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Synthesis, Characterization and Antibacterial Activity Evaluation of New Indole-Based Derivatives

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Abstract

Indole is widely distributed heterocycle found in natural biologically active molecules, drugs, and other substances. Starting from Indole-3-propionic acid (IPA) a metabolite produced by gut's bacteria, new series of N-acyl hydrazones (4a-g) was synthesized. These N-acyl hydrazones were prepared by the reaction of 3-(1H-indol-3-yl) propane hydrazide and aldehyde in the presence of glacial acetic acid as a catalyst. ^{1}HNMR and FT-IR analyses were used to identify the synthesized compounds. *In vitro* study was performed to evaluate the antibacterial activity of the synthesized compounds against six different types of microorganisms by using well diffusion method. All the tested N-acyl hydrazones (4a-g) displayed moderate activity against the Gram-negative *E.coli* which was comparable to Amoxicillin, except compound (4e), which showed high activity. Also, selective moderate activities against other Gram-negative bacteria were shown by compounds (4a, 4c, 4e, 4f and 4g), while, compounds (4b) and (4d) exhibited intermediate activity against Gram-positive *B.subtilis*. All the synthesized compounds exhibited selective lower antibacterial activity compared to Ciprofloxacin. Additionally, no activity was exhibited by any of the examined compounds against the Gram-positive *S. aureus*.

Keywords: N-acyl hydrazone, Indole-3-propionic acid, Antibacterial.

تحضير وتشخيص وتقييم الفعالية المضادة للبكتريا لمشتقات اندول جديدة

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الخلاصة

يعتبر الإندول من اوسع الحلقات الغير متجانسة إنتشاراً في المركبات البايولوجية الطبيعية والأدوية. في هذا البحث تم تحضير سلسلة جديدة من الهيدروزونات (4a-g) تحتوي على مجموعة الاسيل مشتقة من حامض الإندول - ٣- بروبيونيك. هذه المركبات حضرت عن طريق مفاعلة الهايدرازايد المشتق من حامض الإندول - ٣- بروبيونك مع عدة الديهايدات بوجود حامض الخليك الثلجي كعامل مساعد. تم تشخيص جميع المركبات المحضرة عن طريق مطياف الأشعة تحت الحمراء وجهاز الرنين النووي المغناطيسي. أُجريت دراسة مختبرية لتقييم المركبات المحضرة كمضادات بكتيرية لستة انواع مختلفة من البكتريا.جميع المركبات المحضرة (4a-g) أظهرت فعالية متوسطة ضد بكتريا الإشريكية القولونية والتي كانت مماثلة للأموكسيسلين،عدا المركب (4e) حيث كان له فعالية عالية. كما أظهرت المركبات (4a, 4c, 4e, 4f, 4g) فعاليات متوسطة ضد الانواع البكترية الاخرى السالبة لصبغة الغرام. بينما المركبات (4b, 4d) كان لها فعالية متوسطة ضد البكتريا العصوية الرقيقة الموجبة لصبغة الغرام. جميع المركبات المحضرة كان لها فعالية منتقاة ضد البكتريا أقل عند مقارنتها بالسيبروفلوكساسين. إضافة إلى انه لم تظهر ايٌ من المركبات فعالية ضد بكتريا المكورات العنقودية الذهبية الموجبة لصبغة الغرام.

كلمات مفتاحية: الهيدراوزونات المرتبطة بمجموعة الاسيل، حامض الإندول-٣- بروبيونيك، مضاد بكتيري.

Introduction

Indole (benzo[b]pyrrole) is widely distributed heterocycle found in natural biologically active molecules, drugs, and other substances. In the biological system, several indole-based biomolecules occur with different effects such as serotonin (5-hydroxytryptamine) a neurotransmitter, melatonin the sleep hormone, tryptamine and related amino acid tryptophan. Also, natural alkaloids with an important pharmacological activity contain indole base had been isolated, among them; vinblastine and vincristine (vinca alkaloids) with anti-cancer activity [(1)]. Also, many synthetic drugs carrying indole pharmacophore are now available such as Sunitinib an anticancer drug, Delavirdine an antiviral clinically used for HIV, Indomethacin, Etodolac NSAIDs, and many other drugs with various pharmacological activities [(2)]. Indole propionic acid (IPA) is a type of plant auxin (plant hormone) which is involved in plenty of developmental ways through the growth period of the plant [(3)]. In human, IPA is detected in serum and cerebrospinal fluid [(4)], it originates from *Clostridium sporogenes* an intestinal flora, its presence in plasma depends on this type of bacteria [(5)], and recently it was discovered other types of gut bacteria are capable of the production of IPA, which are *Peptostreptococcus anaerobius* and three strains of *Clostridium cadaveris* [(6)].

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These types of bacteria produce IPA through deamination of tryptophan by bacterial enzyme tryptophan deaminase [7].

IPA has antioxidant activity and acts as a free radical scavenger which completely protects nerve cells against the beta-amyloid's toxic effect, so it perhaps applied in the treatment of Alzheimer's disease [8, 9]. Also, IPA is considered a ligand for pregnane X receptor (PXR), and together regulates mucosal homeostasis and integrity of GIT by decreasing tumour necrosis factor-alpha and increases junctional protein-coding mRNAs [10, 11]. Recently, it was observed that IPA has anti-tuberculosis activity *in vitro* and *in vivo* by a mechanism which involves inhibition of bacterial tryptophan biosynthesis through a negative feedback mechanism [12, 13].

Hydrazones constitute a significant group of organic compounds contain (-NH-N=C-) moiety. They are synthesized by the reaction of hydrazine derivatives with carbonyl-containing compound (aldehyde or ketone) [14]. Hydrazones have received considerable interest by many medicinal chemistry researchers owing to the ease of their synthesis as well as the exhibition of wide range of pharmacological effects. They have been notified to have antibacterial, analgesic, anti- anticonvulsant, antiplatelet, anti-tubercular, inflammatory and antitumor activities [15].

N-acyl hydrazone (NAH) is considered a privileged structure, which resembles the smallest essential subunit, shared in many drugs or lead-compounds, capable of interacting with a single receptor or more than one class of receptors. Also, due to its ease of production, the stability to hydrolysis, changing H-bonding (donating ↔ accepting) and altering conformation which results in different molecular property with diverse pharmacological activities [16]. Several drugs containing NAH pharmacophore are now available for clinical use such as Dantrolene the only drug approved for malignant hyperthermia treatment [17], Nitrofurantoin, an antibacterial and Carbazochrome which has been used for the treatment of capillary and parenchymal haemorrhage [18].

The aim of the present investigation was to synthesize new indole derivatives containing N-acyl hydrazone starting from indole-3-propionic acid then to evaluate their antibacterial activity.

Materials and Methods

Starting material 3-indole propionic acid (IPA) and aldehydes were purchased from Himedia (India), Hydrazine hydrate from Merck (Germany), methanol from Thomas Baker (India) and ethanol from Scharlau (Spain). The progress of reactions as well as the purity of newly synthesized compounds was checked out by thin-layer chromatography (TLC) by using silica gel GF (type 60) pre-coated aluminium sheet from Merck (Germany). UV-254 lamp was used to visualize the spots, and the elution system used was (ethyl acetate: toluene: methanol (2:2:1)). Stuart SMP3 melting points device was used to measure the melting points and were incorrected. Infrared spectra were made at the College of Pharmacy/ University of Baghdad using FT-IR (IR Affinity-1) spectrometer (Shimadzu, Japan). ^{1}HNMR spectra were obtained using Bruker (400 MHz) device and DMSO-d6 as a solvent and were performed at the Central instrumental laboratory/ College of Science/ University of Tehran / Iran.

All the synthesized compounds were identified and their structures were determined by FTIR and ^{1}HNMR spectral analyses [19]

Synthesis of ester (methyl 3-(1H-indol-3-yl) propionate) (2) [20]

Ten gm (0.052mole) of indole propionic acid **(1)** was dissolved in (50 mL) of methanol and chilled to 0 ^{o}C using an ice bath. Then 3 mL of concentrated H_2SO_4 was added to the cold solution drop by drop with vigorous stirring, and then the temperature of the solution was raised gradually to 70 C^o and started the reflux. The reaction was monitored by TLC, after 4 hours of reflux the reaction completed and the solution was poured into a crushed ice containing beaker, a precipitate was formed, and then the solution was neutralized with 5% (W/V) $NaHCO_3$. The final precipitate was obtained by filtration and recrystallized from (methanol/water) to yield off-white fine crystals.

Yield 94%; **m.p.** 75-76 ^{o}C; **R*f*** = 0.86; **IR** (*v*, cm^{-1}): 3390: (N-H) str. of Indole. 3086: Ar (C-H) str., 2951, 2916: (C-H) asym. Str. of CH_3 & CH_2, 2897, 2854: (C-H) sym. str. of CH_3& CH_2, 1716: (C=O) str. of ester, 1165: (C-O-C) Str.; **1HNMR** (δ, ppm) **:** 2.66 (2H, t, -CH_2-C$\underline{H}_2$-$COOCH_3$), 2.95 (2H, t, -C$\underline{H}_2$-CH_2-$COOCH_3$), 3.58 (3H, s, -COOC$\underline{H}_3$), 6.96,7.05 (2H,2t, Ar-H), 7.10 (1H, s ,Ar-H), 7.32,7.50 (2H,2 d, Ar-H), 10.79 (1H,s, Indole N-H).

Synthesis of acid hydrazide (3-(1H-indol-3-yl) propanehydrazide) (3) [21]

5gm (0.024 mole) of compound **(2)** was dissolved by heating and stirring in 30 mL ethanol, then (20mL, 0.41 mole) of hydrazine hydrate was added gradually to the hot solution with continuous agitation and started the reflux. After 5 hours, TLC showed hydrazide spot only, the reaction was stopped and left to cool, then poured into a beaker contains (50 mL) of distilled water. Excess ethanol was evaporated and the formed precipitate was obtained by filtration, dried, rinsed with ether then recrystallized from water to obtain white needle shape crystals.

Yield =88%; **m.p** 143 ^{o}C**; *Rf*** = 0.48; **IR** (*v*, cm^{-1}): 3309, 3278: (N-H) str. of Indole and hydrazide, 3082: Ar. (C-H) str., 2897, 2854: (C-H) str. (asym.& sym.) of CH_2, 1666: (C=O) str. of amide, 1508: Ar. (C=C) str.; **^{1}H NMR** (δ, ppm): 2.38 (2H, t, -CH_2-C$\underline{H}_2$-$CONHNH_2$), 2.90 (2H, t, -C$\underline{H}_2$-CH_2-$CONHNH_2$), 4.16 (2H, s, -NH-N$\underline{H}_2$), 6.96, 7.04

(2H,2 t, Ar-H), 7.08 (1H, s ,Ar-H), 7.31, 7.50 (2H, 2d, Ar-H), 8.99 (1H,s, -NH-NH_2), 10.75 (1H,s, Indole N-H) [22].

Synthesis of N-acyl Hydrazones compounds (4a-g) [23].

Equimolar (1mmole) of acid hydrazide **(3)** and appropriate aldehyde were dissolved in suitable solvent (ethanol or water) and reacted, using glacial acetic acid as a catalyst then refluxed for 1-2 hour. Ethanol was used as a solvent except for the reaction with vanillin where water was used instead [24]. When the reaction was finished, the residue was filtered and dried. The quantities of each reactant are listed in table 1.

Table 1. Quantity of reactants that equal to 1 mmole.

Compound	Quantity
Acid hydrazide (3)	203.1 mg
p-OH benzaldehyde	122.1 mg
p-Cl benzaldehyde	140.5 mg
p-Br benzaldehyde	185 mg
Benzaldehyde	0.1 mL
p-NO_2 benzaldehyde	151.1mg
p-$N(CH_3)_2$ benzaldehyde	149.2 mg
Vanillin	152.1 mg

N'-(4-hydroxybenzylidene)-3-(1H-indol-3-yl) propanehydrazide (4a)

White powder, **yield** 73%; **m.p.** 208-209 °C; ***Rf*** = 0.7; **IR** (*v*, cm^{-1}) : 3429: (O-H) str. of phenol, 3302, 3186 : (N-H) str. of indole and hydrazone, 3059: Ar (C-H) str., 2900, 2862: (C-H) str.(asym.& sym.) of CH_2, 1624 : (C=O) str. of amide, 1604: (C=N) str., 1566: Ar. (C=C) str.; **^{1}HNMR** (δ, ppm): 2.53 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 2.97 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 6.79 (2H, t, Ar-H), 6.96, 7.05 (2H,2 t, Ar-H), 7.12 (1H, d ,Ar-H), 7.32 (1H, d, Ar-H), 7.46 (2H, dd, Ar-H), 7.55 (1H, d, Ar-H), 7.87, 8.02 (1H, 2s, -N=C<u>H</u>-), 9.83, 9.87 (1H, s, -OH) , 10.77 (1H,s, Indole N-H), 11.07, 11.16 (1H,2s, -N<u>H</u>-N=C-).

N'-(4-chlorobenzylidene)-3-(1H-indol-3-yl) propanehydrazide (4b)

White grain-like crystals, **yield** 69%; **m.p.** 197-198 °C; ***Rf*** = 0.77; **IR** (*v*, cm^{-1}): 3383, 3170: (N-H) str. of indole and hydrazone, 3055: Ar. (C-H) str., 2958, 2843: (C-H) str. (asym. & sym.) of CH_2, 1654: (C=O) str. of amide, 1612: (C=N) str., 1558: Ar. (C=C) str.; **^{1}HNMR** (δ, ppm): 2.58 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 3.00 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 6.97, 7.06 (2H,2t, Ar-H), 7.13 (1H,d,Ar-H), 7.32 (1H, d, Ar-H), 7.46 (2H, dd, Ar-H), 7.55 (1H,d, Ar-H), 7.66 (2H,dd, Ar-H), 7.96, 8.13 (1H, 2s, -N=C<u>H</u>-), 10.78 (1H,s, Indole N-H), 11.35, 11.45 (1H,2s, -N<u>H</u>-N=C-).

N'-(4-bromobenzylidene)-3-(1H-indol-3-yl) propanehydrazide (4c)

White fine crystals, **yield** 72%; **m.p.** 175-176 °C; ***Rf*** = 0.78; **IR** (*v*, cm^{-1}): 3433, 3174 (N-H) str. of indole and hydrazone, 3062: Ar. (C-H) str., 2970, 2862 : (C-H) str. (asym.& sym.) of CH_2 , 1654 : (C=O) str. of amide, 1608: (C=N)str., 1558: Ar. (C=C) str.; **^{1}HNMR** (δ, ppm): 2.57 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 2.97 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 6.97, 7.06 (2H,2 t, Ar-H), 7.12 (1H,d ,Ar-H), 7.32 (1H, d, Ar-H), 7.54 (2H,dd, Ar-H), 7.56 (1H,d, Ar-H), 7.61 (2H,d, Ar-H), 7.94, 8.11 (1H, 2s, -N=C<u>H</u>-), 10.77 (1H,s, Indole N-H), 11.33,11.44 (1H,2s, -N<u>H</u>-N=C-).

N'- benzylidene- 3-(1H-indol-3-yl) propanehydrazide (4d)

White fine needle-like crystals, **yield** 63%; **m.p.** 173-174 °C; ***Rf*** = 0.74; **IR** (*v*, cm^{-1}): 3294, 3182 (N-H) str. of indole and hydrazone, 3020: Ar (C-H) str., 2900, 2846: (C-H) str. of CH_2 (asym. & sym.), 1620: (C=O) str. of amide, 1600: (C=N) str., 1566: Ar. (C=C) str. **^{1}HNMR** (δ, ppm): 2.57 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 3.00 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 6.97, 7.06 (2H,2t, Ar-H), 7.13 (1H,d ,Ar-H), 7.32 (1H, d, Ar-H), 7.37-7.45(3H, m, Ar-H), 7.55 (1H,d, Ar-H), 7.64 (2H,dd, Ar-H), 7.98, 8.14 (1H, 2s, -N=C<u>H</u>-), 10.78 (1H,s, Indole N-H), 11.28, 11.38 (1H,2s, -N<u>H</u>-N=C-).

3-(1H-indol-3-yl)-N'-(4-nitrobenzylidene) propanehydrazide (4e)

Yellow fine crystals, **yield** 98%; **m.p.** 238-239 °C; ***Rf*** = 0.76; **IR** (*v*, cm^{-1}): 3387, 3190 (N-H) str. of indole and hydrazone, 3055: Ar (C-H) str., 2954, 2835: (C-H) str. of CH_2 (asym & sym.), 1678 : (C=O)str. of amide, 1581: (C=N) str., 1512: Ar. (C=C) str. & asym (NO_2) str., 1334: (NO_2) sym. str.; **^{1}HNMR** (δ, ppm): 2.61 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 3.02 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 6.97, 7.05 (2H, 2t, Ar-H), 7.13 (1H,d ,Ar-H), 7.32, 7.55 (2H,2d, Ar-H), 7.89 (2H,dd, Ar-H), 8.06, 8.22 (1H, 2s, -N=C<u>H</u>-), 8.26 (2H,dd, Ar-H), 10.77 (1H,s, Indole N-H), 11.57, 11.67 (1H,2s, -N<u>H</u>-N=C-).

N'-(4-(dimethylamino) benzylidene)-3-(1H-indol-3-yl) propanehydrazide (4f)

White fluffy crystals, **yield** 83.8%; **m.p.** 206-207 °C; ***Rf*** = 0.74; **IR** (*v*, cm^{-1}): 3163 (N-H) str. of indole and hydrazone overlap, 3043: Ar (C-H) str., 2974, 2908, 2850: (C-H) str. of CH_3 & CH_2 (asym. & sym.), 1643 : (C=O) str. of amide, 1597: (C=N) str., 1527 : Ar.(C=C) str.; **^{1}HNMR** (δ, ppm): 2.52 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 2.93, 2.95 (6H,2s, -N(C<u>H</u>$_3$)$_2$), 2.97 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 6.71 (2H, t, Ar-H), 6.97, 7.05 (2H,2t, Ar-H), 7.12 (1H,d ,Ar-H), 7.32 (1H, d, Ar-H), 7.45 (2H,dd, Ar-H), 7.55 (1H,dd, Ar-H), 7.84,7.98 (1H, 2s, -N=C<u>H</u>-), 10.77 (1H,s, Indole N-H), 10.98, 11.06 (1H, 2s, -N<u>H</u>-N=C-).

N'-(4-hydroxy-3-methoxybenzylidene)-3-(1H-indol-3-yl) propanehydrazide (4g)

White powder, **yield** 65%; **m.p.** 160-165 °C; ***Rf*** = 0.68; **IR** (*v*, cm^{-1}): 3406 : (O-H) str. of phenol, 3383, 3217 (N-H) str. of indole and hydrazone, 3032: Ar. (C-H) str., 2939, 2846: (C-H) str. of CH_2 (asym. & sym.), 1635 : (C=O) str. of amide, 1581 (C=N) str., 1516 Ar.(C=C) str., 1265 : Ar (C-O-C) str.; **^{1}HNMR** (δ, ppm): 2.54 (2H, t, -CH_2-C<u>H</u>$_2$-CO-NH-), 2.98 (2H, t, -C<u>H</u>$_2$-CH_2-CO-NH-), 3.75, 3.80 (3H, s, -OCH_3), 6.79 (1H, dd, Ar-H), 6.94 (1H, t, Ar-H), 7.00 (1H,s, Ar-H), 7.06 (1H,t, Ar-H), 7.13 (1H,d, Ar-H), 7.21 (1H, d, Ar-H), 7.32, 7.55 (2H, 2d, Ar-H), 7.86, 8.01 (1H,2s, -N=C<u>H</u>-), 9.45, 9.49 (1H, s, -OH) , 10.77 (1H,s, Indole N-H), 11.11, 11.18 (1H, 2s, -N<u>H</u>-N=C-) [25,26].

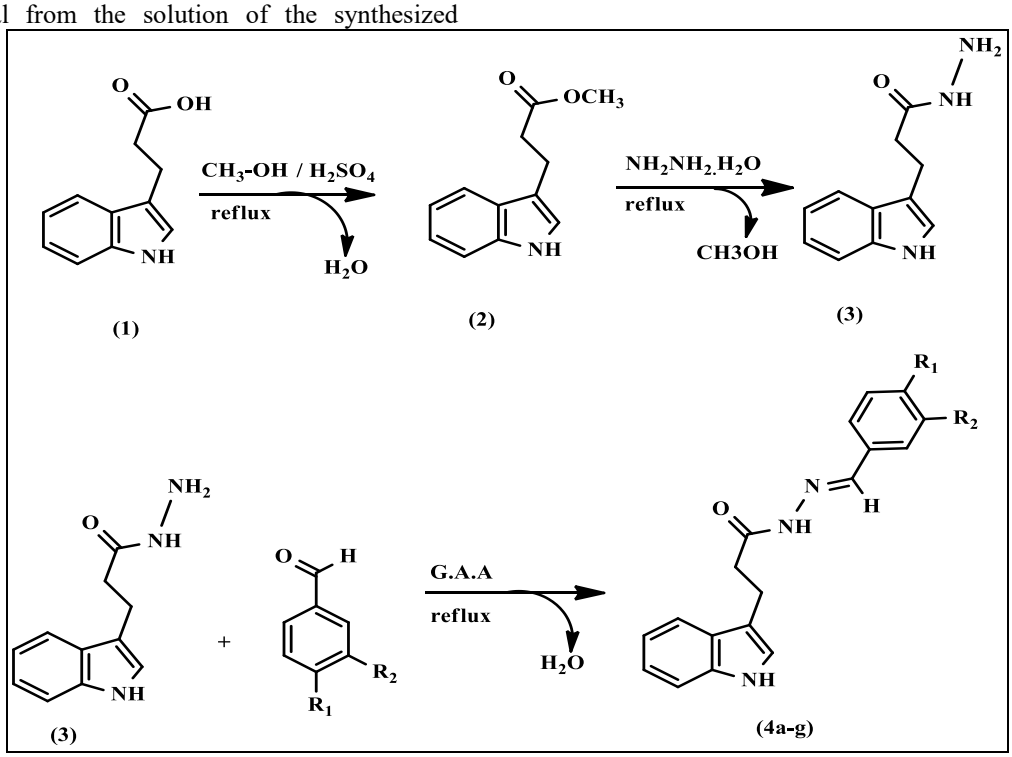
Antibacterial activity

The antibacterial activity was tested by well diffusion method, by using bacterial suspension obtained from McFarland turbidity standard (number 0.5). Mueller-Hinton agar MHA plates were inoculated by this suspension. In each agar plate of examined bacteria, four wells were made, and 80µl from the solution of the synthesized compound was added to them. The plates were incubated at 37 °C for 24 hours, and then the antibacterial activity was evaluated by measuring the diameter of the inhibition zone around the well in (mm) [27]. Four types of Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis*) and two types of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were tested *in vitro* for antibacterial activity. All types of bacteria have already been isolated and identified in the College of the Medicine/ University of Al-Nahrain. Ciprofloxacin and Amoxicillin were used as standards for antibacterial activity. The concentrations of standers were 1mg/mL in Dimethyl sulfoxide (DMSO).

Results and Discussion

Chemistry

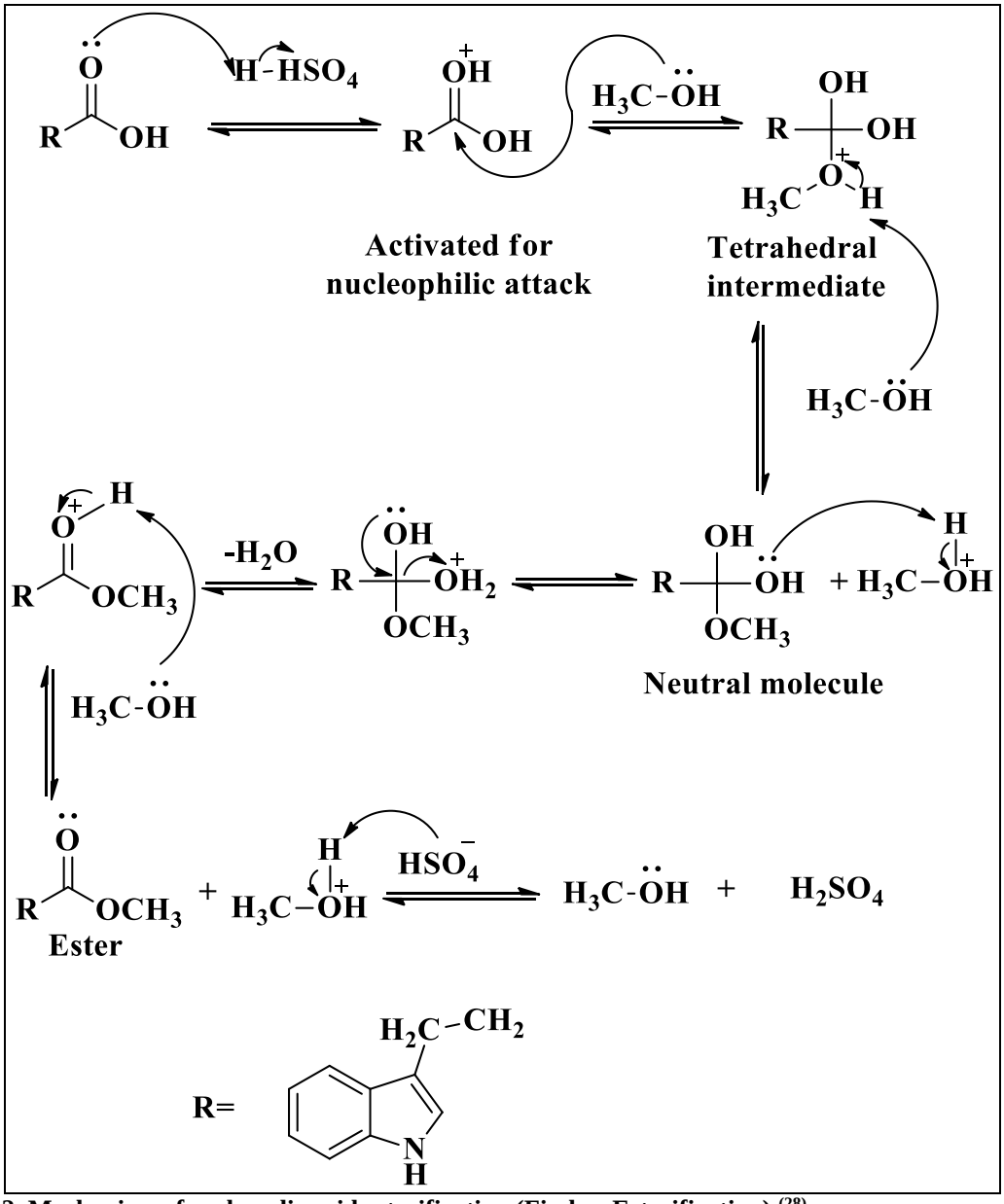
The pathway for the synthesis of the targeted N-acyl hydrazones **(4a-g)** is depicted in scheme 1.



Compound	4a	4b	4c	4d	4e	4f	4g
R1	OH	Cl	Br	H	NO_2	$N(CH_3)_2$	OH
R2	H	H	H	H	H	H	OCH_3

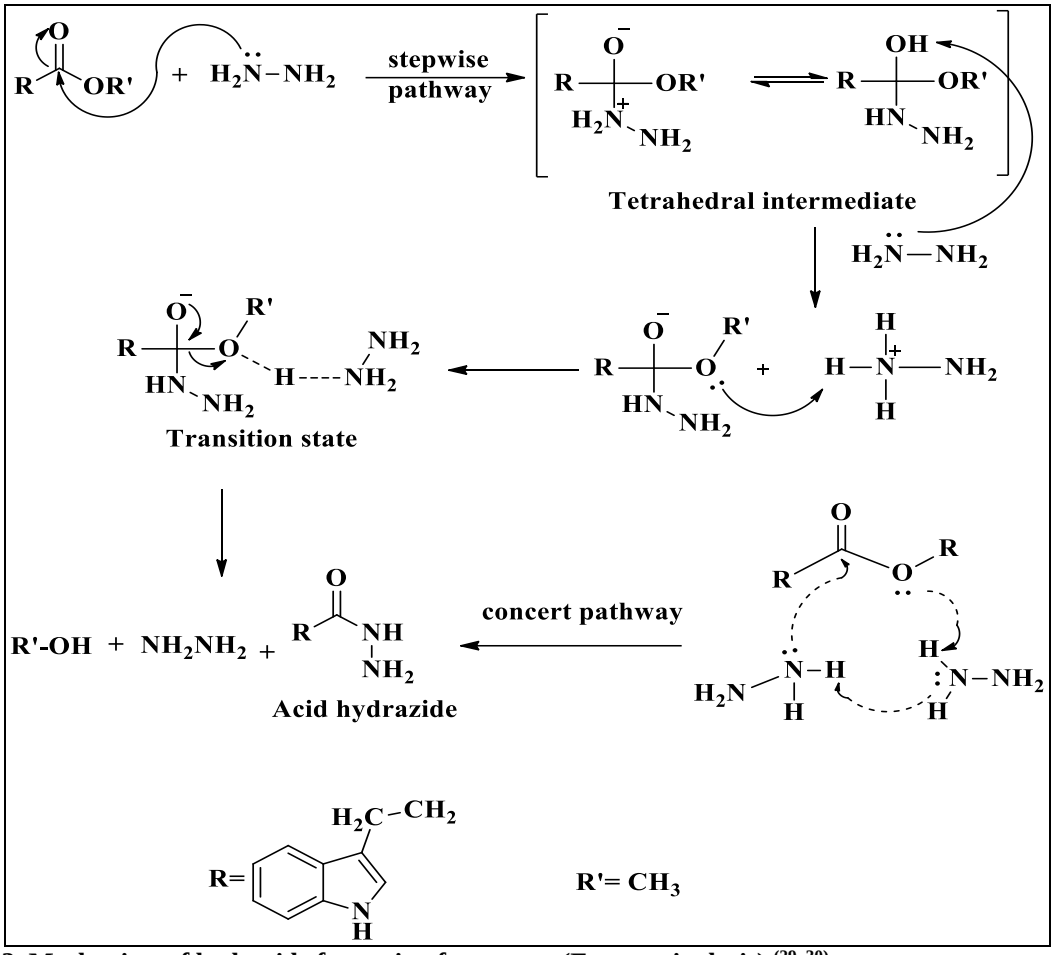
Scheme 1. General synthetic pathway of the titled compounds

The synthetic pathway started by the preparation of methyl ester derivative **(2)** of IPA by Fischer esterification method using H_2SO_4 as a catalyst. The acid protonates oxygen of the carbonyl group and makes carbon more electrophile, subjected to nucleophilic attack by the lone pair of electrons of alcohol. The Nucleophilic attack breaks carbonyl group and forms the tetrahedral intermediate oxonium ion. At this point, the excess of alcohol will deprotonate the tetrahedral intermediate oxonium ion to form neutral molecule. Then, transfer of the proton to hydroxyl group which is eliminated as water and the carbonyl bond is regenerated [28] (scheme 2).This method showed considerable yield with ease of production.



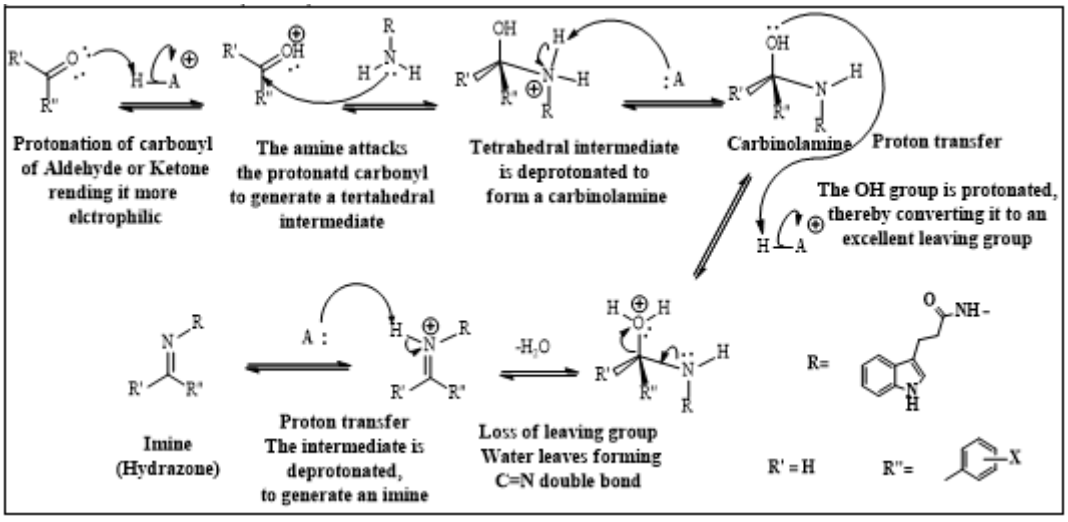
Scheme 2. Mechanism of carboxylic acid esterification (Fischer Esterification) [28]

Hydrazine hydrate ($N_2H_4.H_2O$) was used to prepare the acid hydrazide **(3)** from compound **(2)**. The reaction is base catalyzed ester aminolysis. The mechanism involves two hydrazine molecules either occurred in single step (concert pathway) in which aminolysis occurs through carbonyl attack by amine associate with proton abstraction from the amine, while departure of leaving group associated with proton transfer from ammonium ion, this process happen in one step. The second possibility occurs through multi steps pathway in which the first hydrazine molecule attacks the electrophilic carbonyl group to form tetrahedral intermediate and the second hydrazine molecule will transfer the proton to methoxy group resulting in dissociation of the methoxy group[29-32] (scheme 3).



Scheme 3. Mechanism of hydrazide formation from ester (Ester aminolysis) [29, 30]

Subsequently, the final N-acyl hydrazones **(4a-g)** were synthesized by condensing 3-(1H-indol-3-yl) propanehydrazide compound **(3)** with the corresponding aldehyde in acidic media as a catalyst. In this reaction, one water molecule had been eliminated to form a carbon-nitrogen double bond (imine or Schiff base) [33] (scheme 4). The target compounds **(4a-g)** were obtained in good to excellent yields (63-98%).



Scheme 4. Mechanism of imine formation (Schiff base) [34]

The IR spectra demonstrated shifting of the absorption band of C=O from 1716 cm^{-1} in ester to 1666 in hydrazide (amide formation), in addition, two bands appeared at 3309, 3278 attributed to primary amine N-H stretching overlapping with N-H stretching of indole. The infrared spectra of the synthesized N-acyl hydrazones **(4a-g)** had a characteristic absorption band at 1581-1612 cm^{-1} of carbon-nitrogen double bond (C=N) stretching of imine. Additionally, compounds (**4a-g)** had recognized bands at 1620-1678 cm^{-1}, attributed to the carbonyl group (C=O) stretching of amide.

The ^{1}HNMR spectra of the ester, acid hydrazide and NAHs were consistent with the assigned structures. Two sets of two separated singlets were displayed in the ^{1}HNMR of N-acyl hydrazones (**4a-g)**, attributed to both the –N=CH- and -CONH- protons. These protons appeared as two singlets resonating in the regions (7.84 – 8.22) and (10.98 – 11.67) ppm, respectively. The presence of these paired signals can be explained on the basis of the existence of hydrazones as *E/Z* geometric isomers around the C=N double bonds as well as the *cis/trans* amide conformers [35, 36].

Antibacterial activity

Table 2 shows the activity of the synthesized compounds (NAHs) against the selected bacteria at a concentration of 1mg/mL.

Table 2. *In vitro* antibacterial activity of the synthesized N-acyl hydrazones at 1mg/mL .

Compounds	Zone of inhibition (mm)					
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***	***P. mirabilis***
4a	**-**	**-**	**-**	**14**	**14**	**12**
4b	**-**	**11**	**-**	**12**	**-**	**-**
4c	**-**	**-**	**12**	**14**	**-**	**-**
4d	**-**	**11**	**-**	**11**	**-**	**-**
4e	**-**	**-**	**-**	**18**	**14**	**-**
4f	**-**	**-**	**-**	**12**	**-**	**13**
4g	**-**	**-**	**12**	**12**	**-**	**-**
Amoxicillin	**35**	**-**	**38**	**15**	**10**	**-**
Ciprofloxacin	**52**	**29**	**50**	**30**	**18**	**45**
DMSO	**-**	**-**	**-**	**-**	**-**	**-**

(-) = 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 [37].

The data illustrated in the table 2 reveals that all the synthesized tested compounds showed moderate activity against *E.coli* comparable to that of Amoxicillin, except compound **4e**, which showed high activity. Also, compounds **4a** and **4e** showed moderate activity against *Klebsiella pneumonia*, which was greater than Amoxicillin. In addition, moderate activity was viewed by compounds **4a** and **4f** against *Proteus mirabilis*. Also, moderate anti-*Pseudomonas* activity was seen with compounds **4c** and **4g,** which was lower compared to Amoxicillin. All the tested compounds demonstrated various antibacterial activities against selected bacteria, which were lower compared to ciprofloxacin. Generally, all the tested compounds had no activity against the selected Gram-positive bacteria, except compounds **4b** and **4d**, which showed moderate activity against *Bacillus subtilis*. The using of local isolates and not the control strains (as American Type Collection Culture strains ATCC), may be the cause of low activity of the synthesized compounds due to mutation and resistance [38].

Conclusion

New N-acyl hydrazone derivatives containing indole base were successfully synthesized and the structures of newly synthesized compounds were characterized using IR and HNMR spectral analyses methods. The synthesized compounds had moderate activity against *Escherichia coli* and selective activity against other types of bacteria. Antituberculosis activity evaluation of the synthesized compounds and using the control strains of bacteria instead of local isolates, are recommended for a future study.

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