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ORIGINAL ARTICLE

Synthesis, Characterization and preliminary Anti-Microbial Evaluation of New Ibuprofen Hydrazide Derivatives

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ABSTRACT
To create a novel series of aryl propionic acid with better antimicrobial properties, new Ibuprofen (2-(4-isobutylphenyl) propionic acid) hydrazone was prepared from the reaction of Ibuprofen ethyl ester and hydrazine hydrate in the presences of different aromatic aldehyde. Anti -microbial activity was tested for all compounds against gram positive (S. pyrogen, S.aureus), gram negative (E. coli, Klebsiella pneumoniae) bacterial strains and fungal strains (candida albicans). Compound (3f) display the highest anti-bacterial activity against gram positive bacteria while compound (3e) display highest anti-bacterial activity against gram negative one, the compound (2b) showed highest anti-fungal activity compare with the other prepared compound. All the final compounds were characterized by FT-IR and ^{1}H-NMR spectroscopy.
Keywords: Ibuprofen, hydrazine hydrate, anti-microbial activity.

INTRODUCTION
Non-steroidal anti-inflammatory medicines (NSAIDs) one of the best regularly recommended medications in the world like Ibuprofen, Diclofenac are examples of most regularly utilized medicines. Along with their anti-inflammatory action, they have anesthetic and fever reducing effect. It has also been record, but undervalued, for greater than 20 years [(1),] that NSAIDs bear direct and indirect antimicrobial effects. NSAIDs act by competitive inhibition for both cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) enzymes. [(2)] Using of NSAIDs for long time can lead to different and serious side effect like GIT bleeding and disturbance of renal function, this principally due to the free carboxylic group which cause mucosal irritation, in addition to the inhibition of prostaglandin production that responsible for the protection of mucosal GIT. [(3)] So different attempt was don and till now in order to masking these unwanted effect and enhancing their biological activity [(1)] the ant-bacterial activity of NSAIDs has previously been proven against a wide spectrum of Gram-negative and Gram-positive microorganisms. [(4,5)] The anti-bacterial effect of NSAIDs caused by two different mechanisms either by Their capacity to influence the integrity of bacteria's cytoplasmic membrane or by prevent DNA synthesis, DNA replication, and cell membrane reformation. [(6) (7,8)]

Ibuprofen one of the most important derivatives of aryl-propionic acid which consider one of the important class of NSAIDs (figure-1). [(2) (9)] Ibuprofen is 2- (4-isobutylphenyl) propionic acid, in spite of its common adverse effect like stomach ulcer and bleeding with long term use, Ibuprofen still used and have powerful effect in reducing pain and inflammation. [(10)] Recently, due to increase microbial resistance for commonly used drugs, so various attempt was done to synthesis and developing new non antibiotic drugs, such as Hydrazone/hydrazide derivatives. Hydrazones are chemical molecules having the formula (R1 R2 C = NNH2) that are linked to ketones and aldehydes, which characterized as a class of organic compound, [(11) (12)] with wide range of pharmacological and biological activity like anti-inflammatory, antimicrobial, analgesic and anticancer. [(13)]

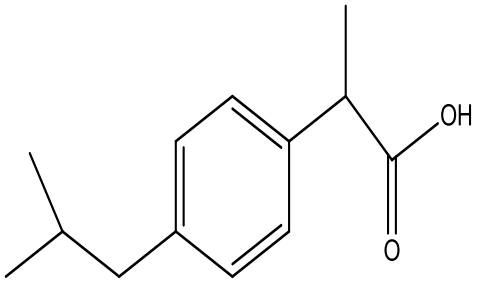
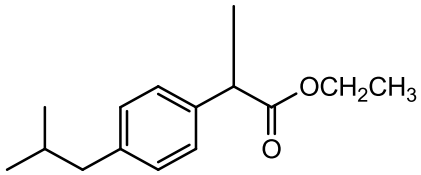


Figure 1: chemical structure of Ibuprofen

Expermantal Section:
Chemistry: All of the compounds used in the synthesis were pick up from Sigma Aldrich Company including hydrazine hydrate, organic solvents and aromatic aldehydes 3-thiophencarboxyaldyhed, 2,4-dichlorobenzyladehyde,4-OH-3-NO2benzylaldehyde, 3-4-Dimethoxy-benzylaldyhed, Syringaldehyde. Ibuprofen was donated by The State Company for Drug Industries (SDI, Samara, Iraq), all solvents and reagents were employed without additional purification. The melting point of the target compounds by using Stuart SMP30 Apparatus and they were determined (uncorrected). The reaction was monitored and the purity of the products was investigated by using thin-layer chromatography (TLC) by using aluminum precoated silica gel sheet (Germany, Merck). The spots of intermediate and the target compounds were seen by UV 254 nm lamp. At the University of Baghdad-College of Pharmacy, the infrared spectra were acquired using the FT-IR (Shimadzu, Japan), the measurement unit (ú, cm-1). ^{1}HNMR spectra were recorded on the NMR 500 spectrometer model.[1] HNMR was don at Tehran University, Islamic Republic of Iran. The antimicrobial study of the synthesized target compounds was done at Chemistry Analysis Center (CAC), Baghdad, Iraq.
Synthesis of Ibuprofen ethyl ester [2-(4- Isobutyl phenyl) Propanote] Compound 1[(14)]: A mixture of Ibuprofen (0.029 moles, 6g) and absolute ethanol was stirred in the presence of H_2SO_4 (3drops) which was added gradually for 5 minute, then the mixture was reflexed for (8) hours at 75ºC. the reaction was checked by the TLC. The resulting mixture was added to beaker containing crushed ice $NAHCO_3$(10%. w/v) was used to neutralized the mixture. Yellowish oil was obtained via separation with ethylacetat, **Yield**: 84%, **Rf =** 0.7 (ethylacetate: n-hexane 3:4), **IR** (v cm^{-1}): 1732:(C=O) stre. ester, 116l:(C-O) stre. ether ,2954: (C-H) asymm.str of CH_3, CH_2, 2870: (C-H) Symme. str. of CH_3, CH_2.

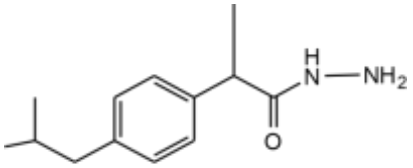


1

Synthesis of Ibuprofen hydrazide [2-(4-Isobutylphenyl) propane hydrazide] Compound 2 [(15)]

Solution of compound **1** (3g ,0.013 mole) was mixed with hydrazine hydrate 99% (3 mL, 0.065 mole) in 30mL of absolute ethanol which then reflexed for 20 hours at 76ºC monitoring by TLC. When reflex time was ended, the resulting mixture was left aside until cooling. After that solvent was evaporated and ice was added and the final product was obtained by Filtrations, washing

several time by D.w, left to dried then recrystallized from ethanol. white powder. **Yield** 83%, **MP** (74-77ºC), **Rf** = 0.49 (Chlorophorm: ethylacetate 7:3), IR (v cm^{-1}): 1685.1(C=O) stretch of amide, 3271, 3209(N-H) str. of NH_2, 3024: (C-H) str. of Aromatic -H, 2954, 2916:((C-H) asymm.str. of CH_3, CH_2, 2870, 2846: (C-H) symm.str of CH_3, CH_2.

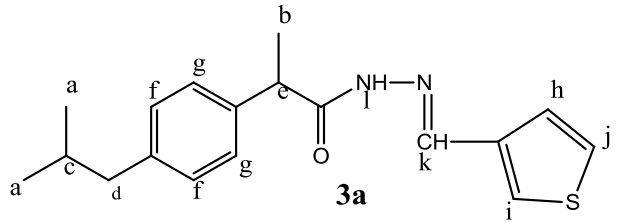


2

Synthesis of Hydrazone (2-(4-Isobutylphenyl) propane hydrazide] derivatives. Compound 3(a-f) [16]

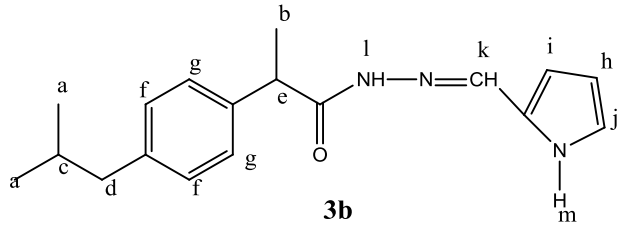
Compound **2** (0.004 moles, 0.8 gm) was mixed with different aldehydes (a-f) (0.5g,0.004 mole in 30mL ethanol with 5 drops of glacial acetic acid. The solution was reflexed for 16 hours at 76ºC. After that the resulting mixture was left for cooling at room temperature, the solid precipitate was obtained by filtration and recrystallization from ethanol.

(E)-2-(4-isobutylphenyl)-N'-(thiophen-3-ylmethylene) propane hydrazide (3a) pale purple powder, **Yield** 75%, **MP** = 123 ºC**,** R_f=0.52 (Methanol, Chloroform, toluene 11:4:3). IR (v cm-1):3186: (N-H) str. of secondary amine, 1512:(N-H) bending vibration,3093, 3043: (C-H) str. of Ar- ring, 2954, 2931(C-H) asymm. str. of CH_2, CH_3, 2912, 2866:(C-H) Symm.str. of CH_3, CH_2, 1662:(C=O) str. Of amide, 1604: (C=N) str. of imin, 636:(C-S) str. ^{1}HNMR: 0.87(6H, d, CH3 (a)), 1.28 (3H, d, CH_3 (b)); 1.82 (1H, m -CH (c)); 2 43(2H, d - CH_2 (d)), 3.52(1H, q - CH (e)), 8.32 (1H, S - CH (k)), 10.57(1H, s - NH (l)),7.05-7.73(7H, m–Ar -H (f, g, h, j, i).



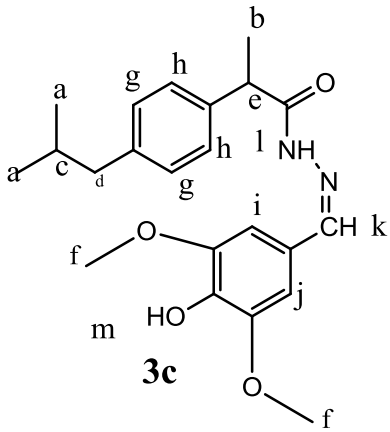
N'-((1H-pyrrol-2-yl) methylene)-2-(4-isobutylphenyl) propane hydrazide (3b)

Off Wight powder**, Yield** 84%, R_f =0.43(ethanol, ethylacetate, n-hexane, toluene (3:3:6:4), **MP** = (145-147), IR (vcm-1), 3285: (N-H) str. of pyrrole,3205,3143:(N-H) str. of secondary amide,3055,3020:(C-H) str. of Ar-H,2951,2908:(C-H) asymm.str. of CH_2, CH_3, 2866,2843: (C-H) symm.str of CH_2,CH_3,1643:(C=O) str. of amide,1604(C=N) str. of imin, ^{1}HNMR, 0.87(6H, D, CH_3 (a)), 1.28 (3H, d, CH_3 (b)); 1.82 (1H, m, CH (c)); 2.43(2H, d, CH_2 (d)), 3.52(1H, q, CH (e)),6.15-7.17(7H, m, Ar-H (f, g, h, i, j)),7.15(1H, s, CH (k)),10,57(1H, s, NH (l)),11.30(1H, s, NH (m)).



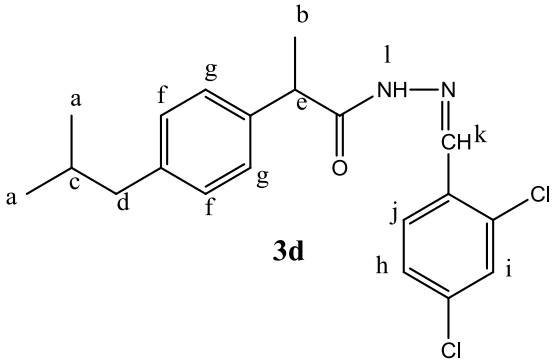
(Z)-N'-(4-hydroxy-3,5-dimethoxybenzylidene)-2-(4-isobutylphenyl) propane hydrazide (3c)

White powder, **Yield** = 82% **MP**= (117 ºC), R_f=0.45 (Methanol, Chloroform, toluene 11:4:3) , IR (v cm-1):3514:(O-H) str. of hydroxyl group, 3194 :(N-H) str. of secondary amide,3051(C-H) str. of Ar, 2966,2931:(C-H) asymm. Str. of CH_3, CH_2, 2863,2842: (C-H) symm. stre. of CH_3, CH_2,1658.2(C=O) stre. of amide, 1620:(C=N) str. of imin,1585:(N-H) bending vibration,1423:(C-H) asymm. bending of CH_3,1373:(C-H) symm. bending of CH_3. ^{1}HNMR, 0.87(6H, d, CH_3 (a)), 1.28 (3H, d, CH_3 (b)); 1.82 (1H, m, CH (c)); 2.43(2H, d, CH_2 (d)), 3.52(1H, q, CH (e)),3.83(6H, s, CH_3(f)),7.05-7.22(6H, m, Ar-H (g, h, i)),8.73(1H, s, of OH(m)),8.47 (1H, s, CH(k)),11.07(1H, s, of NH(l))



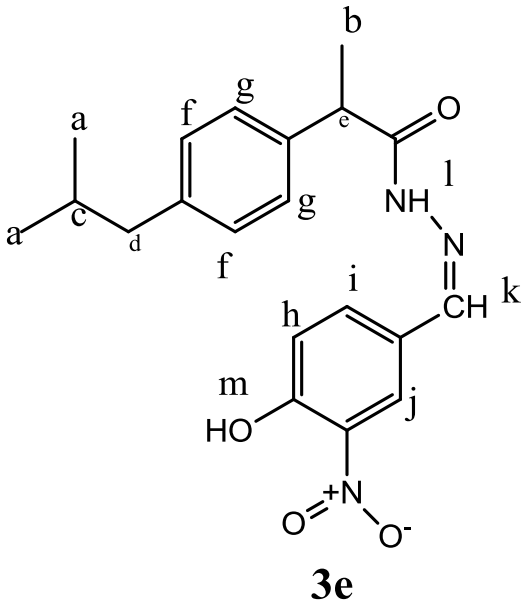
N'-(2,4-dichlorobenzylidene)-2-(4-isobutylphenyl) propane hydrazide (3d)

off-white powder, **Yield**: 67%, **MP**=142 ºC, R_f= 0.48 (ethanol, ethylacetate, n-hexane, toluene 3:3 4:6), IR (v cm-1):3186 :(N-H) str. of secondary amide,3059(C-H) str. of Ar-H, 2954,2927:(C-H) asymm. Str. of CH_3, CH_2, 2870,2824: (C-H) symm. str. of CH_3, CH_2, 1654(C=O) str. of amide, 1593(C=N) str. of imin, 794,536 (C-cl) str. ^{1}HNMR, 0.87(6H, d, CH3 (a)), 1.28 (3H, d, CH_3 (b)); 1.82 (1H, m, CH (c)); 2.43(2H, d, CH_2 (d)), 3.52(1H, q, CH (e)),7.05-7.5(6H, m, Ar-H (f, g,h,i),8,00(1H,m,Ar-H(j)),8.96(1H,s,CH(k)),11.07(1H,s,NH(l)).



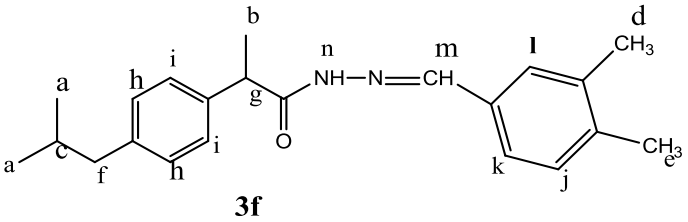
(Z)-N'-(4-hydroxy-3-nitrobenzylidene)-2-(4-isobutylphenyl) propane hydrazide(3e)

Yellow powder, **Yield** 48%, **MP**=142, ºC **Rf**=0.53 (ethanol, ethylacetate, n-hexane, toluene 3:3:4:6), IR (v cm^{-1}):3251:(O-H) str. of OH, 3178 :(N-H) str. of secondary amide,3008(C-H) str. of Ar, 2962,2927:(C-H) asymm. Str.of CH_3, CH_2, 2870,2850: (C-H) symm. str. of CH_3, CH_2,1654(C=O) str. of amide, 1627:(C=N) str. of imin,1577:(N-H) bending vibration,1535,1319(N=O) of NO_2. ^{1}HNMR, 0.87(6H, d, CH_3 (a)), 1.28 (3H, d, CH_3(b)); 1.82 (1H, m, CH (c)), 2.43(2H, d, CH_2 (d)), 3.52(1H,q,CH(e)),7.05-7.18(5H,m,Ar-H(f,g, h)),8.05(1H,m,ArH(i)),8.35(1H,m,Ar-H(j)),8.47(1H,s,CH(k)),11.07(1H,s,NH(l)),14.43(1H,s,OH(m))



N'-(3,4-dimethylbenzylidene)-2-(4-isobutylphenyl) propane hydrazide (3f)

Wight powder, **Yield** 94%, **MP**=147 ºC, **R.$_f$**= 0.56 (ethanol, ethylacetate, n-hexane, toluene 3:3:4:6), IR (v cm-1): 3178: (N-H) str. of secondary amide, 3070:(C-H) str. of Ar , 2947, 2916: (C-H) asymm. Str. of CH_3, CH_2,2866,2846: (C-H) symm.str. of CH_3, CH_2,1666:(C=O) str. of amide, 1604:(C=N) str. of imin,1508:(N-H) bending vibration. ^{1}HNMR, 0.87(6H, d, CH_3 (a)), 1.28 (3H, d, CH_3(b)) ,1.82 (1H, m, CH (c)), 2.31(3H,s,CH_3(d)),2.34(3H,s CH_3(e)),2.43(2H, d, CH_2(f)),3.52(1H, q, CH(g)),7.05-7.22(5H,m,Ar-H(h,I,j)),7.67(1H,m,ArH(k)),7.69(1H,m,ArH(l)),8.41(1H,s,CH(m)), 11.07(1H,s,NH(n)).

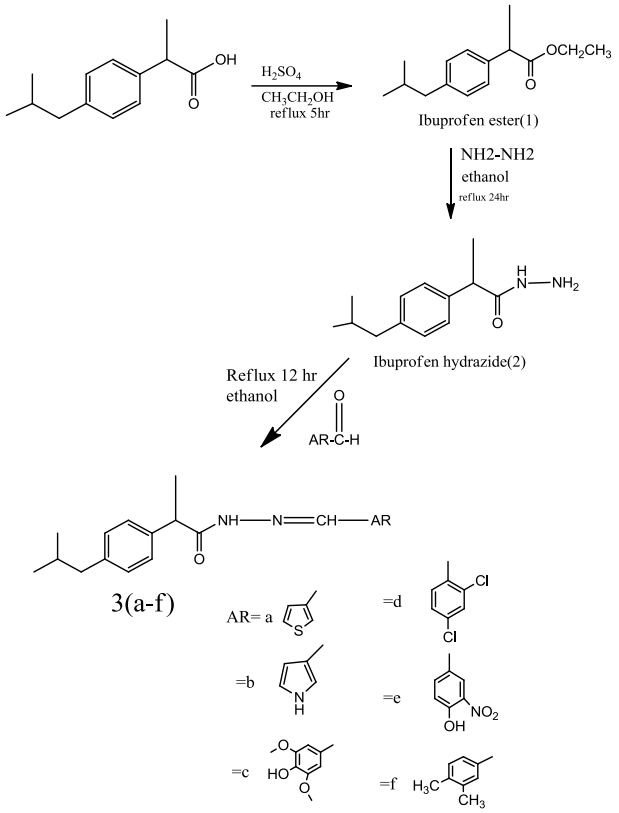


Antimicrobial Activity:[17]

The final prepared compounds (**3a-f**) were tested for their anti-microbial activity by using disc diffusion technique against two different species of "G +ve" bacteria (Staphylococcus aureus, and Streptococcus pyogenes), "G-ve" bacteria (klebsiella pneumonia and E. coli) and one of fungal species (Candida albicans). concentration of final compounds was 100 µg/ML by using DMSO as solvent for all of them, the activity was in-validated by measuring the zone of inhibition in millimeters with comparison to the reference Ciprofloxacin as "antibacterial agent" and Fluconazole as "antifungal agent"

RESULT AND DISCUSSION

Chemistry: Scheme 1 represent the general synthetic pathway of final compounds(3a-f), which starting from Ibuprofen ethyl ester (1) which prepared by reacting of ibuprofen dissolved in ethanol with H_2SO_4.The ATR-FTIR spectra of compound**1**showed absorption bond at (1732 cm$^{-1)}$ due to C=O str. of ester carbonyl and(116lcm^{-1}) due to C-O stretching vibration of ether. Ibuprofen hydrazide **2** was synthesized by the reflux of compound **1**with hydrazine hydrate in absolute ethanol. The ATR-FTIR spectra of comp 2 showed the absorption band at (1685 cm^{-1} of C=O for the stretching vibration of amide and 3271, 3209 due to stretching vibration of N-H of the primary amine. the compound3 (a-f) were prepared by the reflux of 2-(4- Isobutylphenyl) propane hydrazide compound (2) with different aromatic aldehyde include 3-thiophenecarboxyladehyde,3-Hydroxy-3-nitrobenzylaldehyde, Syringaldehyde, pyrrole-2-carboxylaldehyde,3,4 dimethyl benzaldehyde and 2,4 dichlorobenzaldehyde, respectively, in glacial acetic acid as catalyst. ATR-FTIR spectra of compound 3(a-f) show absorption band at (1662-1666 cm^{-1}) of C=O str. of amide and C=N stre. vibration of imin at (1604-1627 cm^{-1}). The ^{1}HNMR spectrum exhibited singlet at (8.32-7.15) resulted from proton of imin. And showed singlet at 10.57-11.07 due to NH proton of amide.



Scheme1: Synthesis of target compounds (a-f)

Anti-microbial activity: Series of new synthesized derivatives (**3a-f**) were evaluated for their anti-microbial activity, the result in the table **1** show that most of target compounds have good activity for both "G-ve" and G+ve " bacteria. The compound 3f showed the highest anti-bacterial activity for the two species of "G-ve" bacteria compare with the other synthesized compound, while compound **3e** show the highest anti-microbial activity for the two species of "G+ve"bacteria, the compounds (**3a, c**) showed moderate activity when compare with the other. The compound 3b show no activity for one species of "G+ve" (Streptococcus pyogenes) while the compound **3d** show no activity for one species of "G-ve" (E. coli) "G+ve" (S. pyrogen), compare with Ciprofloxacin. The compound **3b** show the highest anti-fungal activity compare with and the other synthesized compounds. The compounds (**3a, c, d, f, e**) shows good activity

Table 1: The anti-microbial evaluation of target compounds (3a-f)

Compound	Zone of inhibition in mm					
	Conc.µg/mL	S.aureus	S.pyrogen	K.Pneumonia	E.Coli	C.albicans
3a	10^3	15	16	17	12	17
3b	10^3	12	-	18	-	19
3c	10^3	15	17	15	14	16
3d	10^3	-	14	12	-	13
3e	10^3	16	18	15	14	17
3f	10^3	15	15	20	18	18
Ciprofloxacin		23	26	27	25	
Fluconazole						23
DMSO	Control and solvent					

= No activity, (zone of inhibition between 5–10 mm) = slightly active, (zone of inhibition between 10-15 mm) = moderately active, (zone of inhibition more than 15 mm) = highly active

CONCLUSION

New series of 2-(4- lsobutylphenyl) propane hydrazide derivatives were adequately synthesized using current procedures, all of tested compounds were tested for their anti-microbial activity. The structure of all final compound was identified and confirmed by (ATR-FTIR and ^{1} HMNR). And from anti-microbial evaluation the compounds **3f** and **3e** showed the highest anti-bacterial activity while the compound 3b showed the highest anti-fungal activity.

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