Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3939

DESIGN, SYNTHESIS, AND BIO-EVALUATION OF NEW 2-[(5-SUBSTITUTED ARYL-3-MERCAPTO-1H-1, 2,4-TRIAZOL-1-YL) METHYL]-1H-ISOINDOLE-1,3(2H)-DIONE DERIVATIVES

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Abstract
A new isoindoline-1,3-dione derivatives were synthesized and evaluated for their anti-bacterial activity. The target compounds 2-[(5-substitutedaryl-3-mercapto-1*H*-1,2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione derivatives were obtained by condensation of N-hydroxymethylphthalimide and thiocarbohydrazide to form 2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) methyl] hydrazine-1-carbothioamide and reacted with different aromatic acids. The structures of the synthesized derivatives were confirmed using IR and ^{1}H-NMR spectral data. The antibacterial activity was determined by Mueller–Hinton well diffusion method. The results revealed the importance of the combination of 1,2,4 triazole and phthalimide moieties as a promising antibacterial agent.

Keywords: isoindoline-1,3-dione, *in vivo* anti-bacterial activity.

1. Introduction
Antimicrobial drug discovery research, accompanied by clinical development, has historically been conducted by large pharmaceutical companies. Diseases caused by microbial infection are a serious menace to the health of human beings and often have a connection to some other diseases, whenever the body system gets injured. Several different classes of antibacterial and antifungal agents have been discovered. The extensive use of antibiotics has led to the appearance of multi-drug-resistant microbial pathogens. The incidence of fungal infections has increased significantly in the past two decades. Many reports showed that compounds containing phthalimide have been described as a scaffold to design new prototypes of drugs with different biological activities and are used in infectious diseases, depending upon the type of nucleophile present in the reactants, a variety of products is obtained when such reactants are reacted with phthalimide. Although a lot of work is reported on the reaction of anhydrides with amines there is not much research done on the formation of cyclic products. A valuable functionality that is proven to be having considerable pharmacological activities is cyclic imides. Antimicrobial, antiviral, anti-inflammatory, anti-cancer, antispasmodic, and plant growth regulator activities are the major groups of interest for such kinds of bioactive

compounds. Hence, the syntheses of new heterocyclic derivatives are being reported continuously and many research articles have been published specifying a wide variety of pharmacological activity. After following this survey, it was thought of interest to synthesize indoline moieties which may enhance the pharmacological activity of compounds. So, our research work involves the synthesis of new isoindoline-1,3-dione derivatives followed by in-vitro antibacterial evaluation of the derivatives.

Recently, cyclic imides have been important compounds in medicinal chemistry that possess various broad spectrum of biological activities. Among the existing various N-substituted cyclic imides, isoindoline-1,3-dione derivatives have been identified as one of the most promising scaffolds, which contain the general structure –CO–N(R)–CO–, so that they are hydrophobic and neutral, and can therefore cross biological membranes *in vivo*. N-substituted isoindoline-1,3-dione has been a very interesting and hot research area due to its broad structural diversity and broad-spectrum biological activities. As introduction of nitrogen-containing heterocycle into the isoindoline-1,3-diones, compounds have been found to exhibit potent including antibacterial[1] antimicrobial [2,3], antiviral [4], anti-COVID-19 agent[5], antitumor[6,7,8], analgesic[9], anti-inflammatory[10], anticonvulsant[11,12,13], anti diabetes[14], hypolipidemic[15], anti-alzheimer[16,17], cholinesterase inhibitory[18] and Antileishmanial[19] activities.

It is well-known that the derivatives of 1,2,4-triazole always possess a wide range of biological activities including antimicrobial[20], antifungal[21], antiviral[22], antitumor[23], and insecticidal[24] properties. Some of the 1,2,4-triazole derivatives have also been widely used for agricultural purposes. Therefore, as a part of our research work on the development of novel bioactive nitrogen-containing heterocycles as shown in Scheme 1, we are very interested in the design and efficient synthesis of isoindoline-1,3-diones bearing 1,2,4-triazole substructure, which will be expected to exhibit interesting features due to the co-existence of two kinds of pharmacophore.

2. MATERIALS AND METHODS

All starting materials, reagents, and solvents were purchased from commercial suppliers like Merck (Germany). Analytical thin-layer chromatography (TLC) was conducted using Merck silica gel 60 F254 plates. Infrared (IR) was recorded using a Fourier-transform IR (FT-IR) spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded by a Bruker 400 MHz spectrophotometer and chemical shifts are expressed as ppm with tetramethylsilane (TMS) as the internal standard. Compounds melting points were determined by the melting point apparatus and are uncorrected.

2.1 Synthesis:

2.1.1. Synthesis of 2-(hydroxymethyl)-1*H*-isoindole-1,3(2*H*)-dione (1):

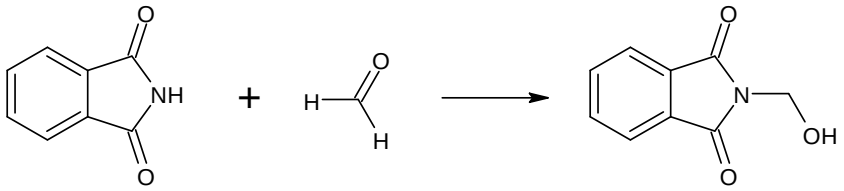
The mixture of phthalimide (0.1mol) and 37 % w/w formaldehyde in water was taken in 250 ml of round bottom flask and refluxed for 4 hours. The progress of the reaction was monitored by TLC. N-hydroxy methyl phthalimide precipitated as white crystals, product was filtered off and purified by recrystallization from hot benzene and dried.

2.1.2. Synthesis of 2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) methyl] hydrazine-1-carbothioamide (2):

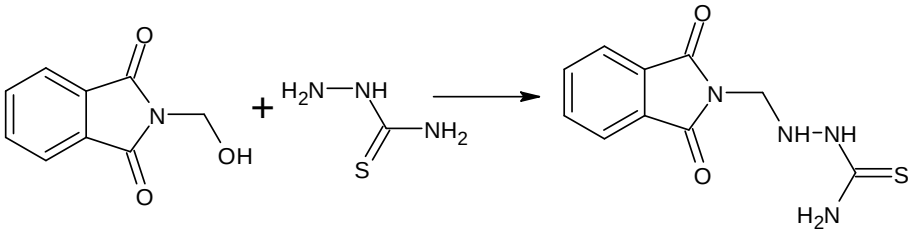
An equimolar mixture o of 2-(hydroxymethyl)-1*H*-isoindole-1,3(2*H*)-dione and thiosemicarbazide in 20 ml methanol along with a few drops of triethylamine was refluxed on a water bath for 7 hours. Progress of the reaction was monitored by TLC. The solids separated were filtered, dried, and recrystallized from ethanol.

2.1.3. Synthesis of 2-[(5-substituted aryl-3-mercapto-1*H*-1,2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a -3e):

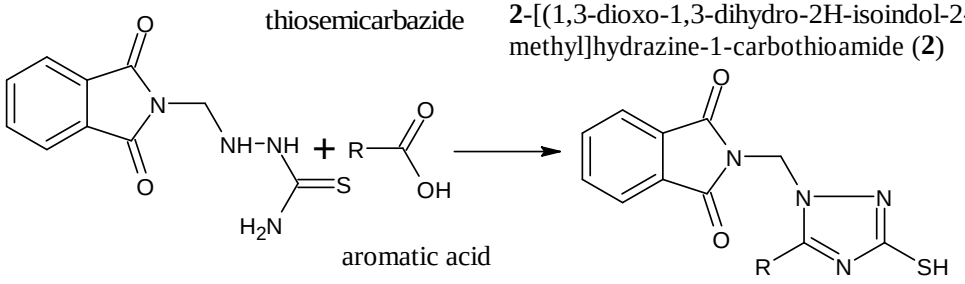
Reflux mixture of 2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) methyl] hydrazine-1-carbothioamide and aromatic acid in 1:1 ratio in presence of ethanol for 4-5 hrs. Progress of the reaction was monitored by TLC. The resulting solution was allowed to stand overnight in a capped round bottom flask during which time product crystallized as a coarse off-white crust on the sides of the flask. The synthesis of target compounds 3a-3e was accomplished using the pathways illustrated in **Scheme 1.**



phthalimide formaldehyde 2-(hydroxymethyl)-1*H*-isoindole-1,3(2*H*)-dione (**1**)



thiosemicarbazide **2**-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl) methyl]hydrazine-1-carbothioamide (**2**)



aromatic acid

2-[(5-substitutedaryl-3-mercapto-1*H*-1,2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (**3a -3e**)

3a = -C_6H_5, 3b = 2(OH)-C_6H_4, 3c = 4(NH_2)-C_6H_4, 3d = 4($N0_2$)-C_6H_4, 3e = C_6H_5CH=CH-

2.2. Physical and Spectral Characterization:

2-(hydroxymethyl)-1H-isoindole-1,3(2H)-dione (1):

Yield: 63%, mp.140-143°C, IR V_{max}, 3448 (OH), 3107-3087 (C-H aromatic), 2979 (C-H aliphatic), 1786 (C=O), 1755 (C=O).

2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl) methyl] hydrazine-1-carbothioamide (2)*:*

Yield: 72%, mp. 154-157 °C, IR V_{max}, 3509, 3340(NH), 3109-3084 (C-H aromatic), 2956 (C-H aliphatic), 1789 (C=O), 1758 (C=O).

2-[(5-phenyl-3-mercapto-1H-1,2,4-triazol-1-yl) methyl]-1H-isoindole-1,3(2H)-dione (3a):

Yield: 66%, mp. 127-130 °C, Molecular formula: $C_{17}H_{12}N_4O_2S$, Mol.wt:336.36,
Composition: C (60.70%) H (3.60%) N (16.60%) O (9.51%) S (9.53%).
IR V_{max}, 3116-3077 (C-H aromatic), 2950 (C-H aliphatic), 1793 (C=O), 1722 (C=O).
^{1}H NMR (400 MHz-$CDCl_3$): δ 5.50 (2H, s), 7.38-7.58 (5H, 7.44 (tt, *J* = 7.4, 1.4 Hz), 7.46 (dddd, *J* = 7.8, 7.4, 1.1, 0.4 Hz), 7.52 (dtd, *J* = 7.8, 1.4, 0.4 Hz)), 7.81-7.95 (4H, 7.88 (ddd, *J* = 7.8, 7.6, 1.3 Hz), 7.88 (ddd, *J* = 7.8, 1.3, 0.5 Hz)).

***2-{[5-(2-hydroxyphenyl)-3-sulfanyl-1H-1,2,4-triazol-1-yl] methyl}-1H-isoindole-1,3(2H)-dione (3b)*:**
Yield: 53%, mp. 194-196 °C, Molecular formula: $C_{17}H_{12}N_4O_3S$, Mol.wt:352.36,
Composition: C (57.95%) H (3.43%) N (15.90%) O (13.62%) S (9.10%).
IR V_{max}, 3518(0H), 3120-3055 (C-H aromatic), 2963 (C-H aliphatic), 1793 (C=O), 1768 (C=O).
^{1}H NMR(400 MHz-$CDCl_3$): δ 5.30 (2H, s), 7.07 (1H, ddd, *J* = 8.2, 1.1, 0.5 Hz), 7.30-7.50 (2H, 7.37 (ddd, *J* = 7.8, 7.6, 1.1 Hz), 7.43 (ddd, *J* = 8.2, 7.8, 1.6 Hz)), 7.80-7.95 (5H, 7.86 (ddd, *J* = 7.6, 1.6, 0.5 Hz), 7.88 (ddd, *J* = 7.8, 7.6, 1.3 Hz), 7.88 (ddd, *J* = 7.8, 1.3, 0.5 Hz))9.95(1H, s).

2-{[5-(4-aminophenyl)-3-sulfanyl-1H-1,2,4-triazol-1-yl]methyl}-1H-isoindole-1,3(2H)-dione (3c):
Yield: 76%, mp. 210-212 °C, Molecular formula: $C_{17}H_{13}N_5O_2S$, Mol.wt:351.38,
Composition: C (58.11%) H (3.73%) N (19.93%) O (9.11%) S (9.13%).
IR V_{max}, 3511(NH), 3122-3073 (C-H aromatic), 2950 (C-H aliphatic), 1792 (C=O), 1776 (C=O).
^{1}H NMR (400 MHz-$CDCl_3$): δ 5.40 (2H, s), 6.74 (2H, ddd, *J* = 8.9, 1.1, 0.4 Hz), 7.67 (2H, ddd, *J* = 8.9, 1.5, 0.4 Hz), 7.81-7.95 (4H, 7.88 (ddd, *J* = 7.8, 7.6, 1.3 Hz), 7.88 (ddd, *J* = 7.8, 1.3, 0.5 Hz)).

2-{[5-(4-nitrophenyl)-3-sulfanyl-1H-1,2,4-triazol-1-yl]methyl}-1H-isoindole-1,3(2H)-dione (3d):
Yield: 58%, mp. 226.5-227 °C, Molecular formula: $C_{17}H_{11}N_5O_4S$, Mol.wt:381.36,
Composition: C (53.54%) H (2.91%) N (18.36%) O (16.78%) S (8.41%).
IR V_{max}, 3120-3067 (C-H aromatic), 2952 (C-H aliphatic), 1793 (C=O), 1771 (C=O).
^{1}H NMR: (400 MHz-$CDCl_3$): δ 5.31 (2H, s), 7.25 (2H, ddd, *J* = 8.8, 1.1, 0.4 Hz), 7.71 (2H, ddd, *J* = 8.8, 1.5, 0.4 Hz), 7.81-7.95 (4H, 7.88 (ddd, *J* = 7.8, 7.6, 1.3 Hz), 7.88 (ddd, *J* = 7.8, 1.3, 0.5 Hz)).

2- [(5-ethenyl phenyl-3-sulfanyl-1H-1,2,4-triazol-1-yl) methyl]-1H-isoindole-1,3(2H)-dione (3e):
Yield: 67%, mp. 218-220 °C, Molecular formula: $C_{19}H_{14}N_4O_2S$, Mol.wt:362.40,
Composition: C (62.97%) H (3.89%) N (15.46%) O (8.83%) S (8.85%).
IR V_{max}, 3108-3071 (C-H aromatic), 3044(alkene),2972 (C-H aliphatic), 1795 (C=O), 1768 (C=O).
^{1}H NMR(400 MHz-$CDCl_3$): δ 5.43 (2H, s), 6.46 (1H, d, *J* = 10.1 Hz), 6.97 (1H, d, *J* = 10.1 Hz), 7.29 (2H, dddd, *J* = 8.0, 7.5, 1.6, 0.5 Hz), 7.41-7.56 (3H, 7.47 (tdd, *J* = 7.5, 1.6, 1.4 Hz), 7.50 (dddd, *J* = 8.0, 1.5, 1.2, 0.5 Hz)), 7.81-7.95 (4H, 7.88 (ddd, *J* = 7.8, 7.6, 1.3 Hz), 7.88 (ddd, *J* = 7.8, 1.3, 0.5 Hz)).

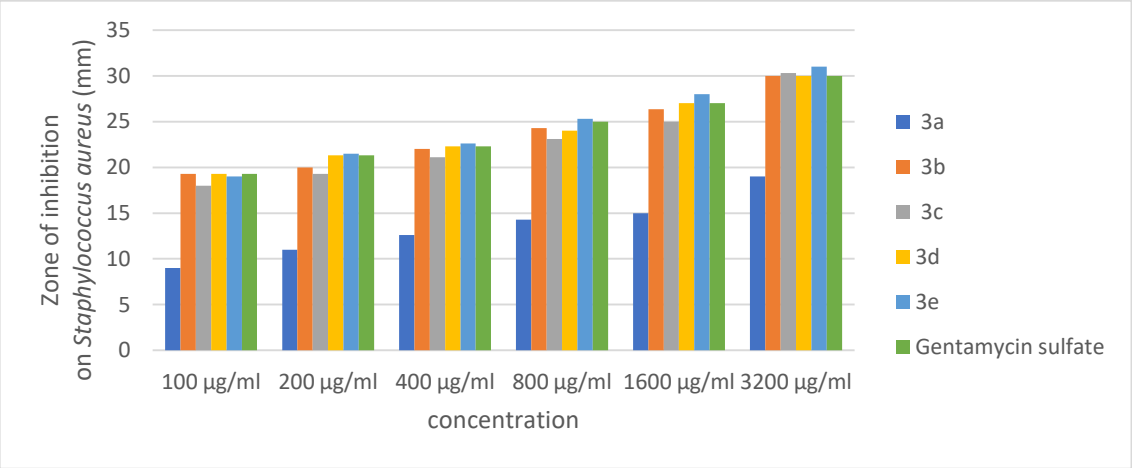
3. Evaluation of Antibacterial Activity:
The antimicrobial activity of prepared compounds against Gram-positive (*Staphylococcus aureus*, ATCC 6538) and Gram-negative (*Escherichia coli*, ATCC 8739) bacteria was investigated by the well diffusion method[25] on Mueller-Hinton agar (MHA). The inhibition zones were reported in millimeters (mm).
The stock solution of each compound (3200 μg/mL) was prepared in N, N-dimethylformamide solvent and used to prepare other concentrations (1600, 800, 400, 200, and 100 μg/mL) through a twofold dilution method.
An overnight culture of each microorganism was prepared and used in sterile conditions, and a few colonies were taken by sterile loop, dispersed in physiological saline serum, and vortexed for a few minutes. The process was repeated to adjust microbial concentration to 0.5 McFarland standards.
Culture media were prepared by the pour plate method, in which Mueller–Hinton Agar culture was prepared, sterilized in an autoclave (temperature 121°C for 15 minutes), and then cooled to temperature 45–50°C with stirring, and then, the microbial suspension was added to culture media at percentage 1%, mixed clockwise and counterclockwise, and then poured in Petri dishes (depth 3-4 mm).
Poured plates were left to cool and solidify in sterile conditions; then, similar wells (8 mm in diameter) were made in the agar and loaded with 100,200,400,800 and 1600 μL of the tested compound solution. Inculcated plates were left at room temperature for 15 minutes without movement and then incubated at 37°C for 24 h. After incubation time, antimicrobial activity was evaluated by measuring the zone

of inhibition (IZ) against the test organisms and compared with that of the standard. Antimicrobial activities were expressed as inhibition diameter zones in mm. Each experiment was carried out in triplicate, and the average zone of inhibition was calculated. Gentamycin sulfate was used as the standard drug for antibacterial activity, and the pure solvent was used as the negative control.

Table 1: In vitro antibacterial activity of synthesized compounds against *Staphylococcus aureus* (Gram-positive microorganism):

S. No	Compounds	Zone of inhibition on *Staphylococcus aureus* (mm)					
		100 μg/ml	200 μg/ml	400 μg/ml	800 μg/ml	1600 μg/ml	3200 μg/ml
1	3a	9.0	11.0	12.6	14.3	15.0	19.0
2	3b	19.3	20.0	22.0	24.3	26.34	30.0
3	3c	18.0	19.3	21.1	23.1	25.0	30.3
4	3d	19.3	21.3	22.3	24.0	27.0	30.0
5	3e	19.0	21.5	22.6	25.3	28.0	31.0
6	Gentamycin sulphate	19.3	21.3	22.3	25.0	27.0	30.0

Graphical representation of antibacterial activity data of synthesized compounds (3a-3e) against *Staphylococcus aureus* (Gram-positive microorganism):

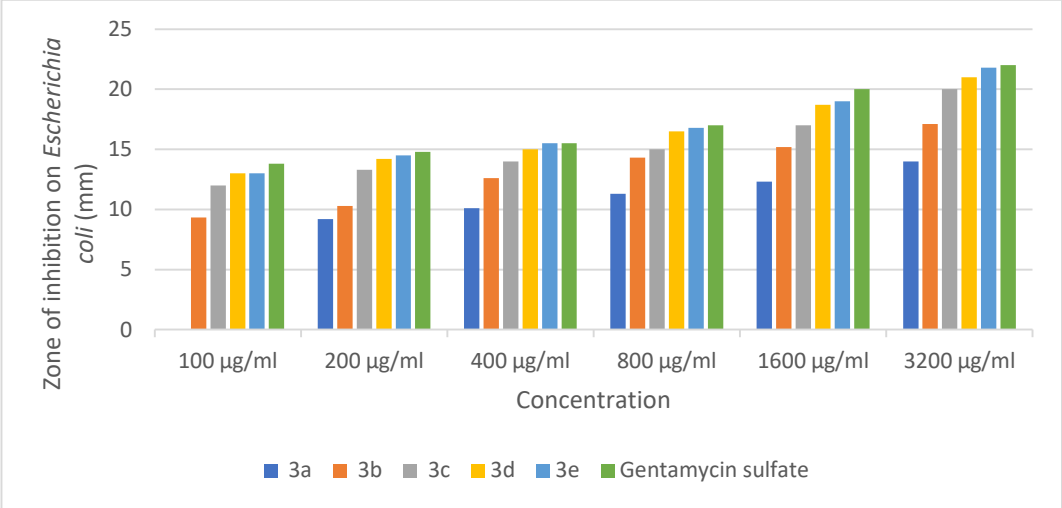


All the synthesized compounds(3a-3e) inhibition against *Staphylococcus aureus* gram-positive bacterial strains, and their results are comparable with those of the standard drug gentamycin sulfate. The investigation of antibacterial screening data revealed that all the tested compounds **3a**–**3e** showed excellent to great inhibition at 100,200,400,800 and 1600 μL µg ml^{-1} in N, N-dimethylformamide. The compound **3a** exhibited less activity than the standard against *S. aureus* gram strain. The active compounds are **3b** and **3c**, which exhibited excellent antibacterial activity against *S. aureus* strain. On the other hand, the antibacterial activity of 3d and 3e on S. aureus was slightly greater inhibition than Gentamycin sulfate.

Table 2: In vitro antibacterial activity of synthesized compounds against *Escherichia coli* (Gram-negative microorganism):

S. No	Compounds	Zone of inhibition on *Escherichia coli* (mm)					
		100 μg/ml	200 μg/ml	400 μg/ml	800 μg/ml	1600 μg/ml	3200 μg/ml
1	3a	0	9.2	10.1	11.3	12.3	13.0
2	3b	9.34	10.3	12.6	14.3	15.2	17.1
3	3c	12.0	13.3	14.0	15.0	17.0	20.0
4	3d	13.0	14.2	15.0	16.5	18.7	20.8
5	3e	13.0	14.5	15.5	16.8	19.0	21.0
6	Gentamycin sulphate	13.5	14.8	15.5	17.0	20.0	22.0

Graphical representation of antibacterial activity data of synthesized compounds (3a-3e) against *Escherichia coli* (Gram-negative microorganism):



All the synthesized compounds 3a-3e (except 3a at 100 μL µg ml^{-1}) inhibit ***Escherichia coli* Gram-negative** bacterial strains, and their results are comparable with those of the standard drug gentamycin sulfate. The investigation of antibacterial screening data revealed that all the tested compounds **3a**–**3e** showed good to excellent inhibition at 100,200,400,800 and 1600 μL µg ml^{-1} in N, N-dimethylformamide. The compound **3a** exhibited less activity than the standard against *S. aureus* gram strain. The compounds are **3b** and **3c**, which exhibited good antibacterial activity against *S. aureus* strain. The active compounds 3d and 3e on S. aureus manifested more inhibition than Gentamycin sulfate.

Results and discussion:

A series of some 2-[(5-substituted aryl-3-mercapto-1*H*-1, 2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione derivatives have been synthesized in three steps. The sequence of the reaction is outlined in Scheme 1, in which initially phthalimide reacted with formaldehyde to form N-hydroxymethyl derivatives after that compound 2-(hydroxymethyl)-1*H*-isoindole-1,3(2*H*)-dione (1) reacted with thiosemicarbazide to give 2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) methyl] hydrazine-1-carbothioamide (2)which reacted with aromatic acid by cyclocondensation reaction to form 2-[(5-substituted aryl-3-mercapto-1*H*-1,2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione(3a -3e) in 53-76 % yield through the formation of an intermediate.

Infra-red (FT-IR) spectroscopy measures the vibrations of the molecules. Each functional group or

structural characteristic of a molecule has a unique vibrational frequency that can be used for its identification in a sample. When the effects of all the different functional groups are taken together, the results are a unique molecular "fingerprint region" that can be used to confirm the identity of the sample.

The FT-IR in the KBr spectrum of compound (3a-3e) exhibited absorption bands in the region at 3518(cm^{-1} (the broadband belongs to the OH stretching) The absorption bands are between 3107 and 3087 cm^{-1} (aromatic C-H Stretching). The absorption bands are between 2972 and 2950 cm^{-1} (aliphatic C-H Stretching). The peaks which splits to two branches bands are between 1793 and 1768cm^{-1} (C=0 Stretching, C=0 Stretching).

The absorption band at 1534.94 bands is caused by the valency vibration of N-O Stretching, the peak at 1299 cm-1 corresponds to =C—N stretching. Finally, the peaks at 761 and 682 cm-1 correspond to the mono substitution of the aromatic ring.

For ^{1}H NMR, spectra of the synthesized compounds were found to be consistence with the suggested structures. Furthermore, the number of integrated protons in the spectra matched the expected number of aromatic protons in each case. The Compound 3a-3e spectra showed a singlet at δ 5.50-5.31 ppm integrated to two protons most likely assigning -CH_2 proton. Compound 3a-3e spectra displayed a multiplet at δ 7.95-6.46 (1H,2H,4H,5H m), ppm integrated to four to five protons corresponding to the aromatic ring respectively. A highly deshielded singlet at 9.95 for compound 3b, corresponding to phenolic OH was observed.

All the synthesized compounds 2- [(5-substituted aryl-3-mercapto-1*H*-1,2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a -3e) showed a zone of inhibition against both gram-positive and Gram-negative microorganism such as *Staphylococcus aureus* and *Escherichia coli* strains, and their results are comparable with those of the standard drug gentamycin sulfate.

Results showed that synthesized compounds are effective against Gram-positive and Gram-negative microorganisms compared to negative control (solvent), with concentrations in the micromolar range. Results showed that Gram-positive microorganisms are more sensitive to studied compounds than Gram-negative microorganisms.

The investigation of antibacterial screening data revealed that all the tested compounds **3a**–**3e** showed good to excellent inhibition at 100,200,400,800 and 1600 μL $\mu g\ ml^{-1}$ in N, N-dimethylformamide. The compounds **3a,3b,3c,3d**, and **3e** are active against *S. aureus* and *E. Coli* and among these compounds, **3a** exhibited less activity than the standard against *S. aureus* and *E. Coli* bacterial strain. The active compounds are 2-hydroxy phenyl (**3b)** and 4-amino phenyl (**3c)**, which exhibited the maximum antibacterial activity. The most active compounds are 4-nitro phenyl (**3d)** and vinyl phenyl (**3e)**, which exhibited great antibacterial activity. On the other hand, the antibacterial activity of **3d** and **3e** S. aureus was slightly stronger than gentamycin sulfate. The results show that the vinyl phenyl derivative of isoindole-1,3(2H) dione enhances the great antimicrobial effect of compounds.

Conclusion:

This study was the report on the antibacterial activity of 2- [(5-substituted aryl-3-mercapto-1*H*-1, 2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a-3e). The advantages of the method are as follows: the synthetic method is simple, cheap, easily available, and reaction in the synthesized of 2-[(5-substituted aryl-3-mercapto-1*H*-1, 2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a-3e) is a time-saving method towards achieving green chemistry. Elemental analysis, FTIR and ^{1}H NMR spectra showed that 2- [(5-substituted aryl-3-mercapto-1*H*-1, 2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a-3e) were produced from the mixture of phthalimide, formaldehyde and thiosemicarbazide showed high antibacterial activity.

The antibacterial activity of 2- [(5-substituted aryl-3-mercapto-1*H*-1, 2,4-triazol-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a-3e) on *S.aureus* was stronger than *E. coli* and antibacterial property was increase with higher concentration of synthesized 1*H*-isoindole-1,3(2*H*)-dione derivatives. The main point of the current study was that the antibacterial activity of 4-nitro phenyl and 5-vinyl phenyl

substituted 1*H*-isoindole-1,3(2*H*)-dione derivatives on S.*aureus* was slightly higher ger than the antibacterial activity of the known antibiotic drugs such as gentamycin sulfate.

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