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ORIGINAL ARTICLE

Synthesis, Characterization, and Antimicrobial Evaluation of New Ibuprofen Derivatives

MOHAMMED MUHSIN JABBER[1], MOHAMMED K. HADI[2]
[1]*Iraqi Ministry of health, Almothana Health Department*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.*
Correspondence to: Mohammed Muhsin Jabber, Email: mohammedmuhsin1988@gmail.com, Cell: 009647802808920

ABSTRACT
A new series of ibuprofen derivatives were synthesized and tested against the microbial activity. P_amino ethyl benzoate (compound I) was prepared, then coupled with ibuprofen acyl chloride (compound II) to give ethyl 4-(2-(4-isobutylphenyl) propanamido) benzoate (compounds III). Hydrazine hydrate 99% was added to compound III to synthesized N-(4-(hydrazinecarbonyl) phenyl)-2-(4-isobutylphenyl) propenamide (compound IV). Finally compound IV coupled with different aldehydes (1. Benzaldehyde (Va) 2. Salicylaldehyde (Vb) 3. 4_hydroxybenzaldehyde (Vc) 4. Vanillin (Vd) respectively to form target compounds. Identifications and characterization of these compounds were done by using Fourier Transform Infrared Spectroscopy (FTIR), and ^{1}HNMR analyses. The antimicrobial results showed that compounds (Va has low activity, Vd has high active, Vb and Vc has the moderate activity) against gram positive bacteria (Staphylococcus aureus and Streptococcus pyogenes) and gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) at concentration 500 µg/mL. Vd was the only compound that has antifungal activity against Candida ablicans compared to fluconazole.
Keywords: Anti-microbial activity, Ibuprofen, Hydrazine hydrate, Schiff base, Aldehyde.

INTRODUCTION
Non-antibiotics can have a direct antimicrobial impact on bacteria and/or fungal cells by a variety of mechanisms, including membrane effects, metabolic modifications, DNA intercalation, adhesion inhibition, and so on. As helper chemicals or immune system modulators, others have an indirect antibacterial effect.[1,2]

In today's pharmaceutical industry, antimicrobial activity of non-antibiotics is frequently discovered during suitability tests conducted prior to routine pharmacopoeial microbiological purity analysis, which is one of the quality requirements for a drug's release to the market and ensures the patient's microbiological safety.[3]

Hersh et al. in 1991 and Sanyal et al. in 1993, respectively, were the first to mention ibuprofen's antibacterial and antifungal activities. As a result, ibuprofen's antibacterial action, both direct and indirect, has been recognized for more than 20 years. Surprisingly, there are only approximately 20 studies on the issue, and several elements of ibuprofen's antimicrobial activity, such as the mechanisms of antibacterial action, have not been studied at all.[4,5]

True, most ibuprofen's antimicrobial effects, as well as those of other non-antibiotics, occur at concentrations significantly higher than those ordinarily obtained in the patient's blood when therapeutic dosages are administered. However, because ibuprofen has been demonstrated to reach inhibitory concentrations in the patient's urine, it has promise as a topical medication and in the treatment of other localized illnesses, such as urinary tract infections.[6]

Ibuprofen might be employed as a coating for prosthetic devices or as a starting point for the development of new antibacterial medicines with unique modes of action.[7,8]

Unfortunately, ibuprofen's antibacterial action is still regarded as a minor adverse effect that is not included in the patient information leaflets for ibuprofen-containing medications. As a result, it may represent an advantage for the patient, a hidden added value of the products of unknown magnitude. However, it may pose extra dangers during use, particularly when ibuprofen products are used with antibiotics[9,10,11] or probiotics[12]or when administered rectally, where it may produce an imbalance in the gut flora.[13,14]

Hugo Schiff, a German scientist and Nobel Prize winner, created Schiff bases, are condensation reactions of primary amines and carbonyl compounds[15]. they (also known as imine or azomethine) is a structural analogue of a ketone or aldehyde that's had the carbonyl group (C=O) exchanged by an imine or azomethine group[16,17].

Schiff bases and their complex are flexible molecules with a wide range of biological activity, including antibacterial, antifungal, and antiviral properties, etc[18].

MATERIAL AND METHODS
Solvents and other chemicals used in the process were purchased from commercial sources. Ibuprofen was obtained from Pioneer (an Iraqi pharmaceutical business). To evaluate the purity of the products and monitor the reactions under UV light, thin-layer chromatography (GF254, Merk, Germany) was utilized (254nm). A total of two solvent systems were used. A and B are Chloroform: Methanol (17:3)[19] and ethyl acetate: n-hexane (4:1)[20]

The melting points were calculated using the Stuart SMP3 melting point instrument in open capillary tubes and are uncorrected. The infrared spectra were created using a Shimadzu FT-IR spectrophotometer from Japan. ^{1}HNMR spectra were obtained on the NMR-500 spectrometer model using tetramethyl silane as an internal standard; chemical shifts (δ) were expressed in ppm.

Synthesis Synthesis of Ethyl P-aminobenzoate (compound I): 6 gm of benzoic acid in 60 ml of absolute ethanol with 6 ml of H_2SO_4 were refluxed for 75 minutes after cooling, cold water was added then sodium bicarbonate (10%) was added, until the bubbles disappear. finally filter and wash with distilled water at least 2 time

synthesis of ibuprofen acyl chloride (compound II): (1 gm 4.8mmol) of ibuprofen was suspended in 1 ml of dry chloroform with addition of excess amount of thionyl chloride (1.2 ml) drop by drop, then the resulting mixture was reflexed for 3 hours.

After that, remove the solvent and rinse the residue twice at least with 3ml of dry chloroform.

Synthesis of ethyl 4-(2-(4-isobutylphenyl) propanamido) benzoate (compound III): (0.825 gm 4.8 mmol) of compound Ⅰ was dissolved in 30 ml of Dichloromethane (DCM) with addition of (1.35 gm 9.6mmol) triethylamine over 3 minutes then stirring for three hours at room temperature.

In other line dissolve ibuprofen acyl chloride in 1ml dry chloroform then added slowly to the above mixture and left stirring at room temperature overnight.

The mixture dried and the residue dissolved in 30 ml of ethyl acetate and washed with 10 ml (1 N HCl), 10 ml D.W., 10 ml NaHCO3 5 %, respectively twice for each one and finally the organic layer was separated and dried with magnesium anhydrous to get oily residue.

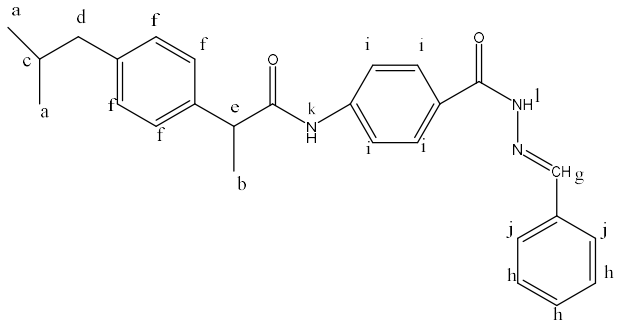
Synthesis of N-(2-hydrazinyl-2-oxoethyl)-2-(4-isobutylphenly) propenamide (compound IV): (0.8 gm 3 mmol) of compound Ⅲ dissolved in 10 ml of absolute ethanol, then hydrazine hydrate 99%

is added and reflexed for 9 hours at 65 C° then the mixture was stirred overnight at room temperature.

The mixture was evaporated by hot air steam then cold water was added a white precipitate was obtained, filtered and finally rinsed with ether.

Synthesis of target compound (Va_Vd): 5 drops of glacial acetic acid were added to an absolute ethanolic solutions (5 ml) containing (4.8mmol) of each of the following aldehydes. benzaldehyde; 1 (1.2 gm), salicylaldehyde; 2 (1.3gm), 4-hydroxybenzaldehyde; 3 (1.22gm), 4-hydroxy-3-methoxybenzaldehyde; 4(1.2gm) respectively. Then, drop by drop, added (4.8mol, 1.2g) of compound IV dissolved in (10ml) of absolute ethanol to the reaction mixtures given above, and reflux for 6 to 8 hours at 80°C. At the end of the reflux time, the mixture was allowed to cool at room temperature or on crushed ice, and the generated ppt. were collected, filtered, and recrystallized with hot ethanol to deliver the necessary target compound.

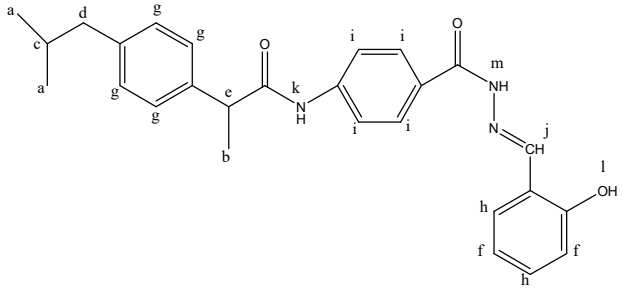
➢ **(E)-N-(4-(2-benzylidenehydrazine-1-carbonyl) phenyl)-2-(4isobutylphenyl) propenamide (compound Ⅴa)**



Light yellow, **Yield**: 85%, **M.P**: (200_202 ℃), **Rf**: 0.88 (B), **IR** (υ cm^{-1}): 3317_3251 (NH) str. of 2 ◦amide, 3059 (CH) str. of aromatic, 2954 (CH_3) str, 1670 O=C-NH-N Asymmetric stretching, 1647 O=C-NH Asymmetric stretching, 1608 C=N.

^{1}H NMR: 0.85 a(6H, d, CH_3), 1.43 b(3H, d, CH_3), 1.81 c(1H, m, CH), 2.42 d(2H, d, CH_2), 3.85 e (1H, m, CH), 7_7.9 f,l,j,h (13 H protons of aromatic ring), 8.46 g(1H, s, HC=N), 10.33 k(1H, s, NH proton), 11.76 l(1H,s, O=C-NH-N).

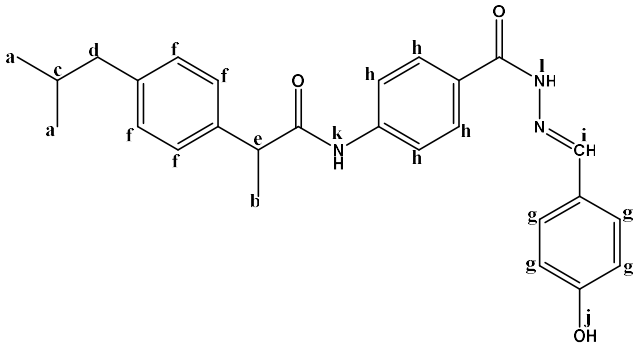
➢ **(E)-N-(4-(2-(2-hydroxybenzylidene) hydrazine-1-carbonyl) phenyl)-2-(4 isobutylphenyl) propenamide (compound Ⅴb)**



Crestal yellow, **Yield**: 50%, **M.P**: (230_233 ℃), **Rf**: 0.88 (B), **IR** (υ cm^{-1}): 3317 Phenolic OH stretching, 3228_3197 (NH) str. of 2 ◦amide, 3055 (CH) str. of aromatic, 2951 (CH_3) str, 1658 O=C-NH-N Asymmetric stretching, 1639 O=C-NH Asymmetric stretching, 1608 HC=N

^{1}H NMR: 0.85 a(6H, d, CH_3), 1.43 b(3H, d, CH_3), 1.81 c(1H, m, CH), 2.42 d(2H, d, CH_2), 3.85 e(1H, m, CH), 6.91_7.85 f,g,h,i(12 H protons of aromatic ring), 8.63 j(1H, s, HC=N), 10.33 k(1H, s, NH proton), 11.76 l(1H,s, OH of aldehyde), 12 m(1H, s, O=C-NH-N).

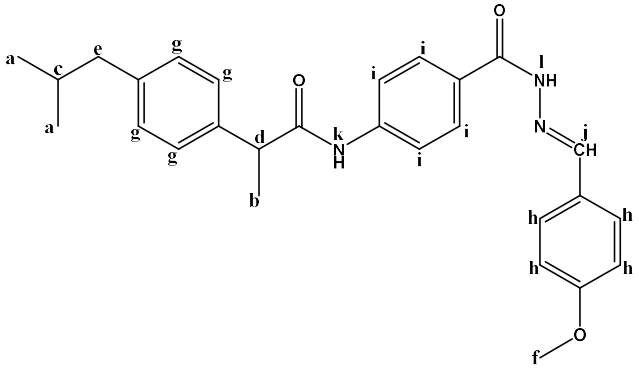
➢ **(E)-N-(4-(2-(4-hydroxybenzylidene) hydrazine-1-carbonyl) phenyl)-2-(4-isobutylphenyl) propenamide (compound Ⅴc)**



White fluffy powder, **Yield**: 63%, **M.P**: (226_228 ℃), **Rf**: 0.88 (B), **IR** (υ cm^{-1}): 3317 Phenolic OH stretching, 3305_3228 (NH) str. of 2 ◦amide, 3084 (CH) str. of aromatic, 2954 (CH_3) str, 1658 O=C-NH-N Asymmetric stretching, 1643 O=C-NH Asymmetric stretching, 1608 C=N.

^{1}H NMR: 0.85 a(6H, d, CH_3), 1.43 b(3H, d, CH_3), 1.81 c(1H, m, CH), 2.10 d(2H, d, CH_2), 3.85 e(1H, m, CH), 6.86_7.89 f, g, h(12 H protons of aromatic ring), 8.31 i(1H, s, HC=N), 9.80 j(1H,s, OH of aldehyde), 10.31 k(1H, s, NH proton), 11.45 k(1H, s, O=C-NH-N).

➢ **(E)-2-(4-isobutylphenyl)-N-(4-(2-(4-methoxybenzylidene) hydrazine-1-carbonyl) phenyl) propenamide (compound Ⅴd)**



Off white powder, **Yield**: 65%, **M.P**: (220_222 ℃), **Rf**: 0.88 (B), **IR** (υ cm^{-1}): 3321_3248 (NH) str. of 2 ◦amide, 3084 (CH) str. of aromatic, 2951 (CH_3) str, 1666 O=C-NH-N Asymmetric stretching, 1647 O=C-NH Asymmetric stretching, 1608 C=N, 1253 C-OCH_3 Asymmetric stretching

^{1}H NMR: 0.85 a(6H, d, CH_3), 1.43 b(3H, d, CH_3), 1.81 c(1H, m, CH), 2.10 d(2H, m, CH), 3.41 e(2H,d, CH_2), 3.82 f(3H, s, aromatic of aldehyde), 7.10_7.85 g, h, i(12 H protons of aromatic ring), 8.39 j(1H, s, HC=N), 10.32 K(1H, s, NH proton), 11.62 l(1H, s, O=C-NH-N).

Antimicrobial Assay: The antibacterial effect was measured by measuring the diameter of the inhibition zone (IZ) all over the disc in millimetres after 24 hours of incubation at 37 °C. The antibacterial and antifungal effect was determined by measuring the width of the inhibitory zone generated all around the well.[21]

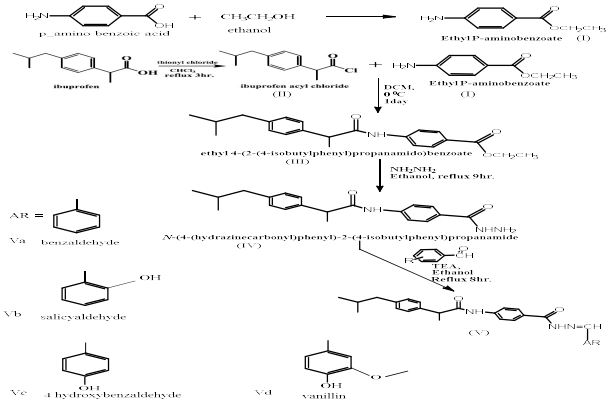
RESULTS AND DISCUSSION

Chemistry: The steps of the synthesis of target compound **(Ⅴa-Ⅴd)**, starting from ibuprofen are demonstrated in scheme1. In the first, synthesis the ibuprofen acyl chloride by interacting ibuprofen with thionyl chloride. the other line prepare of ethyl ester of

p_amino benzoic acid was addition to compound II to form compound III. Then added hydrazine hydrate 99% to compound III to form compound IV. Finally, target compound (Va-Vd), were prepared by refluxing different aromatic aldehydes **(1. benzaldehyde (Va)2. Salicylaldehyde (Vb) 3. 4_hydrxy benzaldehyde (Vc) 4. Vanillin (Vd))** with compound IV in absolute ethanol in presence of acetic acid as catalyst.

The FTIR spectra of the target compounds recorded characteristic bands in cm-^{1}. Compound (II) was identified by the appearance of the absorption band of carbonyl group C=O stretching of (II) at 1708 cm-1 due to ester formation and appearance of the absorption band of carbonyl group C=O stretching of amide at 1658 cm-1. In compound (III), carbonyl group C=O stretching was shifted to 1627 cm-1due to amide hydrazide formation, also compound (III), showed two characteristic absorption bands at 3313 and 3224 cm-1attributed to primary amine N-H stretching (asymmetric and symmetric str. respectively) of hydrazide formation. Carbon-nitrogen double bond formation (imine) in N-acyl hydrazones (NAH) (IV a-d) was confirmed by the appearance of absorption bands (C=N) stretching at 1600-1608 cm-1, while N-H stretching vibration of secondary amide appeared at 3325-3197 cm-1. Additionally, the carbonyl C=O stretching of amide showed absorption bands at 1639-1670 cm-^{1}. Also, the N-acyl hydrazones (NAH) formation were confirmed by the appearance of disappearance of absorption band of primary amine at (3313-3224) cm-^{1}.

Interpretation of the results of 1H-NMR spectra the ester compound (II) was characterized by the appearance of singlet signal at δ= 10.37 ppm due to (O=CNH) proton of NH group and quartet signal at δ= (4.30) ppm due to methylene (OCH2CH3) protons of CH2 group and triplet signal at δ= (1.41) ppm due to methyl (CH3), protons of CH3 group. The acid hydrazide compound (III) displayed two characteristic singlet peaks at δ= 4.45 and 9.64 ppm of -NH-NH2 and -NH-NH2 respectively. The 1HNMR spectra of N-acyl hydrazones (NAHs) were consistent with the assigned structures. Two sets of two separated singlets were displayed in the 1HNMR of N-acyl hydrazones (V a-f), attributed to both the –N=CH- and -CONH- protons. These protons appeared as two singlets resonating in the regions (8.31 - 8.63) and (11.45 - 12) ppm, respectively. Moreover, the NAHs spectra showed the disappearance of singlet peak at 4.34 ppm of NH-NH2 protons of acid hydrazide. The aromatic protons of substituted phenyl groups displayed different chemical shifts between (6.86-7.97) ppm, due to variations in the electronegativity as well as differences in the electron donating or the electron withdrawing characteristic of each substituent.



Scheme 1: Pathway synthesis of target compounds (Va-Vd)

Table 1: Antimicrobial activity of target compounds (Va-Vd) with concentration 500 µg/mL

Compound No.	Inhibition zone (IZ) in mm				
	Escherichia coli (G-ve)	Pseudomonas aeruginosa (G-ve)	Staphylococcus aureus (G+ve)	Streptococcus pyogenes (G+ve)	Candida ablicans
Ciprofloxacin*	22		18	15	
Fluconazole**					25
DMSO					
Va	9	6	9	10	10
Vb	21	12	19	20	18
Vc	20	14	20	20	17
Vd	22	15	25	23	27

* Standard for bacterial strains, ** Standard for fungi.

= No activity, slightly active (inhibition zone between 5-10 mm), moderately active= (inhibition zone between 10-20 mm), highly active= (inhibition zone more than 20 mm).[22]

Antimicrobial evaluation: Using a well diffusion approach, the antibacterial activity of target compound (Va_Vd) were investigated in relation to ciprofloxacin and fluconazole using G(+ve Staphylococcus aureus and Streptococcus pyogenes) and G(-ve Escherichia coli and Pseudomonas aeruginosa) bacteria and fungal strain Candida albicans. As indicated in Table 1, DMSO served as both a solvent and a negative control.

compounds synthesized derivatives show acitvity against 4 types of bacteria, especially compound (Vd) has the greatest activity against 4 type of bacteria tested. While Vb and Vc have moderate activity against bacteria tested.

When the antifungal activity of the novel compounds was tested against Candida albicans species, the majority of them were shown to be extremely active when compared to Fluconazole; in fact, compound (Vd) outperformed Fluconazole.

CONCLUSION

The novel target compounds were produced effectively. FT-IR spectroscopy and 1HNMR were used to validate their chemical structures. The well-diffusion method was used to investigate the antibacterial activity of target compounds (Va_Vd). The synthesized compounds had antibacterial activity, (Vd) compounds has more antibacterial activity, while (Va) has slightly antibacterial activity when compared to standard pharmaceuticals (ciprofloxacin). (Vd) has greater antifungal activity compared to fluconazole.

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