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Functionalization of ibuprofen core structure compound: Part 1 Synthesis of potential chemotherapeutic agents incorporated ibuprofen sub-structure and their *in vitro* antimicrobial study

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ABSTRACT

*A series of overfifteen newly potential chemotherapeutic agents of hydrazides, hydrazones and imides incorporated ibuprofen sub-structure have been synthesized in a very good yield under conventional heat and irradiation conditions. The microbial inhibitory effect of the new agents has been assessed in vitro against Gram-positive and Gram-negative bacteria as well as antifungal activity. Compound **15** showed the highest antibacterial as well as antifungal activities compared to other compounds with some antifungal activity higher than standard drugs. However compound **14** showed the lowest antibacterial activity but similar antifungal activity compared to other compounds. All compounds have been characterized by IR, ^{1}H-NMR, ^{13}C-NMR and x-ray analyses.*

Key words: Ibuprofen, anti-inflammatory, NSAID, biologically active compounds.

INTRODUCTION

Ibuprofen, 2-(4-isobutylphenyl) propionic acid is one of essential drug list of basic health care system according to the World Health Organization (WHO) and counted as one of non-steroidal anti-inflammatory drugs (NSAID) which are commonly used for the relief of a wide range of illnesses such as headaches, backache, period pain, dental pain, neuralgia, rheumatic pain, muscular pain, migraine, cold and flu symptoms and arthritis.Ibuprofen was used in combination with antibiotics in the treatment of bacterial infections.It was reported that ibuprofen could interfere with microbial growth *in vitro* and a possible antimicrobial effect of these drugs might add to the effect of conventional antimicrobial drugs in vivo [1,2].Ibuprofen itself found to be limited the effect of E. coli endotoxin on several physiological activities of rabbits [3] and human. [4].Incorporation of ibuprofen with other pharmacological compounds such as imidazoles [5],oxadiazoles [6] and triazoles[7] has showed a significant increase in the antibacterial and anti-inflammatory effect of such compounds.

It has also been reported that compounds containing hydrazide-hydrazone or imide moiety possess good analgesic, anti-inflammatory [8-13] and antimicrobial activities [14-23]. In addition, some evidences showed the hydrazone moiety that present in the anti-inflammatory drug structure of COX is behind its inhibition character [24].

Further to our interest in synthesis of biologically active compounds [25-27], and in view of the above observations, it was therefore considered worthwhile to manipulate the carboxylic acid group of 2-(4-isobutylphenyl) propanoic

acid into hydrazidic and imidic group via condensation reactions with different reagentsin order to obtain more effective chemotherapeutic agents.

EXPERIMENTAL SECTION

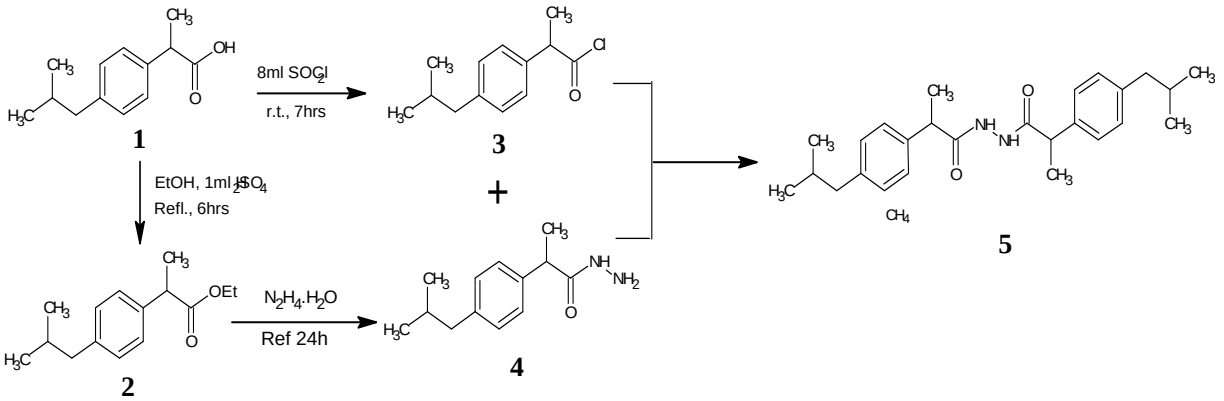
Melting points are uncorrected and determined by the open capillary method using Gallen Kamp melting point apparatus. The IR spectra were recorded with Perkin-Elmer FT-IR instrument using potassium bromide pellets. ^{1}HNMR, ^{13}CNMR Spectra were recorded in deuterated chloroform ($CDCl_3$),acetone CD_3COCD_3or dimethylsulphoxide (DMSO-d_6) with TMS as an internal standard on a Joel 400 MHz instrument.Chemical shifts are expressed as [ppm], s for singlet, d for doublet, t for triplet, q for quartet, m for multiplet, and b for broad.X-ray has been measured at National Crystallography Services (NCS), Southampton, United Kingdom.

Materials:
Ibuprofen has been purchased from Aldrich chemical supplier. Compounds such as ethyl 2-[4-(2-methylpropyl)phenyl]propanoate**2**[28], 2-[4-(2-methylpropyl)phenyl]propanoyl chloride**3**[29], 2-[4-(2-methylpropyl)phenyl]propanehydrazide**4**[30],and 2-(4-Isobutyl-phenyl)-N-(4-oxo-2-phenyl-4*H*-quinazoline-3-yl)-propionamide**9**[31],5-{1-[4-(2-methylpropyl)phenyl]ethyl}-1,3,4-oxadiazole-2(3H)-thione **10**[6]and 4-amino-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione**11**[7] were synthesized according to the cited literature.

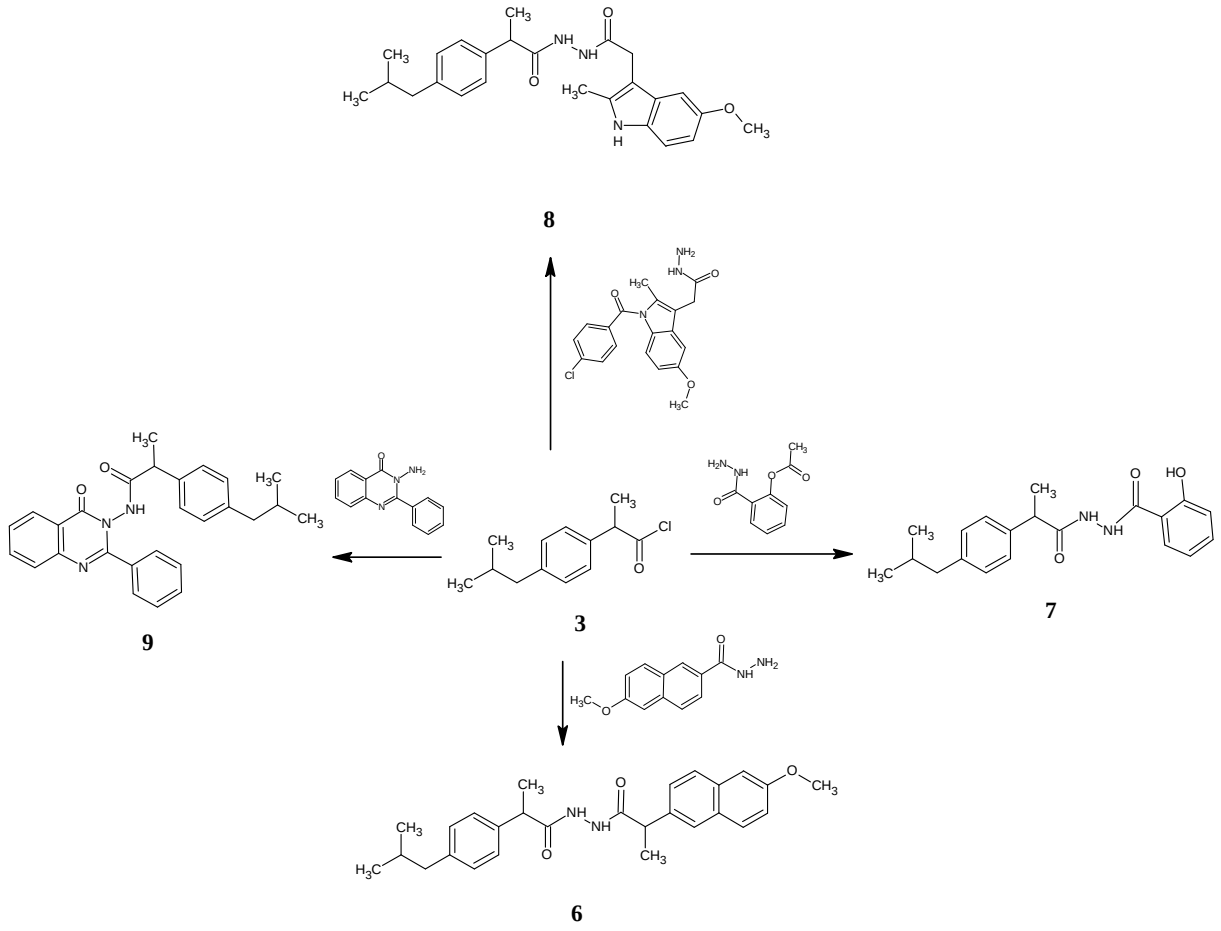
The physical properties of the synthesized compounds are tabulated in table1.

Table 1: physical properties of synthesized compounds

No.	M.F	M.wt	solvent	Yield%	M.P. (^{0}C)
2	$C_{15}H_{22}O_2$	234.33398	ether	78%	104-107
3	$C_{13}H_{17}ClO$	224.72648	-----	85%	117-119
4	$C_{13}H_{20}N_2O$	220.3107	ethanol	81%	66-68
5	$C_{26}H_{36}N_2O_2$	408.57624	ethanol	86%	185-188
6	$C_{27}H_{32}N_2O_3$	432.55458	ethanol	78%	200-203
7	$C_{20}H_{24}N_2O_3$	340.41616	ethanol	81%	182-184
8	$C_{25}H_{31}N_3O_3$	421.53194	ethanol	87%	210-212
9	$C_{27}H_{27}N_3O_2$	425.52218	ethanol	87%	170-172
10	$C_{14}H_{20}N_4S$	276.4004	ethanol	83%	82-84
11	$C_{14}H_{20}N_4S$	276.4004	ethanol	76%	166-167
12a	$C_{20}H_{24}N_2O$	308.41736	ethanol	87%	138-140
12b	$C_{20}H_{24}N_2O_2$	324.41676	ethanol	91%	163-165
12c	$C_{20}H_{23}BrN_2O_2$	403.31282	ethanol	90%	161-163
12d	$C_{21}H_{26}N_2O_2$	338.44334	ethanol	89%	146-148
12e	$C_{22}H_{29}N_3O$	351.48516	ethanol	92%	152-154
13	$C_{34}H_{42}N_4O_4$	570.72168	ethanol	76%	165-167
14	$C_{34}H_{42}N_4O_2$	538.72288	Ethanol/DMF	81%	240-242
15	$C_{21}H_{23}N_3O_2$	349.42622	ethanol	92%	164-166
16	$C_{33}H_{41}N_5O_2$	539.71094	ethanol	77%	182-185



Scheme 1



(Scheme 2)

On treatment of the ibuprofen acid chloride **3** with ibuprofen hydrazidic acid **4** in THF under reflux afforded white crystals of 2-(4-Isobutyl-phenyl)-propionic acid N'-[4-isobutyl-phenyl)-propionyl]-hydrazide **5**(Scheme 1). Similarly, reaction of ibuprofen acid chloride with the hydrazidic acids of naproxen, aspirin and indomethacin individually in THF under conventional heat furnished formation of the corresponding hydrazide compounds of 2-(6-Methoxy-naphthalen-2-yl)-propionic acid N'-[2-(4-isobutyl-phenyl)-propionyl]-hydrazide**6**,2-hydroxy-N'-{2-[4-(2- methylpropyl)phenyl]propanoyl}benzohydrazide **7**, and(5-Methoxy-2-methy-1H-indol-3-yl)acetic acid N'-[2-(4-isobutyl-phenyl)-propionyl]-hydrazide **8**respectively (Scheme 2).

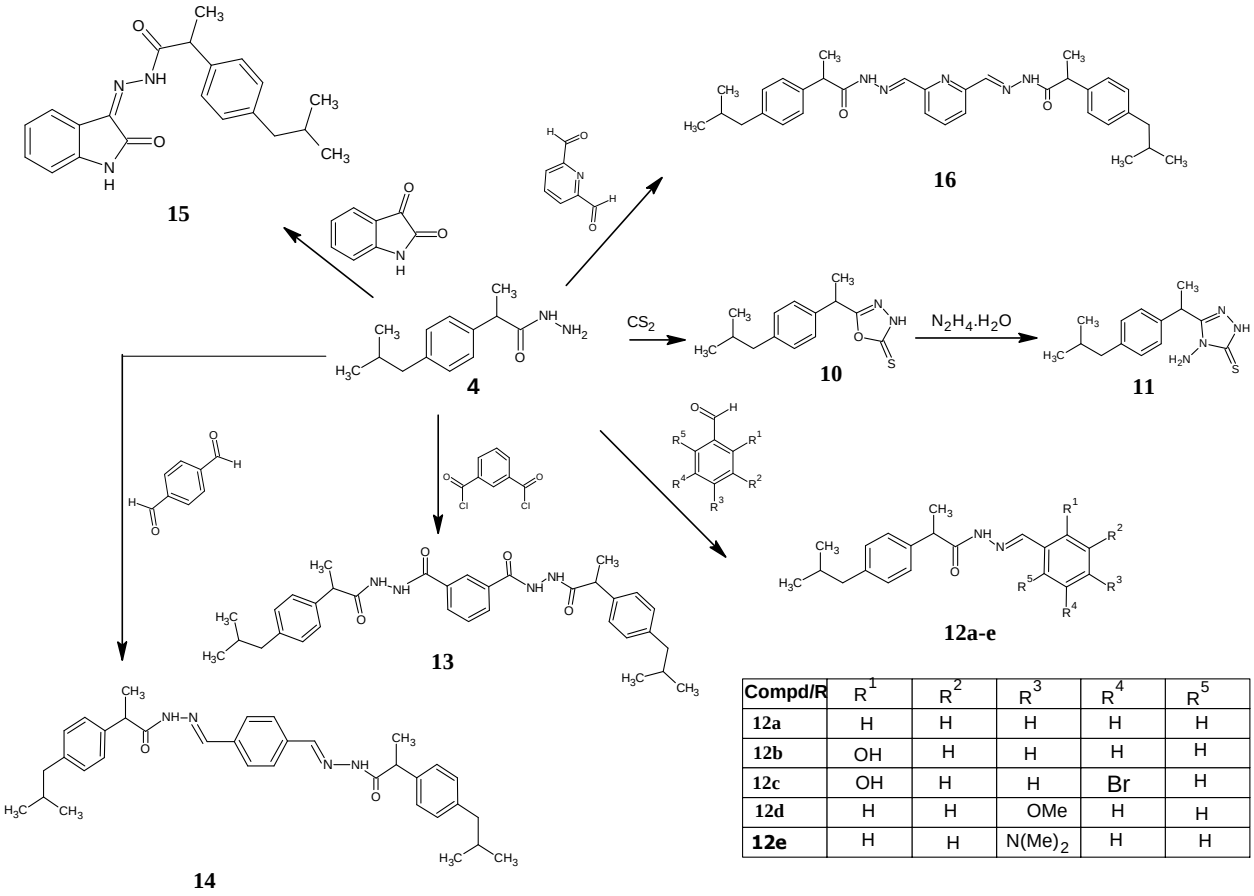
Reaction of ibuprofen hydrazide**4** with aromatic aldehydes, bis aldehydes and isatin under different reaction conditions afforded formation of the corresponding hydrazides**12a-e**, bishydrazides**13** and **16**, and indolidenehydrazide derivative **15** respectively(Scheme 3).

Synthesis of 2-(4-Isobutyl-phenyl)-propionic acid N'-[4-isobutyl-phenyl)-propionyl]-hydrazide 5:
A mixture of 0.01 mol (2.2gm) of **4** and 0.01mol of **3** (2.24gm) was refluxed for two hr in 50ml dry THF. The mixture was cooled and poured onto crushed ice, the precipitate was collected and recrystallized from the appropriate solvent(see table 1) to get **5** as white crystals. IR(KBr cm^{-1}): (C=O diamide, 1599), (C-H aliphatic, 2866), (Ar, 2951), (NH-NH, 3196).^{1}H-NMR: ($CDCl_3$**)** δ,0.9 (d, $(CH_3)_2$), 1.9(m, 1H aliphatic), 2.5(d, -CH_2-), 1.5(d, 3H aliphatic), 3.6(q, 1H), 7.1(dd, Ar, J=8.24), 7.4(s, NH). ^{13}C NMR: 170 (C=O), 18(CH_3), 22($CH3)_2$, 45(CH_2 aliphatic), 43(C-H aliphatic), 30(C-H aliphatic tertiary), 128, 129(C=C,Ar), 141, 136(=C- Ar).

General synthetic method of the thehydrazides of 6,7and8:
Equimolar of **3** (0.01mol) and Naproxen hydrazide, salicylic acid hydrazide, Indomethacin hydrazide (0.01mol) respectively were refluxed for two hour in 50ml dry THF. The mixture was cooled and poured onto crushed ice. The precipitate that formed was collected and recrystallized from ethanol to get the pure compounds **6, 7** and **8**.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid N'-[2-(4-isobutyl-phenyl)-propionyl]-hydrazide 6**:** This product was obtained as yellow crystals. IR(KBr cm^{-1}) (C=O diamide 1603), (C-H aliphatic, 2868-2952), (Ar, 3023), (NH-

NH, 3201).[1]H-NMR (DMSO-d6) δ at0.9 (d, $(CH_3)_2$), 1.8(m, 1H aliphatic), 2.5(d, $-CH_2-$), 1.6(d, 3H aliphatic), 3.4(quartet, 1H), 3.6 (s, $O-CH_3$), 7.0(dd, Ar, J=8.14 for ibuprofen ring), 7.43, 6.9(s, Ar),7.18, 7.54(d, Ar for naphthalene ring), 7.57, 7.03(d, Ar for naphthalene ring) 7.2(s, NH).[13]C-NMR**:** 170, 171(C=O), 19(CH_3), 23$(CH_3)_2$, 44(CH_2 aliphatic), 42(C-H aliphatic), 31(C-H aliphatic tertiary),54(CH_3-O), 130, 131(C=C Ar for ibuprofen ring), 140, 137(=C- Ar for ibuprofen ring), 128.1, 128, 135, 130(C=C Ar for naproxen attached to isopropyl), 131, 135(C=C Ar for two fused ring in naproxen), 127, 117, 105 (C=C Ar), 156(C-O Ar).



Compd/R	R^1	R^2	R^3	R^4	R^5
12a	H	H	H	H	H
12b	OH	H	H	H	H
12c	OH	H	H	Br	H
12d	H	H	OMe	H	H
12e	H	H	$N(Me)_2$	H	H

Scheme 3

***2-hydroxy-N'-{2-[4-(2- methylpropyl)phenyl]propanoyl}benzohydrazide 7*:** This product was obtained as white crystals. IR(KBr cm^{-1}): (C=O diamide 1604, 1683), (C-H aliphatic, 2870), (Ar, 2957), (NH-NH, 3221). [1]H-NMR**:**(DMSO-d6), δat1.0 (d, $(CH_3)_2$), 2.0(m, 1H aliphatic), 2.4(d, $-CH_2-$), 1.6(d, 3H aliphatic), 3.5(q, 1H), 6.9(s, NH), 10.3(s, OH phenolic), 6.9(dd, Ar, J=0.92), 7.38(m, Ar, J=7.3), 7.80(d, Ar 1H), 7.82(d, Ar 1H).[13]C NMR: 172, 167(C=O), 19(CH_3), 23$(CH_3)_2$, 44(CH_2 aliphatic), 43(C-H aliphatic), 31(C-H aliphatic tertiary), 127, 129(C=C, Ar for ibuprofen ring), 141, 137(=C- Ar for ibuprofen ring), 115, 117, 119, 128, 134 (C=C, Ar), 159(C-OH phenolic).

(5-Methoxy-2-methy-1H-indol-3-yl)acetic acid N'-[2-(4-isobutyl-phenyl)-propionyl]-hydrazide 8: This compound was obtained as black crystals. IR(KBr cm^{-1}) (C=O diamide, 1683), (C-H aliphatic, 2865), (Ar, 2954), (NH-NH, 3271).[1]H-NMR**:**(Acetone-d6), δat 0.9(d, 6H,(CH3)2), 1.8(m, 1H, C-H), 1.6(d, 3H, CH_3), 2.5(d, 2H, $-CH_2-$), 3.7(q, 1H, J=2.75), 2.8(s, 3H, CH_3), 3.2(s, 2H, $-CH_2-$), 3.9(s, 3H, $-OCH_3$),6.9(dd, 4H, C-H Ar), 7.1-7.5(3H, Ar of indol ring), 7.8(s, NH), 7.9(s, NH).

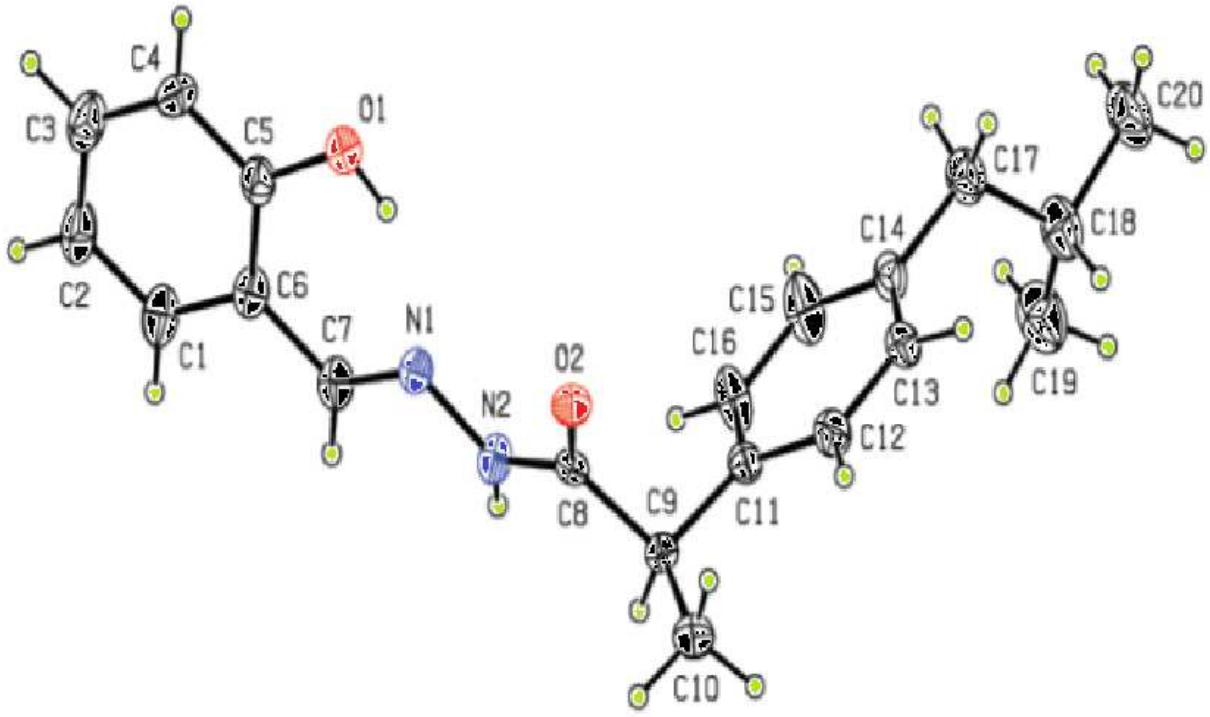
[13]C-NMR: 172, 173 (C=O amide and cyclic amide), 18(CH_3), 22$(CH_3)_2$, 45(CH_2 aliphatic), 43(C-H aliphatic), 30(C-H aliphatic tertiary), 128, 127(C=C,Ar), 141, 136(=C-,Ar), 150, 129(Ar, two fused rings disappeared in dept), 161(=C- between 2 nitrogen atoms in quinazoline ring disappeared in Dept), 135(-C=, Ar, of phenyl group in position 2 of quinazoline ring disappeared in Dept), 126, 126, 129,129,131(C=C,Ar, of phenyl group), 129, 127, 135, 122(C=C , Ar of quinazoline).

General synthesis of 2-[4-(2-methylpropyl)phenyl]-N'-[(E)-arylmethylidene]propanehydrazide 12a-e:
Microwave irradiation method:
A mixture of an equimolar ratio of aldehydes **12a-e**(0.01mol) irrespectively (0.01mol) and the hydrazidic acid of ibuprofen**4**along with few drops of catalytic glacial acetic acid was transferred to a conical flask and subjected to microwave irradiation for 2minutes.The solid mass that obtained was collected and recrystallized from ethanol.

Conventional heat method:
An equimolar ratio of aldehydes**12a-e**(0.01mol) irrespectively and the hydrazidic acid of ibuprofen**4** (0.01mol) with few drops of glacial acetic acid as a catalyst was refluxed in 20ml of absolute ethanol for 4hr. The mixture was concentrated to half and the precipitate that formed was collected and recrystallized from ethanol.

2-[4-(2-Methylpropyl)phenyl]-N'-[(E)- phenylmethylidene]propanehydrazide 12a:
This product was obtained as white amorphous crystals**.** IR(KBr cm^{-1}): (C=O amide, 1672), (C=N, 1610), (NH, 3182), (C-H aliphatic, 2867), (Ar, 2958). ^{1}H-NMR: (Chloroform –d**)**δ at 0.9 (d, $(CH_3)_2$), 1.8(m, 1H aliphatic), 2.4(d, $-CH_2-$), 1.5(d, 3H aliphatic), 4.5(q, 1H), 7.2(dd,4H, Ar, J=7.7), 8.8(s, NH), 8.0(s, -CH=N), 7.5(m, 2H,Ar, J=1.83), 7.3(q,Ar, J=8.2), 7.4(d, Ar). ^{13}C-NMR : 176 (C=O amide), 19(CH_3), 22$(CH_3)_2$, 41(CH_2, aliphatic), 45(C-H, aliphatic), 30(C-H aliphatic tertiary), 127, 126(C=C Ar), 140, 133(=C- Ar), 147(-C=N), 132(-C= Ar), 129, 129(C=C, Ar), 128, 128(C=C, Ar), 130(C=C, Ar).

N'-[(E)-(2-Hydroxyphenyl)methylidene]-2-[4-(2-methylpropyl)phenyl]hydrazide 12b:
It was obtained as white crystals. IR(KBr cm^{-1}): (C=O amide 1660), (C=N 1609), (NH 3194), (C-H aliphatic, 2865), (Ar, 2947). ^{1}H-NMR:(DMSO-d6**)**δ at0.9 (d, $(CH_3)_2$, 1.9(m, 1H aliphatic), 2.4(d, $-CH_2-$), 1.5(d, 3H, aliphatic), 4.0(q, 1H), 9.1(s, OH phenolic), 7.0(dd,2H, Ar, J=7.3), 8.3(s, NH), 8.0(s, -CH=N), 7.3(d,1H, Ar), 6.9(d, 1H, Ar), 7.1(t, 1H, Ar), 6.9(t, 1H, Ar). ^{13}C-NMR: 177 (C=O amide), 20(CH_3, aliphatic), 24$(CH_3)_2$, 41(CH_2, aliphatic), 44(C-H, aliphatic), 33(C-H, aliphatic tertiary),155 (C-OH phenolic), 131, 132(C=C,Ar), 144, 133(=C-,Ar), 156(-C=N, schiff base), 120(-C=,Ar), 155(C-OH, Ar, phenolic), 117, 134, 122, 130 (4C, Ar).

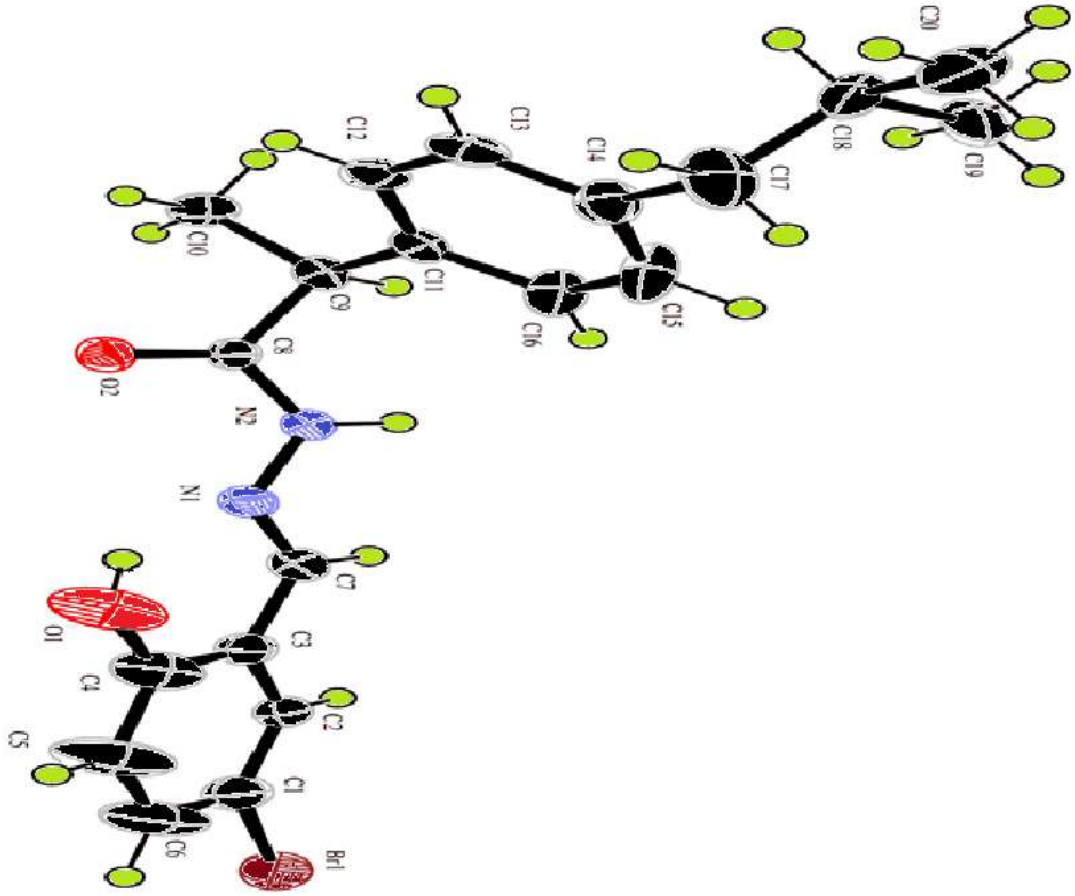


The crystal structure of 12b

X-Ray analysis of **12b** showed that the terminal and central aromatic rings inclined to each other by a dihedral angle being 79.74(10)°, form dihedral angles of 26.05 (11)° and 83.77 (9)°, respectively, with the propenone-hydrazide unit. In the crystal, molecules are connected into infinite chains paralel to c axis, generating S(6) $R^2_2(14)$ ring motifs by intramolecular O—H...N and intermolecular N—H...O and C—H...O hydrogen bonds. C—H...π interactions further consolidate the structure.

N'-[(E)-(5-Bromo-2-hydroxyphenyl)methylidene]-2-[4-(2-methylpropyl)phenyl]propanehydrazide 12c:
This compoundwas obtained as green crystals. IR(KBr cm^{-1}): (C=O amide, 1665), (C=N, 1610), (NH, 3226), (C-H aliphatic, 2866), (Ar, 2950).^{1}H-NMR: (DMSO-d6)δ at 1.1 (d, $(CH_3)_2$), 2.0(m, 1H aliphatic), 2.5(d, $-CH_2-$), 1.6(d, 3H aliphatic), 4.1(q, 1H), 921(s, OH phenolic), 7.0(dd,2H, Ar, J=7.0), 8.4(s, NH), 8.2(s, -CH=N), 7.8(s, Ar, 1H), 6.8(d,Ar, 1H), 7.4(d, Ar, 1H). ^{13}C-NMR**:** 177 (C=O amide), 20 (CH_3), 23$(CH_3)_2$, 40 (CH_2 aliphatic), 46(C-H aliphatic), 31(C-H aliphatic tertiary), 128, 129(C=C Ar), 141, 134(=C- Ar), 150(-C=N), 124(-C=C, Ar), 158(C-OH, Ar, phenolic), 118(C-Br, Ar), 120, 137, 1134(3C, Ar).

X-Ray analysis proved that **12c** is containing an ibuprofen core, and crystallizes with three independent molecules of similar conformation in the asymmetric unit. In these three molecules, the two benzene rings make dihedral angles of 82.7 (2), 71.2 (2) and 78.0 (3)_ with respect to each other. The atoms of the isobutyl groups in two of the molecules are disordered over two positions, with site-occupancy ratios of 0.516 (8):0.484 (8) and 0.580 (8):0.420 (8). In the crystal, molecules are linked by N—H_ _ _O, C—H_ _ _O and O—H_ _ _N hydrogen bonds. Furthermore, C—H_ _ π interactions are also observed.The crystal structure of the single crystal of **12c** is shown below [32].



X-ray structure of 12c

2-(4-Isobutyl-phenyl)propionicacid(4-methoxy-benzylidene)-hydrazide 12d**:** This compoundwas obtained as a white powder .IR(KBr cm^{-1}): (C=O amide 1666), (C=N 1604), (NH 3186), (C-H aliphatic 2839), (Ar2954). ^{1}H-NMR:(DMSO-d6)δ at 1.1 (d, (CH3)2), 1.9(multiplate, 1H aliphatic), 2.5(d, -CH2-), 1.6(d, 3H aliphatic), 4.3(quartet, 1H), 3.9(s, O-CH3), 7.1(dd,4H, Ar, J=7.6), 8.6(s, NH), 8.2(s, -CH=N), 7.6(d, 2H, Ar), 6.9(d, 2H, Ar). ^{13}C-NMR**:** 173 (C=O amide), 18(CH3, aliphatic), 23(CH3)2, 43(CH2 aliphatic), 47(C-H aliphatic), 31(C-H aliphatic tertiary),52(O-CH3), 129, 131(C=C Ar), 140, 133(=C- Ar), 150(-C=N), 125(-C= Ar), 131, 131(C=C, Ar), 117,117(C=C, Ar), 160(=C-O-, Ar).

2-(4-Isobutyl-phenyl)-propionic acid (4-dimethyl amino-benzylidene)-hydrazide 12e: It was obtained as a yellow powder. IR(KBr cm^{-1}): (C=O amide 1666), (C=N 1610), (NH 3435), (C-H aliphatic 2851-2952), (Ar3041) . ^{1}H-NMR:(DMSO-d6)δ at 1.0 (d, $(CH_3)_2$), 1.8(multiplate, 1H aliphatic), 2.6(d, $-CH_2-$), 1.5(d, 3H aliphatic), 4.4(q, 1H), 2.9(s, $N(CH_3)_2$), 7.0(dd,4H, Ar, J=7.3), 8.4(s, NH), 8.2(s, -CH=N), 7.4(d, 2H, Ar), 6.9(d, 2H, Ar). ^{13}C-NMR: 169

(C=O amide), 19(CH3, aliphatic), 25(CH3)2, 42(CH2 aliphatic), 46(C-H aliphatic), 32(C-H aliphatic tertiary),44($CH_3)_2$), 130, 131(C=C Ar), 142, 132(=C- Ar), 151(-C=N), 123(-C= Ar), 132, 132(C=C, Ar), 115,115(C=C, Ar), 144(=C-N-, Ar).

Synthesis of Benzen-1,3-dicarbo hydrazide-1,3-Bis-2-(4-Isobutyl-phenyl)-propionic acid 13:
A mixture of 0.001 mol of isophthaloyl chloride with 0.002 mol of **4**was refluxed in 20ml of THF for 3hr. The reaction mixture was poured onto crushed ice. The obtained precipitate was collected and recrystallized from ethanol to get **13** as a white powder. IR(KBr cm^{-1}): (C=O amide 1681),(NH 3206), (C-H aliphatic 2868-2953), (C-H Ar3014). ^{1}H-NMR: (DMSO-d6),δ at0.86(d, $(CH_3)_2$), 12H, aliphatic), 1.9(m, C-H, 2H, aliphatic), 2.4(d, CH_2, 4H, aliphatic), 3.7(q, 2H, aliphatic), 1.5(d, 6H,C-H aliphatic), 7.1(dd, 8H, Ar, J=7.7), 7.3(s, 4H, NH-NH), 8.7(s, 1H, Ar), 7.8(dd, Ar, J=7.7), 8.0(d, 2H,Ar).^{13}C-NMR:163,172(C=O amide), 22(4C, aliphatic,(CH3)2), 30(C-H, 2C, aliphatic), 45(-CH_2, 2C, aliphatic), 18(CH3, 2C, aliphatic), 44(C-H, 2C, aliphatic), 127, 129(C=C, 8C,Ar), 131.1, 141.1(-C=C, 4C,Ar), 137(-C=C, 2C, Ar), 132(C=C, 2C, Ar), 125, 127(C=C, 2C, Ar).

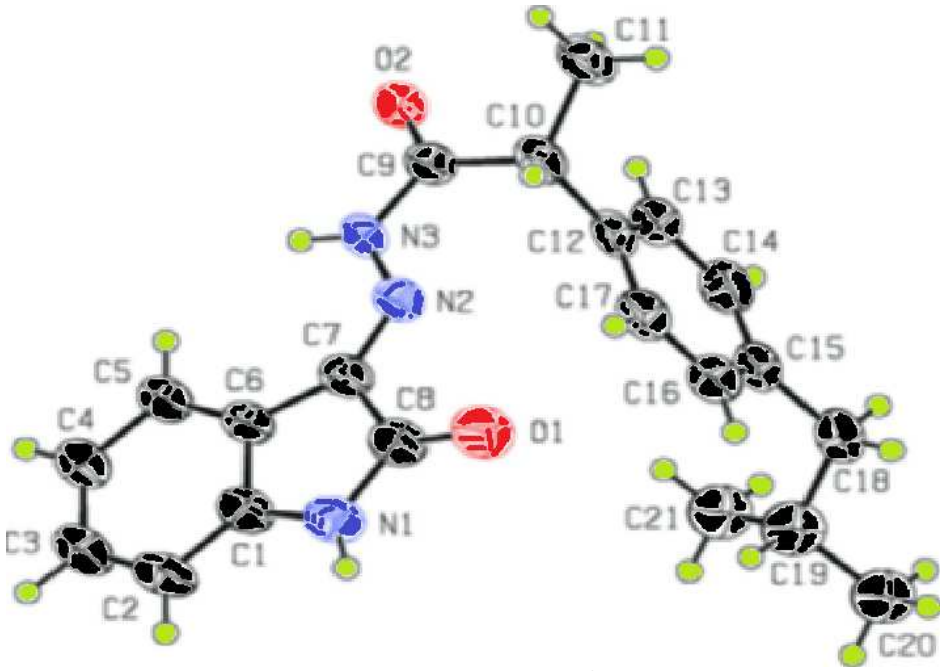
Synthesis of 2-(4-Isobutyl-phenyl)-propionic acid (4-{[2-(4-isobutyl-phenyl)-propionyl]-hydrazon methyl}-benzylidene)-hydrazide 14:
A mixture of 0.001mol of benzene-1,4-dicarbaldehyde and 0.002mol of **4** in 50ml absolute ethanol with few drops of glacial acetic acid was refluxed for 4hr. The mixture was concentrated to half and the precipitate was collected and recrystallized from the appropriate solvent to give**14** as a white powder. IR(KBr cm^{-1}): (C=O amide 1662), (C=N 1600), (NH 3248), (C-H aliphatic 2868-2952), (Ar, 3052).

^{1}H-NMR: (DMSO-d6)δ at0.85 (d, (CH3)2, 12H), 1.95(m, 2H aliphatic), 2.4(d, -CH_2-, 4H), 1.6(d, 6H aliphatic), 3.7(q, 2H), 7.15(dd,8H, Ar, J=8.24), 8.3(s, NH, 2H), 8.2(s,2H, CH=N). ^{13}C-NMR: 172(C=O, 2C), 19(CH3, 2C), 22((CH3)2, 4C) , 44(CH2,2C, aliphatic), 42(C-H aliphatic, 2C), 30(C-H aliphatic tertiary, 2C), 128, 129(C=C,8C, Ar of ibuprofen ring), 141, 136(=C- , 4C, Ar of ibuprofen), 130(4C, Ar of benzene ring). 145(1C of –C=N).

Synthesis of 2-[4-(2-methylpropyl)phenyl]-N'-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]propanehydrazide 15:
An equimolar ratio mixture of **4** (0.01mol) and isatin (0.01mol) was transferred to beaker along with few drops of glacial acetic acid as a catalyst. The mixture has been irradiated under microwave for 2 minutes then left to cool at room temperature.The product that obtained was collected and recrystallized from ethanol to furnish**15**as yellow crystals. IR(KBr cm^{-1}): (C=O amide 1691), (C=O cyclic 1733),(C=N, 1616), (NH 3224), (C-H aliphatic 2866-2953), (Ar3044). ^{1}H-NMR:(Chloroform-d6)δ at 0.85(d, $(CH_3)_2$), 2.0(m, 1H aliphatic), 2.4(d, -CH_2-), 1.6(d, 3H aliphatic), 3.7(q, 1H), 7.1(dd, Ar, J=8.24), 8.2(s, NH), 7.9(s, NH of cyclic amide), 7.5(d, 1H, Ar), 7.6(d, 1H,Ar, 7.6), 7.4(triplet, 2H, Ar). ^{13}C-NMR: 177, 160(2C, C=O), 19(CH3), 23(CH3)2, 44(CH_2 aliphatic), 43(C-H aliphatic), 31(C-H aliphatic tertiary), 127, 129(C=C Ar), 140, 135(=C- Ar), 151(C=N), 117, 129, 121,127,122, 140 (6C for benzen ring).

X-Ray analysis 0f 15 showedthe indolin-2-one group is essentially planar, with a maximum deviation of 0.016 (2) A ° for the N atom, and makes a dihedral angle of 84.38 (14) with the benzene ring. The N—N(H)—C(O)—C– torsion angle is 0.9 (3)_. In the crystal, molecules are linked into a three-dimensional network via N—H_ _ _O and C—H_ _ _O hydrogen bonds. In addition, a C—H_ _ π interaction was observed. The crystal structure of the single crystal of **15** is shown below [33].

Synthesis of 2-(4-Isobutyl-phenyl)-propionic acid (6-{[2-(4-isobutyl-phenyl)-propionyl]-hydrazonmethyl}-pyridin-2-ylmethylene)-hydrazide 16:
A mixture of 0.001mol of pyridine-2,6-dicarbaldehyde and 0.002mol of **4** in 50ml absolute ethanol with few drops of glacial acetic acid was refluxed for 4hr. The mixture was concentrated to half and the precipitate was collected and recrystallized from the appropriate solvent to afford**16** as a brown powder.IR(KBr cm^{-1}): (C=O amide 1669), (C=N 1579), (NH 3187), (C-H aliphatic 2868-2954), (Ar 3031). ^{1}H-NMR: (DMSO-d6)δ at0.9 (d, (CH3)2, 12H), 2.0(m, 2H aliphatic), 2.6(d, -CH_2-, 4H), 1.7(d, 6H aliphatic), 3.8(quartet, 2H), 7.0(dd,8H, Ar,), 8.4(s, NH, 2H), 7.7(s, CH=N, 2H),8.2,8.2(d, 2H,Ar of Pyridine), ^{13}C-NMR: 174 (C=O, 2C), 18(CH3, 2C), 21((CH3)2, 4C) , 45($CH_2)_2$, aliphatic), 43(C-H aliphatic, 2C), 30(C-H aliphatic tertiary, 2C), 127, 129(C=C,8C, Ar of ibuprofen), 140, 134(=C- , 4C, Ar of ibuprofen), 151(C=N, 2C of Schiff base), 153(-C=N, 2C, Ar of Pyrideine), 128, 128(C=C, Ar of Pyridine), 137(C=C, 1C, of Pyridine).



The x-ray crystal structure of 15

Anti bacterial and anti fungal study:

Methodology

Antibacterial activity of the synthesized compounds has been tested using Gram-negative strains such as *Escherichia coli, Salmonella enteric, Pseudomonas aeruginosa* and *Pseudomonas putida*; and Gram-positive strains such as *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* and *Micrococcus luteus*. Additionally, antifungal activity has been tested using different Candida strains such as *Candida albicans, Candida glabrata* and *Candida krusei*.

The Synthesized compounds were dissolved in DMF followed by dilution in buffer to obtain final concentrations of 60,000, 30,000 and 15,000 ppm. Final concentration of DMF was less than 2% in the final buffer solutions. Negative control was performed using DMF diluted to similar final concentrations of compound solutions. Ampicillin and Geneticin were used as positive controls with similar dilutions and final concentrations.

Using cup-plate agar diffusion method [34], a definite volume of the microbial suspension (inoculums) was mixed with sterilized agar media. Plates were prepared and left to solidify and then wells were prepared using a sterile cork borer. The wells were filled with equal volume of a solution of synthesized compounds and standard drugs. The plates were incubated for a definite time under specified conditions. The zones of inhibition were measured in cm as a parameter of antimicrobial activity.

Table 2: Antimicrobial activity of compounds 5, 8, 9, 12a-c and 13-16measured in cm

Compound	5			8			9		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Escherichia coli	0.2	--	--	0.3	--	--	0.2	--	--
Salmonella enterica	0.3	--	--	0.2	--	--	0.3	--	--
Pseudomonas aeruginosa	0.1	--	--	0.2	--	--	0.1	--	--
Pseudomonas putida	0.2	--	--	0.1	--	--	0.3	--	--
Staphylococcus aureus	0.8	0.5	0.1	0.5	0.2	--	0.6	0.2	--
Staphylococcus epidermidis	0.7	0.2	--	0.7	0.6	0.3	0.7	0.6	0.3
Bacillus subtilis	0.7	0.3	--	0.7	0.2	--	0.7	0.3	--
Micrococcus luteus	1.4	1.0	0.7	2.2	1.9	1.3	2.6	2.0	1.3

Compound	12a			12b			12c		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Escherichia coli	0.3	--	--	0.4	--	--	0.2	--	--
Salmonella enterica	0.2	--	--	0.3	--	--	0.1	--	--
Pseudomonas aeruginosa	0.2	--	--	0.2	--	--	0.3	--	--
Pseudomonas putida	0.1	--	--	0.2	--	--	0.3	--	--
Staphylococcus aureus	0.7	0.4	--	0.9	0.6	0.2	0.7	0.4	--
Staphylococcus epidermidis	0.7	0.5	0.1	0.8	0.4	--	0.6	0.2	--
Bacillus subtilis	0.7	0.3	--	0.7	0.4	0.1	0.8	0.5	0.1
Micrococcus luteus	2.8	2.4	1.9	2.9	2.4	2.1	1.5	1.1	0.6

Compound	13			14			15		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,00	60,000	30,000	15,000
Escherichia coli	0.4	--	--	0.3	--	--	0.1	--	--
Salmonella enterica	0.3	--	--	0.4	--	--	0.3	--	--
Pseudomonas aeruginosa	0.2	--	--	0.1	--	--	0.2	--	--
Pseudomonas putida	0.2	--	--	0.1	--	--	0.2	--	--
Staphylococcus aureus	0.8	0.4	--	0.7	0.4	--	1.0	0.7	0.2
Staphylococcus epidermidis	0.7	0.4	--	0.8	0.6	0.3	0.8	0.5	0.1
Bacillus subtilis	0.6	0.2	--	0.5	0.1	--	0.8	0.4	--
Micrococcus luteus	2.4	1.8	1.2	0.5	0.2	--	3.2	2.7	2.0

Compound	16			Ampicillin			Geniticin		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Escherichia coli	0..2	--	--	1.2	0.9	0.5	1.8	1.3	1.1
Salmonella enterica	0.3	--	--	1.6	1.4	0.8	1.0	0.7	0.3
Pseudomonas aeruginosa	0.2	--	--	1.2	0.8	0.5	1.8	1.3	1.0
Pseudomonas putida	0.1	--	--	1.0	0.6	0.3	2.0	1.2	0.8
Staphylococcus aureus	0.8	0.5	0.2	4.00	3.5	3.0	1.4	1.2	0.6
Staphylococcus epidermidis	07	0.3	--	3.8	3.5	2.8	1.9	1.5	1.2
Bacillus subtilis	0.9	0.7	0.2	2.4	2.0	1.6	1.1	0.7	0.2
Micrococcus luteus	2.8	2.4	1.8	3.6	3.3	2.5	1.5	1.2	0.5

Table 3: Anti-fungal activity of compounds 5, 8, 9, 12a-c and 13-16 measured in cm.

Compound	0013			0014			5		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.4	0.1	--	0.3	--	--	0.9	0.6	0.2
Candida glabrata	0.3	--	--	0.7	0.3	--	0.7	0.4	--
Candida Krusei	0.9	0.7	0.2	0.8	0.6	0.2	0.8	0.6	0.3

Compound	12a			12b			12c		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.8	0.3	--	0.9	0.5	--	0.6	0.2	--
Candida glabrata	0.6	0.2	--	0.6	0.2	--	0.6	0.2	--
Candida Krusei	0.8	0.6	0.1	0.8	0.6	0.1	0.8	0.6	0.1

Compound	13			14			15		
	Conctn.			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.4	--	--	0.6	0.1	--	0.7	0.3	--
Candida glabrata	0.8	0.5	--	0.9	0.5	0.1	0.8	0.6	0.2
Candida Krusei	0.9	0.7	0.4	0.8	0.6	0.2	0.9	0.6	0.1

Compound	16			Ampicillin			Geniticin		
	concentration			concentration			concentration		
Organism	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.7	0.4	--	0.8	0.4	--	1.0	0.6	0.2
Candida glabrata	0.9	0.5	--	0.7	0.5	--	0.8	0.3	--
Candida Krusei	0.8	0.3	--	0.7	0.6	0.1	0.8	0.6	0.1

RESULTS AND DISCUSSION

Compound **5** was proofed by ^{1}H-NMR which showed a significant signal(dd at 7.1 , J=8.2) which belongs to 8H of the aromatic ring in two ibuprofen molecules and the same signals of the aliphatic protons in each moiety of the symmetric molecule . In addition, the NH-NH signal was appeared at (s,7.4ppm).^{13}C-NMR of **5** showed one signal at (170ppm) for each C=O amide and disappeared in DEPT. Also, the DEPT showed the disappearance of two signals in ^{13}C-NMR belongs to the two carbon atoms in ibuprofen which are not attached to the hydrogen atoms. Compound **6** was proofed by IR which showed νC=O at 1603, the νC-H aliphatic in the range of 2868-2952 and ν NH at 3201. ^{1}H-NMR showed the aromatic protons of Ibuprofen at (7.0ppm, dd, J=8.1) and the aromatic protons of naphthalene ring in the range of (6.9-7.5ppm). ^{13}C-NMR of **6** showed two signals at 170, 171ppm belong to the C=O in the molecules and these two signals were disappeared in DEPT. The structure of compound **7** was confirmed by IR which showed two νC=O at 1604 and 1683, the νC-H aliphatic in the range of 2870-2957 and νN-H at 3221.^{1}H-NMR of **7** showed the signal of phenolic OH at (s,10.3ppm,), NH at (s, 6.9ppm) and the aromatic protons of ibuprofen at (dd, 6.9,J=0.92) and the aromatic protons of benzene ring at(m, 7.3, J=7.3) , (d,7.80,Ar) and (d,7.82,Ar). ^{13}CNMR of **7** showed two signals at 172, 167ppm belong to the C=O and these two signals have disappeared in DEPT. Compound **8** was confirmed by IR which showed the νC=O at 1683, νNH 3271 and the νC-H aliphatic in the region of 2865-2954.^{1}H-NMR of **8** showed the signal of aromatic protons of ibuprofen at (dd, 6.9ppm) and the aromatic protons of indol ring at (7.1-7.5ppm). Also, there is a characteristic signal at (s, 3.9ppm, 3H) belongs methoxy protons, (s, 7.8ppm) for NH and (s, 7.9ppm) for NH.

Compound **12a** was proofed by IR which showed the νC=N at 1610 and νC=O at 1672. ^{1}NMR of **12a**showed the signal of –CH=N- at (s, 8.0ppm) , the signal of –NH- at (s, 8.8). The signal of aromatic protons of Ibuprofen was appeared at (dd, 7.2, J=7.7). ^{13}C-NMR of **12a** showed two characteristic signals the first one at 176 ppm which belong to C=O and disappeared in DEPT, and the second one was at 147ppm which belong to –CH=N- and appeared in DEPT. The signals of aliphatic carbon in ibuprofen were five signals and $–CH_2$- was appeared in DEPT. Compound **12b** was confirmed by IR which showed the νC=N at 1610 and C=O at 1662. ^{1}H-NMR of **12b**showed the signal of –CH=N- at (s, 8.0ppm), NH at (s, 8.8ppm). ^{13}CNMR showed the signal of C=O at 176 which was disappeared in DEPT and the signal of –C=N at 156 which was appeared in DEPT. The structure also was confirmed by X-Ray.Similarly, compound **12c** was confirmed by IR, NMR and X-ray. Compound **12d** was proofed by IR which showed theνC=N at 1604 and νC=O at 1666. 1HNMR of **12d** showed the signal of –CH=N- at (s, 8.2ppm), the signal of $–OCH_3$ at (s,3.9), NH(s,8.6) and the aromatic protons of ibuprofen ring at (dd, 7.1,J=7.6) and the aliphatic protons of ibuprofen were appeared in the range of 1.1-4.3ppm. ^{13}C-NMR of **12d** showed the amide signal at 173ppm but the signal of –C=N- was appeared at 150ppm. Compound **12e** was proofed by IR which showed the νC=N at 1610 and C=O at 1666νC-H aliphatic was appeared in the region of 2851-29551. ^{1}H-NMR of **12e** showed the –CH=N- at (s, 8.2ppm), and all the aliphatic protons of ibuprofen were in the range of (1-4.4ppm). ^{13}C-NMR of **12e** showed the signal of –C=N- at 151ppm, and the C=O signal at 169 which was disappeared in DEPT.

The structure of **13** was confirmed by IR which showed νC=O at 1681, νNH at 3206 and νC-H aliphatic in the region of 2868-2953. ^{1}H-NMR of **13** showed the characteristic signal (s, 1H,8.7ppm) which belongs to one proton in the benzene ring, (s,4H, 7.3ppm) for NHNH and the aromatic protons of ibuprofen (8H) was appeared at(dd, 7.1ppm, J=7.7).In addition, the signals of aliphatic protons(26H) was appeared in the range of 0.86-3.7ppm. ^{13}C-NMR of **13** showed two signals at 163, 172ppm which belong to the two amide groups and they disappeared in DEPT. Compound **14** was proofed by IR which showed νC=O at 1662 , νC=N at 1600, νNH at 3248 and νC-H aliphatic in the region 2868-2952. ^{1}H-NMr of **14** was showed the signal of –CH=N- at (s, 2H, 8.2ppm), the signal of –NH- at (s,2H, 8.3ppm) and the protons of aliphatic moiety in ibuprofen(26H) were appeared in the range of 0.85-3.7ppm.^{13}C-NMR of **14** showed one signal at (2C) 172ppm which belongs to the amide group and the signal of C=N was appeared at 145ppm. Compound **15** was proofed by IR which showed two νC=O at 1691 and 1733 belong to the amide and cyclic amide, νNH at 3224 and νC-H aliphatic at 2866-2953. ^{1}H-NMR of **15**showed all the signals of the aliphatic protons in ibuprofen in the range of 0.85-3.7ppm and the signals of aromatic protons of indole ring were appeared in the range of 7.1-7.6. In addition the structure of **15**was confirmed by X-ray. Compound **16** was proofed by IR which showedνC=O at 1669, νC=N at 1579, νNH at 3187 andνC-H aliphatic at 2868-2954. ^{1}H-NMR of **16** showed the signal of –CH=N- at (s, 2H, 7.7), the signal of NH at (s, 2H, 8.4) and all the aliphatic protons in ibuprofen were in the range of 0.9-3.8ppm. ^{13}C-NMR of **16** showed one signal for 2C of C=O at 174 and this signal was disappeared in DEPT. The signal –C=N of 2C was appeared at 151 and appeared in DEPT. The signal of –C=N in pyridine 2C was appeared at 153.

Biological activity

Compared to the standard drugs; Ampicillin and Geniticin; all synthesized compounds showed different ranges of antibacterial activity against all tested strains. Higher activity was clear against Gram positive bacteria when

compared to Geniticin, but similar to lower activity when compared to Ampicillin. For Gram-negative bacteria, antibacterial activities of all synthesized compounds were lower than that of Ampicillin and Geniticin (Table 2, Figure 1). Antifungal activity was significantly similar to what achieved compared with standard drugs Ampicillin and Geniticin which recommend further comparison against antifungal drugs (Table 3, Figure 2).

All compounds at high concentration; 60,000ppm; showed general antibacterial and antifungal activities against all strains tested with inhibition zones ranged from 3.2cm for compound **15** against *Micrococcus luteus* down to 0.1cm for some compounds mainly against Gram-negative bacteria.

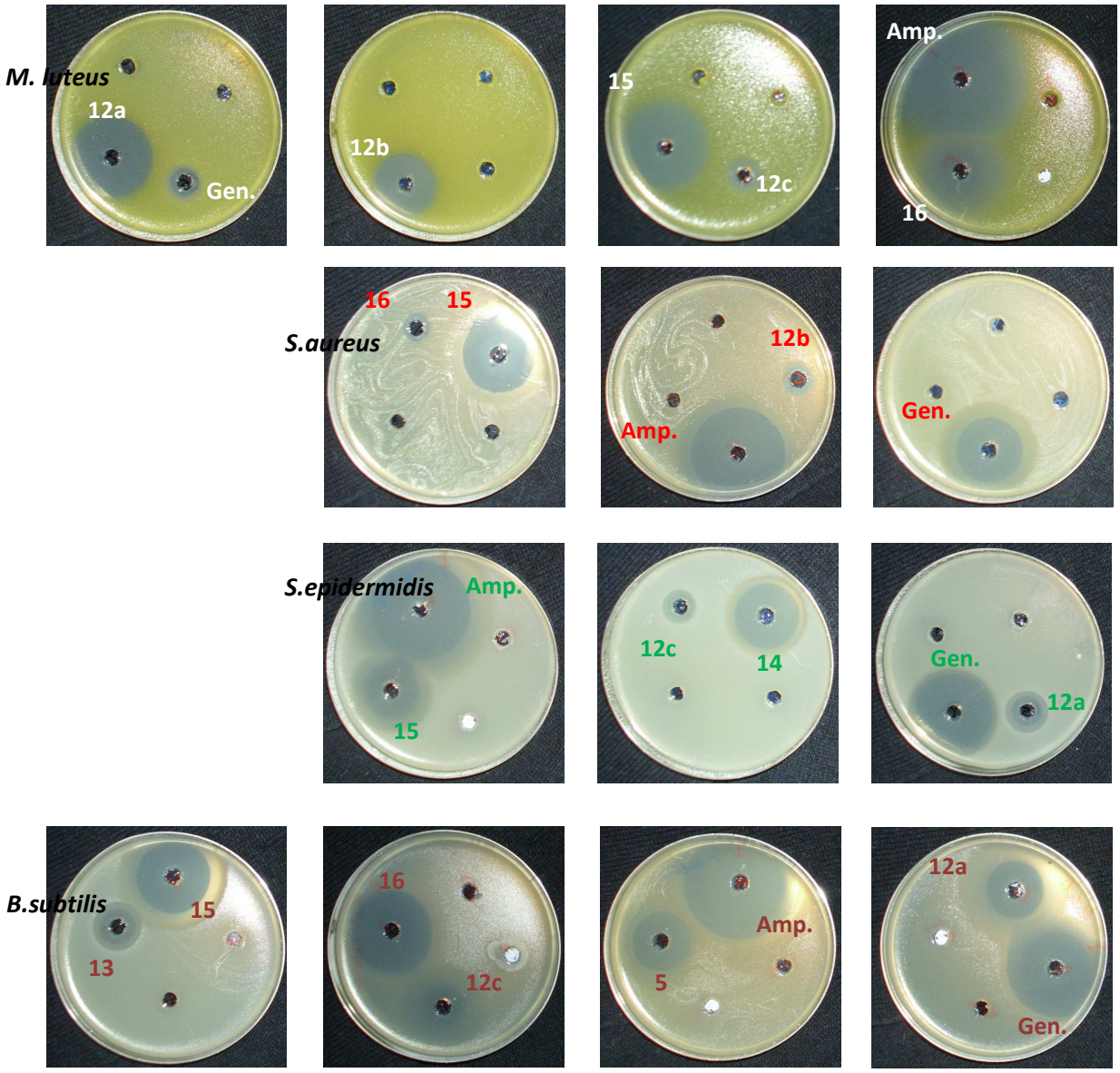


Figure 1: Antibacterial activity of labeled compounds on different bacterial strain

All compounds showed high activity against Gram-positive bacteria represented by large inhibition zones against *Micrococcus luteus* and moderate zones against other Gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis.*

Compounds showed also extended antifungal activity by having moderate to low inhibition against Candida species including *Candida krusei, Candida albicans* and *Candida glabrata*.

Very limited activity against Gram-negative bacteria was determined through all compounds. Among such organisms, *Micrococcusluteus* showed the highest inhibitory effect towards with inhibition zones as high as 3.2cm for

compound **15** at 60,000ppm down to 0.6cm for compound **12c** at 15,000ppm compared to standard drugs 3.6cm for Ampicillin at 60,000ppm down to 0.5 for Geniticin at 15,000. Compound **14** showed limited antibacterial activity against *Micrococcus luteus* with inhibition zones of 0.5cm for 60,000ppm and no inhibition zone at 15,000ppm.

Compound **15** showed the highest antibacterial as well as antifungal activities compared to other compounds with some antifungal activity higher than standard drugs. This significant activity of **15** might be due to its possessing of isatin moiety which complies with literature reports that stating,"the level of endogenous isatin may influence the in vivo pharmacological activity of compounds possessing the isatin moiety" [35]. Moreover, it was reported thatisatin and its analogues act on a large number of biological targets and have a wide variety of actual and potential pharmacological actions [36]. However compound **14** showed the lowest antibacterial activity but similar antifungal activity compared to other compounds.

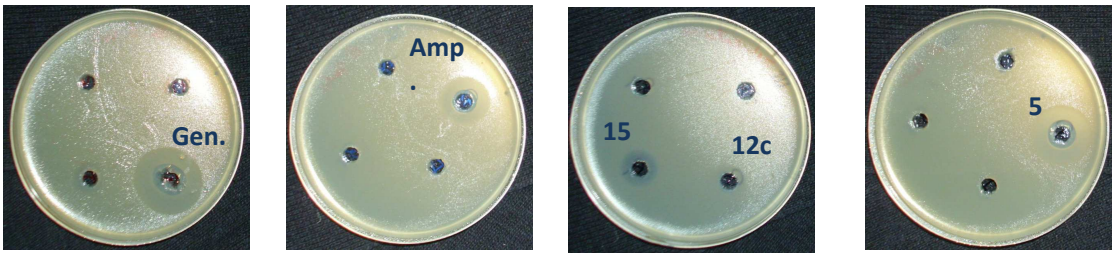


Figure 2: Antifungal activity of labeled compounds on *C.albicans*

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