appearance to normal state.

SIGNIFICANCE STATEMENT

This study discovers the ability of promising newly synthesized chemical compounds 2-(biphenyl) imidazo [1,2-a] pyrimidine-3-carbaldehyde, (2E)-3-[2-(biphenyl) imidazo [1,2-a] pyrimidine-3-yl]-1-(4-nitro phenyl)-prop-2-en-1-one and 6-[2-(bi phenyl) imidazo [1,2-a] pyrimidine-3-yl]-4-nitro pyrimidine-2(1H)-one to have hepatoprotective activity against CCl_4 damage in albino male mice.

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