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**Pharmaceutical Sciences** **Review Article……!!!**

# ANTIMICROBIAL ACTIVITY OF AMIDES USING DIFFERENT SYNTHETIC PROCEDURE WITH DIFFERENT MICROBIAL STRAINS

**DEVI HIMANI*[1], SWETA JOSHI[2]**

[1] M.Pharm (Pharmaceutical Chemistry), Himalayan Institute of Pharmacy and Research, Dehradun, Uttarakhand, India.

[2] Assistant Professor (Pharmaceutical Chemistry), Himalayan Institute of Pharmacy and Research, Dehradun, Uttarakhand, India.

**KEYWORDS:**

Antimicrobial; Peptide linkage;

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**FOR CORRESPONDENCE:**

**DEVI HIMANI ***

**ADDRESS:**

M.Pharm (Pharmaceutical Chemistry), Himalayan Institute of Pharmacy and Research, Dehradun, Uttarakhand, India.

**ABSTRACT**

An antimicrobial is an agent that kill microorganism or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act mainly against bacteria. For the treatment of diseases inhibitory chemicals employed to kill micro-organism or to prevent their growth, are called antimicrobial agents An amide also known as an acid amide is used as antimicrobial agent. Their peptide linkage in amide group is responsible for inhibiting microbial growth. Amides have nitrogen which is directly attached to a carbon in a carbonyl group which shows antibacterial activity. So we are studied about antimicrobial activity of amide using different types of different synthetic procedure with different microbial strains.

**INTRODUCTION:**

An antimicrobial is an agent that kill microorganism or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act mainly against bacteria. [1]. The science dealing with the study of the prevention and treatment of diseases caused by micro-organisms is known as medical microbiology. It is subdivided into virology (study of viruses), bacteriology (study of bacteria), mycology (study of fungi), phycology (study of algae) and protozoology (study of protozoa). For the treatment of diseases inhibitory chemicals employed to kill micro-organism or to prevent their growth, are called antimicrobial agents[70].Antibacterial agents are used to treat bacterial infections. The drug has toxicity in the humans and other animals, antibacterial is usually considered as low. Prolonged use of some certain antibacterial can reduce the number of gut flora, which may have been a negative impact on the health.[2] Consumption of probiotics and realistic eating can help to replace destroyed gut flora. Stool transplants may be consider for patients who are having trouble recovering from prolonged antibiotic treatment, as for persistent *Clostridium difficile* infections.[3] The discovery and development of the use of antibacterials during the 20th century has condensed mortality from bacterial infections.[4] The antibiotic period began with the pneumatic application of the nitroglycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970. When a number of structurally various and highly effective agents were discovered and developed.[5] Since 1980 the introduction of some new antimicrobial agents for clinical use has been declined, because of the huge expense of developing and testing new drugs. In parallel there has been an increase in antimicrobial resistance of bacteria, fungi, parasites and some viruses to multiple active agents.[6]

For example, antibiotics are used beside bacteria and antifungal are used against fungi. Agents that kill microbes are called microbicidal, they mostly inhibit their growth are called biostatic. The utilization of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, whereas the use of antimicrobial medicines to stop infection is known as antimicrobial prophylaxis. [7]Antimicrobial agent shows disinfectant properties which is used to kill a large range of microbes on non-living surfaces and used to prevent the spread of illness, but is now also useful to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones.[8] Antibacterial agents can be promoting subdivided into bactericidal agents, who kill bacteria, and bacteriostatic agents, which slow down or stop bacterial growth. [9]

The nitrogen and sulfur containing compounds plays important role in medicine for the treatment of various kinds of bacterial and fungal diseases caused by different pathogenic bacterial and fungal strains [10].The sulphur containing compounds also used essentially for the treatment of different kinds of tumors along with gastric ulcer caused by *Helicobacter pylori* [11]. Presence of nitrogen and sulphur

generally enhances the efficacy of the compound against the infection [12].Recently our group reported the synthesis and bio-medicinal importance of some novel amides of 5-sulphosalicylic acid [13].

In continuation of our research work on amides of sulphosalicylic acid, the present communication reveals the synthesis and biomedicinal importance of some novel amides of 5-sulphosalicylic acid. Chemotherapy has revolutionized the treatment of infectious diseases since the discovery of antibacterial dyes by Ehrlich earlier in the 20th century and paved the way to a great victory for human health and longevity. The emergence of resistance against currently used antimicrobial drugs led to a revitalized interest of the researchers in infectious diseases to develop new chemical entities to combat them [14]

Tuberculosis (TB), caused by Mycobacterium tuberculosis (M. tuberculosis), remains a pivotal cause of high mortality worldwide despite the handiness of highly potent antitubercular drugs due to the development of resistance by the mycobacterium as a result of gene mutation to first-line antitubercular drugs [15]. To combat the mycobacterial resistance, there is a need to identify novel targets unique to M. tuberculosis which are absent in humans whose blockage would either prove lethal to the bacterium or render it extremely susceptible to the host immune response [16]. pantothenate from D-pantoate and b-alanine for the biosynthesis of coenzyme A and acyl carrier protein in mycobacterium [17].

### 1.1. Amide

Amides have nitrogen which is directly attached to a carbon in a carbonyl group.[6]

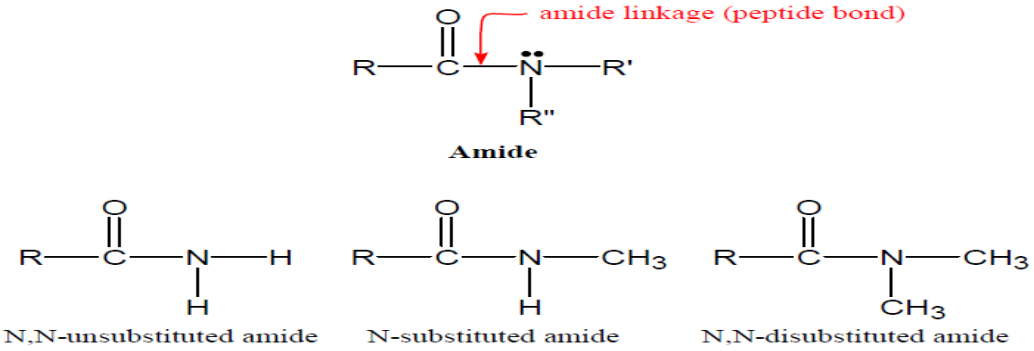


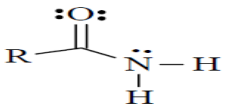

**Figure 1**

Structures of three kinds of amides: an organic amide, a sulfonamide and a phosphoramide. [5]

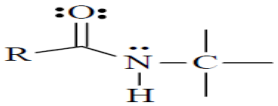
An **amide** also known as an **acid amide**, is a compound with the functional group $R_nE(O)_xNR'_2$ (R and R′ refer to H or other functional groups). Most common are carboxamides (n = 1, E = C, x = 1), but many previous significant types of amides are known, as well as phosphoramides (n = 2, E = P, x = 1) and sulfonamides (E = S and x = 2). The term amide refers together to classes of compounds and to the functional group within those compounds.

Amides having one of the 3 bonds as carbonyl carbon. Therefore amides may be viewed as "acylated amines" or as derivatives of carboxylic acids in which the -OH of the acid has been replaced by -NR2 where R=H, alkyl and aryl, etc. [7]

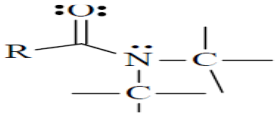
Amides can be classify as "primary", "secondary" or "tertiary" depends upon the degree of carbon which is substituted on nitrogen.



Primary Amide



Secondary Amide



Tertiary Amide

Amines are derivative where one nitrogen substituent is carbonyl moiety. These structural modifications produce a major change in physicochemical properties of amides and amines. Most important amides are characterized by a "conjugated system". In which the NBEs of nitrogen can delocalized into adjacent carbonyl group (C=O). The carbonyl group having strong electron withdrawing nature by resonance (due to the presence of the double bond involving an electronegative oxygen atom) allow for delocalization of the NBEs of nitrogen.[9]

The simple amides are derivative of ammonia where one hydrogen atom has been replaced by an acyl group.[116,17] The group is generally represented as $RC(O)NH_2$ and is describe as a primary amide. It is directly related and even more frequently are secondary amides which can be derived from primary amines ($R'NH_2$) and they have the formula RC(O)NHR′. Tertiary amides are generally derived from secondary amines (R′R″NH) and they have the general structure RC(O)NR′R″.[13] Amides may be generally regarded as derivatives of carboxylic acids group, in which the hydroxyl group has been replaced by an amine or ammonia.[18]

The lone pair of electrons on the nitrogen is delocalized into the carbonyl group, they mainly forming a partial double bond between the nitrogen and the carbonyl carbon.[17] Therefore, the nitrogen in amides is not pyramidal. It is estimated that the acetamide is describing by resonance structure.[19]

Amide has a conjugated system spread over the oxygen, carbon and nitrogen atoms, They consisting of molecular orbitals occupied by delocalized electrons.[18,19] One of the π molecular orbitals in formamide.

**ANTIBACTERIAL ACTIVITY**

The amides of 5-sulphosalicylic acid were tested for antibacterial activity against three pathogenic bacterial strains viz. Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae, using 10 µg/ml concentration of the compound. The compounds show positive antibacterial activity. It was found that the positive activity of these entire compounds was due to

presence of different kinds of amino group. The activity of compound 1 found higher against Pseudomonas aeruginosa while compound 3 show higher efficacy against Staphylococcus aureus and Klebsiella pneumonia respectively. The efficacy of compound 6 against Klebsiella pneumoniae was also found higher. The biological activity of these entire compounds generally occurs due to presence of nitrogen and sulphur contents in the molecule, presence of hydrogen bonding in 5- sulphosalcylic acid, presence of polar OH

groups which increases the water solubility. These compounds generally reacted with some groups of bacterial cell wall and damage it is such a manner that the aromatic ring gets entered inside the cell wall followed by death of bacterial cell.

All the newly synthesized compounds were screened for their antifungal activity against Aspergillus fumigatus, Syncephalastrum racemosum, Geotriucum candidum and Candida albicans by poisoned food technique.[18] Ampicillin, Gentamicin and Amphotericin B were used as standards for antibacterial and antifungal studies respectively. The inhibition zones of bacteria were recorded after 24 h by visual observations while that of fungi after 72–96 h of incubation. Results of antimicrobial studies and the activity percent for each compound compared with the reference standard and Comp. No. Chemical structure Tested fungi Inhibition zone (mm) Percent activity %Amphotericin B* As. fumigatus 23.7 100.00 S. racemosum 19.7 100.00 G. candidum 28.7 100.00 C. albicans 25.4 100.00* Amphotericin B was taken as standards for antifungal microbes.

The antibacterial activity of these compound was carried out by disc diffusion method [19],

Using Gentamycin as standard. In this technique the filter paper (Whatman No.1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 µg/ml of ethanol) along with standard were placed on the nutrient agar plate at 37oC for 24 hrs in BOD incubator. The inhibition around dried impregnated disc was measured after 24 hrs. The bacterial activity was classified as highly active (dia = > 15 mm), moderate active (dia = 10-15 mm) and partially active (dia = 5-10 mm).

Bacterial cells were suspended in sterile culture medium; the inoculum size was 1 × 104 cells/mL in Müller–Hinton broth (DIFCO), confirmed by the use of the McFarland scale. From this culture, 50 µL was spread onto a microplate previously containing 50 µL ofMüller–Hinton broth, resulting in a final cell density of 1.5 × 103 cells/mL. Cells were incubated at 37 °C for 18 h in the presence of 100 µL of each peptide solution, at concentrations ranging from 0.24 to 500 µg/mL. After incubation, 10 µL of a triphenyltetrazolium chloride (TTC) (Mallinckrodt) solution (final concentration 0.05% w/v) was added to each plate. The plates were incubated at 37 °C for 24 h, and, after incubation, 20 µL of a triphenyltetrazolium chloride solution (TTC) 0.5% (w/v) was added. The plates were then incubated for an additional period of 2 h at 37 °C. The minimal inhibitory concentration was defined as the

concentration at which the dye level was not reduced; oxytetracycline, at concentrations ranging from 0.24 to 500 μg/mL, was used as a control.

The antimicrobial activity of [I5, R8]MPwas evaluated against several bacterial strains and fungi, [I5, R8]MPwas potent against Gram-positive and -negative bacteria, as well as against fungi (MIC in the range 3–25 μM). This peptide was, however, poorly active against Listeria ivanovii (MIC = 50 μM) and inactive against Enterococcus faecalis (MIC higher than 100 μM). A previous study of Moerman and collaborators indicated thatMICs ofMP against Gram-negative bacteria were 2-fold (Escherichia coli and Klebsiella pneumoniae) or 8-fold (Pseudomonas aeruginosa) lower than [I5, R8] MP [20]. It is probably even higher considering the fact that a bacterial suspension of 5 × 105 cfu·ml−1was used forMP (106 cfu·ml−1 in our antimicrobial assay). MP was shown to be potent (MIC = 2.7 μM) against colistinresistant Acinetobacter baumannii clinical isolates [21]. A same activity was observed for the analog [I5, R8] MP against the sensitive strain ATCC 19606 . Regarding Gram-positive bacteria, a MIC in the range of 12.5–25 μMwas reported forMP against Staphylococcus aureus ATCC 29213 and E. faecalis ATCC 19433 (assays with 5 × 105 cfu·ml−1) [22]. Considering this lower bacterial concentration (as mentioned above) and the fact that different strains were used in our assay, the activity of [I5, R8] MP toward S. aureus appears to be similar to that of MP, with also a low activity/inactivity of MP against E. faecalis . In order to analyze the influence of C-terminal amidation in the sequence of [I5, R8] MP, we measured the potential changes in the antimicrobial activity of [I5, R8] MP-amide against a Gram-negative (Klebsiella pneumoniae) and a Gram-positive (Staphylococcus aureus) bacterial strain. When amidated, [I5, R8] MP displayed higher activity (MIC = 3–6.25 μM), with 2-fold or 4-fold better activity against K. pneumoniae (MIC=3 μM) and S. aureus(MIC=6.25 μM), respectively. Interestingly, the potency of [I5, R8] MP-amide was conserved (MIC=3 μM) against antibiotic-resistant strains of these bacterial species, such as carbapenem-resistant K. pneumoniae (3259271) and methicillin-resistant S. aureus (7133623).

The results of antibacterial screening of the synthesized compounds are show modest antibacterial activity is observed with most of the tested compounds. Some of synthesized compounds showed good to moderate activity with MIC value in the range of 62.5–100 lg/ml. Particularly, compound 5h, 5j, 6b and 6d showed good activity (MIC value 100 lg/ml) against E. coli and 6h showed significant activity (MIC value 62.5 and 100 lg/ml, respectively) against E. coli and P. aeruginosa, respectively. Moreover, 5a, 5b, 5d, 5e, 5g, 5i, 5j, 6a, 6b, 6c, 6f, 6g, 6h and 6j displayed comparable activity (MIC value 100–250 lg/ml) against S. aureus relative to the reference drug ampicillin.

The antimicrobial activity of the synthetic compounds (4a–4i, 6a–6h) was evaluated by in vitro agar diffusion and micro well dilution assay, and results are summarized in all synthetic compounds were

active against all tested gram positive bacteria, Bacillus subtilis, Staphylococcus epidermidis and Staphylococcus aureus, except compound 6e. This compound had no antimicrobial activity. Compound 4g is active against gram positive bacteria as well as against fungi. Furthermore, All compounds had no antibacterial activity against gram negative bacteria. Compounds 4a and 4g were most effective against B. Subtilis with MIC value of 62.5 mg/mL compared with other compounds . Compounds 4a, 4f, 4g, 5a and 5 h revealed the lowest MIC value (<31.25 mg/mL) against S. epidermidis, while compounds 4e, 4f and 4g were most effective against S. aureus (MIC value of <31.25 mg/mL). The results indicated that only compound 4g has antifungal activity against A. brasiliensis and A. niger with MIC value 1000 and 2000 mg/mL respectively. The results showed that compound 4g in comparison with other compounds has better antimicrobial

activity.

Compounds were tested for in vitro antimicrobial activity against microorganisms representing Gram-positive bacteria (Staphylococcus aureus), Gram-negative bacteria (Escherichia coli) and fungi (Aspergillus flavus and Candida albicans), using agar well diffusion assay [23] at 20 lg/ml. Tetracycline was used as antibacterial agent and amphotericin B as antifungal agent standards. Zones of inhibition around the disc were observed. The screening results indicate that all compounds have antibacterial activities against the tested microorganisms except compound 22 against S. aureus. While, the antifungal activity was recorded only for compound 14 which showed pronounced activity against C. albicans.

All of the newly synthesized derivatives were evaluated as antimicrobial against two Gram-positive and two Gramnegative bacteria as well as many fungal strains. All of the test compounds showed promising activities as antimicrobial. Agar diffusion method [24] was carried out to determine the in vitro antibacterial activity of the newly synthesized derivatives. In this work, two Gram-positive organisms (Streptococcus pneumoniae, Bacillus subtilis) and two Gram-negative organisms (Pseudomonas aeruginosa, and E. coli) were used to investigate the antibacterial activity.

Many of the newly synthesized compounds are found to exhibit good to excellent antimicrobial activity. From antimicrobial activity data, it is observed that compounds 3c (3-Cl–C6H4), 3d (4-Cl–C6H4), 3f (4-F–C6H4), 3h (4-NO2–C6H4) and 3j (3-OH–C6H4) are most active compounds. For antibacterial activity, it has been observed that compounds 3a (–C6H5), 3e (2,5-(Cl)2–C6H3), 3g (3-NO2–C6H4), 3i (2- OH–C6H4) and 3j (3-OH–C6H4) possess good activity, while incorporation of chloro group at 3rd position of phenyl ring in compound 3a to 3c increases the activity and it possesses very good activity against Escherichia coli. It has been observed from the data that when we replace chloro group at 4th position of phenyl ring in 3a we get compound 3d, and when we replace nitro group

at 4th position of phenyl ring in 3a we get compound 3h and it enhances the activity and possesses excellent activity against E. coli. Compounds 3c (3-Cl– C6H4), 3d (4-Cl–C6H4), 3f (4-F–C6H4), 3g (3-NO2–C6H4), 3i (2-OH–C6H4) and 3k (2-OH,4-Cl–C6H3) possess good activity against Pseudomonas aeruginosa. When we substitute hydrogen in 3a by nitro group and hydroxyl group, it leads to corresponding compounds 3h (4-NO2–C6H4) and 3j (3-OH–C6H4) which possesses very good activity against P. aeruginosa. Compounds 3b (C6H5–CH2), 3c (3-Cl–C6H4), 3d (4-Cl–C6H4), 3h (4-NO2–C6H4), 3k (2-OH,4-Cl–C6H3) and 3l (–C5H4N) possess good activity against Staphylococcus aureus, while compounds 3f (4-F–C6H4) and 3i (2-OH–C6H4) possess very good activity against S. aureus. Compounds 3h (4-NO2– C6H4) and 3l (–C5H4N) possess good activity against Streptococcus pyogenes and compound 3d (4-Cl-C6H4) possesses very good activity against S. pyogenes. For antifungal activity, compounds 3a (–C6H5), 3i (2-OH– C6H4) and 3k (2-OH,4-Cl–C6H3) possess good activity against Candida albicans. When we replace hydrogen by chloro, nitro and pyridine substituent in 3a it will give 3d, 3g, 3l respectively and it possesses very good activity against C. albicans. Furthermore, replacement of hydrogen in 3a by 3-Cl–C6H4, 2,5-(Cl)2- C6H3, 4-F-C6H4 and 4-NO2-C6H4 and enhances the activity and it possesses excellent activity against C. albicans. Compounds 3b (3-Cl–C6H4), 3d (4-Cl–C6H4), 3h (4-NO2–C6H4) and 3j (3-OH-C6H4) possess good activity against A. niger. Compounds 3c (3-Cl–C6H4), 3f (4-F–C6H4), 3g (3-NO2– C6H4) and 3l (–C5H4N) possess good activity against Aspergillus clavatus. Enhancement of activity of these compounds is due to the presence of hydroxy, nitro and halogen groups in title compounds. Thus, we have discussed and compared antibacterial and antifungal activities based on standard drugs ampicillin and griseofulvin, respectively.

The antimicrobial activity was determined using the disc diffusion method [25] by measuring the zone of inhibition in millimetre (mm). All the compounds (10a–u) were screened in-vitro concentration of 10 lg/disc for their antibacterial activity against two Gram-positive strains (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative strains (Escherichia coli and Pseudomonas aeruginosa). The antifungal evaluation was carried out against Candida albicans and Aspergillus niger at a concentration of 10 lg/disc. Standard antibacterial drug ciprofloxacin (10 lg/disc) and antifungal drug fluconazole (10 lg/disc) were also tested under similar conditions against these organisms. All the synthesized compounds exhibited significant antibacterial and antifungal activities. Each experiment was performed in triplicate and the average reading was taken. The activity was classified as highly active (P26 mm), moderately active (11–25 mm) and least active (<11 mm). In view of the values obtained, compounds 10b, 10c, 10g, 10h, 10m, 10n, 10r and 10s are the most potent in the series. The active molecules depict a trend wherein halogen substitutions mainly consist of fluoro or chloro groups at either position-3 or 4

of the phenyl ring. On the other hand, bromo, methoxy and methyl groups did not impart much enhancement in the activity. Furthermore, an overall comparison between compounds 10a–10k and 10l–10v hints at slightly improved activity of the latter set which may be due to the presence of bioisostere fluoro group on the indole core (R1). It can also be seen that the potency of the active molecules is comparable with that of the standard drugs.

## MATERIAL AND METHOD

### Reaction of 5-sulphosalicylic acid with pmethoxy aniline [26]

An ethanolic solution of 5-suphosalicylic acid (2.5 gm; 1 mole) and *p*-methoxy aniline (2.46 gm; 2 m mol) was refluxed together at room temperature for 6-7 hrs, followed by their evaporation and concentration in vacuum gives a crystalline solid which was recrystalized in ethanol to give respective amide of 5-sulphosalicylic acid.

### Reaction of 5-sulphosalicylic acid with alpha- naphthylamine [27]

An ethanolic solution of 5-sulphosalicylic acid (2.5 gm; 1 m mole) and salicylic acid alpha-naphthylamine (2.86 gm; 2 m mole) was refluxed together at room temperature for 6-7
hrs, followed by their evaporation and concentration in vacuum afforded a crystalline solid mass which was further recrystalized in ethanol to gives respective amides.

### Reaction of 5-sulphosalicylic acid with hydrazine [28]

An ethanolic solution of 5-sulphosalicylic acid (2.5 gm; 1 m mole) and hydrazine (0.64 gm; 2 m mole) was refluxed together at room temperature for 6 hrs, followed by evaporation and concentration in vacuum afforded a crystalline solid which was further recrystalized in ethanol to give respective amides.

### Mast cell degranulation

Degranulation was determined by measuring the release of the granule marker, β-D-glucosaminidase, which co-localizes with histamine, as previously described [29].Mast cells were obtained by the peritoneal washing of female adult Wistar rats. Mast cells were washed three times by re-suspension and centrifugation in a mast cell medium [150 mM NaCl (Merck), 4 mM KCl (Merck), 4 mM NaH2PO4 (Synth), 3 mM KH2PO4 (Synth), 5 mM glucose (Synth), 15 µM BSA (Sigma), 2 mM CaCl2 (Merck), and 50 µL Liquemine (5000 UI/0.250 mL) (Roche)]. The cells were incubated with various peptide concentrations for 15 min at 37 °C, and, following centrifugation, the supernatants were assayed for β-D-glucosaminidase activity. Briefly, 50 µL of substrate (5mMp-nitrophenyl-N-acetyl-β-D-glucosaminidine in 0.2Mcitrate, pH 4.5) and 50 µL of medium samples were incubated in 96-well plates for 6 h at 37 °C to yield the chromophore p-nitrophenol. After incubation, 50 µL of the previous solution was added to 150 µL of 0.2 M Tris, and the absorbance was measured at 405 nm. The values

were expressed as the percentage of total β-D-glucosaminidase activity from rat mast cell suspensions, determined in lysed mast cells in the presence of 0.1% (v/v) Triton X-100 (considered to be the 100% reference point). The results were compared to the activities measured for the standard mast cell degranulating peptide HR2 (Sigma). The cell suspensions in the absence of any peptide were used as negative controls.The results are expressed as the means ± SD of five experiments.

**Mast cell lysis**

Mast cell lysis was assayed by measuring the leakage of lactate dehydrogenase (LDH) from the mast cell cytoplasm; the LDH assay was performed as previously described [30]. The cells utilized in this assay were the same as those used in mast cell degranulation assay, described above. LDH activity was assayed by using the UV-LDH Assay Kit (Biobras Diagnostics); 20 µL of each supernatant was pre-incubated with 800 µL of LDH buffer (50 mM Tris pH 7.4, containing 1.2 mM pyruvate and 5 mM EDTA) for 5 min, at 37 °C. The reaction was initiated by the addition of 200 µL of LDH substrate (0.15mMNADH); the kinetics of NADH consumption were monitored by recording the decrease in absorbance at 340 nmover 3 min (Δ A340) at 37 °C. The results were initially calculated as catalytic units (µmol NADHmin−1 at 37 °C and pH7.4) and then converted into relative activity, compared to the total LDH activity of rat mast cells lysed in the presence of 0.1% (v/v) Triton X-100 (considered to be the 100% reference point). The results are expressed as the means ± SD of five experiments.

**Hemolysis**

Five hundredmicroliters ofwashed rat red blood cells (WRRBC)was suspended in 50 mL of physiological saline solution [0.9% (w/v) NaCl]. Ninety microliters of this suspension was incubated with 10 µL of peptide solution at different concentrations, at 37 °C for 2 h. The samples were then centrifuged, and the supernatants were collected; the absorbance values of the supernatants were measured at 540 nm. The absorbance measured fromlysed WRRBC in the presence of 1% (v/v) Triton X- 100 was considered to be 100%. The results are expressed as the means± SD of five experiments.

**Circular Dichroism (CD) measurements**

CD spectra were obtained using a 20 µMpeptide concentration in different environments: in water; in Tris/H3BO3 buffer, containing 0.5 mM EDTA and 150 mM NaF, pH 7.5; in 40% (v/v) 2,2,2-trifluoroethanol (TFE)/bi-distilled water; in sodium dodecyl sulphate solutions (SDS), above and below the critical micellar concentration (CMC) (8 mM and 165 µM, respectively); in 100 µM PC; and in 100 µM PCPG SUVs. CD spectra were recorded from 260 to 190 nm or 200 nmwith a Jasco-815 spectropolarimeter (JASCO International Co. Ltd., Tokyo, Japan), which was routinely calibrated at 290.5 nm using d-10-camphorsulfonic acid solution. Spectra were acquired at 25 °C using 0.5 cm path length cell and averaged over nine scans, at a scan speed of 20 nm/min, bandwidth of 1.0 nm, 0.5 s

response and 0.1 nmresolution. Following baseline correction, the observed ellipticity, θ (mdeg) was converted to mean residue ellipticity [Θ] (degcm2/ dmol) using the relationship: [Θ] = 100 h/(lcn); where l is the path length in centimeters, c is the peptide millimolar concentration, and n is the number of peptide bonds. Assuming a two-state model, the observed mean residue ellipticity at 222 nm([Θ]222 obs) was converted into an α-helix fraction (fα), using the method proposed by Rohl and Baldwin [32].

**Molecular modeling**

The modeling procedure began with an alignment of the target peptide sequence with Mastoparan-X (PDB ID: 2CZP), a related peptide of known three-dimensional (3D) structure, as a template [33]. Homologous structures for the peptides Protonectarina-MP-NH2 and Protonectarina-MP-OH, among known structural templates in the PDB (Protein Data Bank), were identified using BLASTP [34,35].This alignment was formatted to be the input for the program MODELER.The output is a 3D model for each target sequence containing all main chain and side-chain non-hydrogen atoms. Several slightly different models can be calculated by varying the initial structure. To build the models, MODELLER 8 v.2 was used [36]. For each peptide, a total of 1000 models were created, and the stereochemical quality of the models was assessed by the program PROCHECK [37]. The finalmodelswere selectedwith 100% residues in favored regions of the Ramachandran plot, with the best values for the overall G factor and the lower values of energy minimization. The images were constructed using the graphics programs MolMol [38] and VMD 1.8.6 [39].

**ESI mass spectrometry**

Mass spectrometric analyses were performed in a triple quadrupole mass spectrometer (Micromass, Mod. Quattro II). The samples were injected into the electrospray transport solvent with a microsyringe (250 μL) coupled to a microinfusion pump (KD Scientific) at a flow rate of 4 μL/min. The mass spectrometer was calibrated with intact horse heart myoglobin and its typical cone-voltage induced fragments to operate at a resolution of 4000. The samples were analyzed by positive electrospray ionization (ESI) using typical conditions: a capillary voltage of 3.5 kV; a cone-voltage of 52 V; a desolvation gas temperature of 80 °C; and flow of nebulizer gas (nitrogen) of approximately 20 L/h and drying gas (nitrogen) at 200 L/h. The ESI mass spectra were obtained in the continuous acquisition mode, scanning from m/z 50 to 4000 at a scan time of 10 s. Typical conditions used to perform the CID MS experiments were: argon as the collision gas; a capillary voltage of 1.78 kV.

**Determination of minimum inhibitory concentration (MIC)**

The microorganisms used to determine the MIC of [I5, R8] MP included the Gram-positive bacteria Staphylococcus aureus (ATCC 25923), a clinical isolate of methicillin-resistant S. aureus (7133623), and Enterococcus faecalis (ATCC 29212), the Gram-negative bacteria Escherichia coli (ATCC 25922),

Pseudomonas aeruginosa (ATCC 27853), Acinetobacter baumannii (ATCC 19606), Klebsiella pneumoniae (ATCC 13883), and a clinical isolate of carbapenem-resistant K. Pneumonia (3,259,271). These strains were cultured in Lysogeny Broth (LB). The two Gram-positive species, Streptococcus pyogenes (ATCC 19615) and Listeria ivanovii (Li 4pVS2), were cultured in Brain Heart Infusion (BHI), whereas the fungi Candida albicans ATCC 90028 and C. parapsilosis ATCC 22019 were cultured in Yeast Dextrose medium (YPD).MICwas determined in 96-wellmicrotitration plates by growing the microorganisms in the presence of two-fold serial dilution of the peptide, as previously described [40]. Briefly, logarithmic phase cultures of bacteria and fungi were centrifuged and suspended in MH (Muller Hinton) broth to an absorbance at 630 nm (A630) of 0.01 (106 cfu·ml−1), except for S. pyogenes, L. ivanovii, E. faecalis and Candida species which were suspended in their respective growth medium. Microtiter plate wells received aliquots of 50 µl each of the culture suspension followed by the addition of 50 µl of the diluted peptide (200 to 1 µM, final concentrations). After overnight incubation at 37 °C (30 °C for fungi), antimicrobial susceptibility was monitored by measuring the change in A630 value using a microplate reader. MIC was determined as the lowest peptide concentration that completely inhibits the growth of the microorganism and corresponds to the average value obtained from three independent experiments, each performed in triplicate with positive (0.7% formaldehyde) and negative (without peptide) inhibition control [41].

**Molecular modeling**

One hundred molecular models for each variant were constructed by comparative molecular modelling through MODELLER 9.14 [42], using the structure of MP with detergents (PDB ID: 1D7N) [43]. This score assesses the energy of the models and indicates the best probable structures. The best models were evaluated through PROSA II [44] and PROCHECK [45]. PROCHECK checks the stereochemical quality of a protein structure through the Rama chandran plot, where good quality models are expected to have N90% of amino acid residues in most favoured and additional allowed regions, while PROSA II indicates the fold quality. Structure visualization was done in PyMOL.

**Antimicrobial screening using broth dilution method**

The building blocks 2-amino-6-methoxybenzothiazole [46] and 2-[N-(6 methoxy benzothiazolyl) amino] pyridine-3-carboxylic acid [47] were prepared according to the reported procedures [48].

**Preparation of building block 2-[N-(6-methoxybenzothiazolyl)**

amino] pyridine-3-carboxo hydrazide [4] 2-[N-(6-methoxybenzothiazolyl)amino] pyridine-3-carboxylic acid 3 (0.01 mol) in SOCl2 (7.0 ml) was refluxed on water bath until the reaction was completed. The mixture was protected from humidity with CaCl2 guard tube. The excess of SOCl2 was removed by vacuum distillation. The solid material 2-[N- (6-methoxybenzothiazolyl)amino] pyridine-3-carbonyl

chloride was obtained, which was directly used in the next step. Hydrazine hydrate (0.02 mol, 80%) in chloroform (5 mL) was added dropwise in a mixture of acid chloride (0.01 mol) in chloroform (10 mL) and triethylamine (2–3 drops) while stirring at 0–5 _C for half an hour and the stirring was continued at room temperature for 3–4 h. The solvent was evaporated and the product obtained was collected, dried and recrystallized from methanol. m.p. – 245–247 _C, yield, 68%; IR (KBr) cm_1: 3339 (–NH str.), 1648 (CONH, amide-I), 1542 (amide-II), 1224 (amide-III), 1469 (C–N, C–C ring str.), 1337 (C–N str.), 1025, 1205 (C–O–C sym., asym.). 1H NMR (DMSO-d6) d (ppm): 8.30–6.90 (6H, m, pyridine and aromatic), 9.35 (1H, s, –NH ), 8.95 (1H, s, –CONH–), 4.10 (2H, s, –NH2), 3.88 (3H, s, OCH3).

**General method for preparation of hydrazones [5a–j]**

To a solution of 4 (0.01 mol) in 10 ml of DMF; appropriate aldehyde a–j (0.012 mol) and 3–4 drops of glacial acetic acid were added. The reaction mixture was refluxed for 5–6 h. The reaction mixture was cooled and poured onto crushed ice. The reaction was monitored by TLC on silica gel using toluene:ethyl acetate (3:1). The separated solid was isolated, washed with water and recrystallized from ethanol to give 5a–j.

**Preparation of aminated carbon nanotubes as catalyst**

Raw carbon nanotubes are chemically neutral species because there are no active groups in CNT structure. Functionalization of carbon nanotubes enables them to be active in chemical reactions. In this research, we prepared animated MWCNTs through a three step process.

**Oxidation of MWCNTs**

0.5 g of MWCNTs was added to 200 ml of H2SO4/HNO3 (in ratio of 3:2) and sonicated in an ultrasonic bath with a frequency of 50 Hz for 15 min. The reaction was continued by

reflux at 80 _C for 3 h. After that, the reaction mixture was filtered and washed with distilled water to neutral pH and finally dried at 95 _C for 24 h.

**Preparation of aminated carbon nanotubes**

0.3 gr of acylated carbon nanotubes and 6 gr of methyl amine were added to 15 ml of dimethyl formamide and refluxed for 20 h at 100 _C under nitrogen atmosphere. Then the reaction mixture was cooled to room temperature, washed with ethanol and dried at 80 _C.

**General procedure for synthesis of 1,4-dihydropyridines**

A mixture of 1 mmol arylaldehyde derivative, 1 mmol ammonium acetoacetate, 2 mmol ethyl acetoacetate and 0.001 gr of aminated carbon nanotubes as catalyst, (or 1 mmol arylaldehyde

derivative, 1 mmol ammoniume acetoacetate, 1 mmol ethyl acetoacetate, 1 mmol dimedone and 0.001 g of catalyst) in 2 ml EtOH was stirred at 80 _C for appropriate time. Reaction progress was assessed by thin layer chromatography (petroleum ether/ethyl acetate 3:1). After completion of the reaction, the

catalyst was separated with simple filtration. Finally, the crude product was obtained from filtrate after evaporation of ethanol. In order to obtain more purification, the product was recrystallized in EtOH. Products were characterized, using FTIR, 1H NMR and 13C NMR techniques.

**IR spectra analysis**

All melting points are uncorrected. IR spectra (KBr) were run on a Unicam SP 1200G infrared spectrophoto meter. 1H NMR and 13C NMR spectra (DMSO-d6) were run on a Varian spec-trometer (300 MHz) with a T.M.S. as internal standard. Mass spectra (MS) were recorded on a Shimadzu Qp-2010 Plus mass spectrometer at 70 eV. Elemental analyses and in vitro antimicrobial activities were carried out at the Micro Analytical Center, Cairo University. Compounds 1, 6 and 7 were prepared by the procedures described in the literature.

**Procedure for the synthesis of 4-((2-chloroquinolin-3- yl)methylene)-2-phenyloxazol-5(4H)-one (2)**

**Conventional method. A mixture of 2-chloroquinoline-**

3-carbaldehyde (1) (47.75 g, 0.25 mol), hippuric acid (44.75 g, 0.25 mol), acetic anhydride (28.28 mL, 0.30 mol) and anhydrous sodium acetate (20.50 g, 0.25 mol) was taken in a

250 mL round bottom flask and heated on an electric hot plate with constant stirring. As soon as the mixture liquefied completely, the flask was transferred to a water bath and further

heated for 4 h, ethanol (100 mL) was added slowly to the flask and the mixture was allowed to stand overnight in refrigerator. The crystalline product obtained was filtered and washed with ice-cold alcohol and then with boiling water, the crude product was recrystallized from benzene.

**Microwave method.**

A mixture of compound 2-chloroquinoline-3-carbaldehyde (1) (47.75 g, 0.25 mol), hippuric acid (44.75 g, 0.25 mol), acetic anhydride (28.28 mL, 0.30 mol) and anhydrous sodium acetate (20.50 g, 0.25 mol) was taken in a reaction vessel and mixed thoroughly. The mixture was irradiated under microwave for 3 min at 300W with constant shaking and intermittent radiation of 30 s interval. Progress of the reaction was monitored by TLC. Upon completion of reaction the vessel was cooled, ethanol was added and the mixture was kept overnight in the refrigerator. The crystalline product obtained was filtered and washed with ice cold alcohol and then with boiling water, crude product was recrystallized from benzene. Yield – 68%; m.p. 232–234 _C; IR (KBr, cm_1): 3057, 3080 (C–H stretching, aromatic ring), 3011 (C–H stretching, – CH,C–), 1682 (C,O stretching, imidazoline ring), 1609, 1570, 1527 (C,N, C,C, aromatic ring stretching), 1204 (C–O stretching), 961 (C–H bending, –CH,C–), 743 (C–Cl stretching); 1H NMR (400 MHz, DMSO-d6, d, ppm): 7.37– 8.30 (m, 10H, Qui-H, Ar-H), 7.61 (s, 1H, –C,CH–); 13C NMR (400 MHz, DMSO-d6, d, ppm): 114.7, 126.2, 126.4,

127.5, 127.6, 127.9, 128.1, 128.5, 128.8, 129.3, 131.3, 132.6, 136.1, 145.6, 152.4, 159.5, 166.3; LCMS (m/z): 334 (M+). Anal. Calcd. for C19H11ClN2O2: C – 68.17%, H – 3.31%, N – 8.37%; Found: C – 68.24%, H – 3.25%, N – 8.43%.

**Results and Discussion**

The amides of 5-sulphosalicylic acid were synthesized by the general procedure as shown below. All the compounds were crystalline solids and quite stable at room temperature with good yield (70-75%). The compounds were soluble in polar solvents. They have sharp melting points. The compounds were also characterized on the basis of elemental analysis and IR spectra.

**I.R. Spectra**

The solid state FTIR spectra of all these compounds were recorded in the spectra range of 4000-400 cm–1 and significant frequencies were observed in this region. The IR spectra of the entire compound shows absorption band due to phenyl group of 5- sulphosalicylic acid. The absorption frequencies of carbonyl group in the amide derivative have been assigned. Clearly, confirming the proximal geometrical arrangement.

**Antimicrobial activities of [I5, R8] mastoparan**

In order to analyze the influence of C-terminal amidation in the sequence of [I5, R8] MP, we measured the potential changes in the antimicrobial activity of [I5, R8] MP-amide against a Gram-negative (Klebsiella pneumoniae) and a Gram-positive (Staphylococcus aureus) bacterial strain. When amidated, [I5, R8] MP displayed higher activity (MIC = 3–6.25 μM), with 2-fold or 4-fold better activity against K. pneumoniae (MIC=3 μM) and S. aureus(MIC=6.25 μM), respectively. Interestingly, the potency of [I5, R8] MP-amide was conserved (MIC =3 μM) against antibiotic-resistant strains of these bacterial species, such as carbapenem-resistant K. pneumoniae (3259271) and methicillin-resistant S. aureus (7133623).

**Cytotoxicity toward human cells**

We investigated the hemolytic activity of [I5, R8] MP on human erythrocytes and also its cytotoxicity toward other human cells, such as THP-1-derived macrophages and HEK-293 cells. LC50 values, corresponding to the concentration of peptide producing 50%of cell death, indicate that [I5, R8] MP was neither haemolytic against human erythrocytes nor cytotoxic against HEK-293 cells (LC50 N 200 μM). By contrast, [I5, R8] MP showed cytotoxicity toward THP-1-derived macrophages, as revealed by the much lower LC50 value (24.5 ± 12.9 μM). In contrast to MP for which 40% hemolysis is already observed at a concentration of 10 μM in rat erythrocytes, our results indicate that [I5, R8] MP is not hemolytic at antimicrobial concentrations.

**Therapeutic index of [I5, R8] mastoparan**

The therapeutic index (TI) is an important parameter to evaluate the balance between the toxic and the desired effect of a drug. Taking this into account, the TI of [I5, R8] MP was calculated. We found a value of 10.29, which means that a tenfold administration of this peptide would be necessary to achieve a toxic effect.

**Structure prediction**

Molecular modelling of [I5, R8]MP indicated that this peptide adopts an α-helical structure, where a segregation between charged and hydrophobic amino acids is observed, as denoted by the helical wheel diagram. It was possible to observe that the structure of [I5, R8]MP tends to unfold in a water environment, either with a carboxyl or a carboxamide terminal. The root-mean-square-deviation (RMSD) of [I5, R8] MP along the simulations shows a variation of 2–7 Å, while for [I5, R8] MP-amide the variation is of about 2–6 Å. The residues' RMS fluctuation indicated that in the amidated peptide the N-terminal end is more stable than the C-terminal one, while for the C-terminal carboxylated peptide the whole structure showed a similar behaviour. In contrast, when placed in a DOPC bilayer, [I5, R8] MP presents little variation in its structure, with a RMSD variation of about 1 Å, which is reflected in the residues' RMS fluctuation (below 1.5 Å)

**IR Spectra**

The structures of synthesized compounds 5a–j were confirmed by elemental analyses and IR spectra (cm_1) absorption bands at 3419 (NH), 1646 (amide-I), 1612 (–N,CH–), 1556
(amide-II), 1340 (C–N), 1225 (amide-III) 1036, 1186 (C–O– C). Some additional peaks appear due to substitution in aromatic ring showing absorption band at 3410 (O–H), 1370,
1512 (–NO2 sym., asym.) 758 (CCl). In 1H NMR spectra common signals that appear are: dH (ppm): a singlet at d 8.85 corresponds to >CONH, a singlet at d 5.83 for –N,CH–, a
multiplet at d 6.84–8.57corresponds to aromatic proton. A singlet at d 3.85 for –OCH3, and due to the substitution on aromatic ring, a singlet appeared at d 5.15 corresponding to –OH.

The structure of compounds 6a–j were supported by elemental analyses and IR spectra as observed in 5a–j with disappearance of 1612 cm_1 for –N,CH– band with 1710 cm_1 for
>C,O of thiazolidinone. The 1H NMR singlet signals of cyclised thiazolidinone was observed at d 3.58–3.64, corresponding to –CH2– in the ring, and d 6.09–6.15, corresponding to
C2–H. The other signals observed were same as 5a–j.

In FTIR of chlorinated MWCNT (MWCNT-COCl), absorption in the region of 1725 and 726cm_1 proved the existence of ACOACl groups. Also peaks at 500 and 1024 cm_1 are from CACl and CAO groups on MWCNT surfaces show the FTIR of aminated MWCNTs. There is an absorption peak in the

region of 3437 cm_1 which is from NAH groups and absorption at 1632 cm_1demonstrated the existence of C,O amidic groups on CNT surfaces. SEM images of raw and functionalized MWCNTs are presented in. Images from functionalized CNTs indicated that methylamine groups were successfully attached on CNT surfaces due to increasing the diameter of treated CNTs. Also, SEM images from aminated CNTs, show that no damage occurred on the morphology of CNT structure after functionalization.

The results showed that 85_C can be considered as optimized temperature. At temperatures higher than 85 _C, the yield of product become lower because of decomposition of product.

The synthesis of 1,4-dihydropyridines from aldehyde derivatives, 1,3-diketo compounds and ammonium acetate in the presence of CNT-CONHCH3 was performed in optimized reaction conditions. As shown in, the yield of products was high to excellent and in case of aldehyde with electron withdrawing groups, the yield of reaction was higher than aldehydes with electron donating groups.

For estimation of catalyst activity in large scale, the model reaction was done using 2 mmol 4-nitrobenzaldehyde, 2 mmol ammoniume acetoacetate and 4 mmol ethyl acetoacetate in the presence of 0.002 gr of aminated carbon nanotubes. The yield of product was 92% after 5 h. Consequently,the catalyst has a high performance in the synthesis of DHPs and can be used in large scale reactions. The reusability of catalysts is one of the most important advantages. Presented CNT based catalyst can be reused in 3,4-DHP synthesis easily. After completion of the reaction, the catalyst was separated, washed with ethanol and dried. The recovered catalyst was used in the same reaction and this cycle was repeated four times. Results in demonstrate the high efficiency of catalyst even after 4 times of recycling.

IR spectrum of 8 showed an absorption band at 2211 cm_1 corresponding to C,,N group. Its 1H NMR spectrum showed two singlets at d =2.71 and 4.49 ppm for SCH3 and CH2, respectively. Base-induced intramolecular cyclization of 8 afforded thienopyrimidine derivative 9. IR spectrum of 9 showed absorption bands at 3324, 3221 cm_1 corresponding to NH2 group, in addition to a band at 2197 corresponding to C,,N group. 1H NMR spectrum of 9 showed two singlets at d= 2.69 and 6.09 ppm for SCH3 and NH2, respectively. Hydrazinolysis of 2-(methylsulfanyl)-6-oxo-1,6-dihydropyrimidine derivative 6 with hydrazine hydrate resulted in rearrangement affording diaminopyrimidine 10. IR spectrum of 10 showed absorption bands at 3429, 3283 corresponding to NH2, in addition to bands at 2204 and 1668 cm_1 for C,,N and C,O groups, respectively. While its 1H NMR spectrum showed two broad singlets at d =3.29 and 7.09 ppm corresponding to two NH2, and its Mass spectrum showed M+ at 261 (100%). Diaminopyrimidine derivative 10 seemed to be suitable for further heterocyclization

affording azolo, azino and triazepino ring joined to pyrimidine nucleus. Thus, diazotization of compound 10 provided tetrazolopyrimidine 11. IR spectrum of 11 showed absorption bands at 2227 and 1656 cm_1 corresponding to C,„N and C,O groups, respectively. Its 1H NMR spectrum showed a singlet at d =8.92 ppm for NH. 13C NMR spectrum of 11 showed signals at d = 83.86, 154.13, 154.48 ppm for pyrimidine carbons, 116.37 ppm for C,„N, 164 ppm for C,O and 128.86, 130.5, 132.81, 136.57 ppm for aromatic carbons.

Cyclocondensation of compound 10 with formic acid and phthalic anhydride yielded triazolopyrimidine derivatives 12 and 13, respectively. IR spectrum of 12 showed absorption

bands at 2223 and 1694 cm_1 corresponding to C,„N and C,O groups, respectively. Its 1H NMR spectrum showed two singlets at d = 8.64 and 9.36 for N,CH and NH, respectively.

13C NMR spectrum of 12 showed signals at d =83.32, 150.06, 155.16 ppm for pyrimidine carbons, 116.56 ppm for C,„N, 168.27 ppm for C,O, 128.48, 130.31, 133.16, 135.00 ppm for aromatic carbons and 136.07 ppm for AN,CA in the triazole ring. IR spectrum of 13 showed

absorption bands at 2221, 1741 and 1648 cm_1 corresponding to the C,„N, carboxylic C,O and amidic C,O groups, respectively. 1H NMR spectrum of 13 showed two singlets at d= 7.60 and 10.04 ppm for NH and COOH, respectively.

The new 3-Substituted-6-Methoxy-1H-pyrazolo[3,4-b]quinoline derivatives were prepared through reaction of 6- methoxy-1H-pyrazolo[3,4-b]quinolin-3-amine with acid anhydrides.

The yields obtained by this method were 60% or more. Structure confirmation of pyrazolo[3,4-b]quinoline derivatives 7–9 was performed based on their spectral data (IR, 1H NMR, and MS). The IR spectra of these compounds showed the characteristic C,O amide absorption bands of amide at the range of 1660–1670 cm_1 while NH stretching bands were observed in the range of 3260–3270 cm_1. 1H NMR spectra of these amides are found characterized by the presence of D2O exchangeable singlet of the NH proton at d 12.8–13.8 in addition to another singlet of three protons of methoxy group at 3.8 ppm. In the present study, chalcone 10 was prepared through the reaction of compound 4 with pmethoxyacetophenone in the presence of alcoholic NaOH. The reaction occurred by ratio (1:1) to form chalcone which crystallized from ethanol, the structure of compound 10 was established on the basis of its elemental and spectral data. The IR spectrum of compound 10 was characterized by a strong absorption band of C,O which appeared at low absorption value because of extended conjugation with the double bond. The synthetic pathway of the target compounds 7–9 through reaction of 6-Methoxy-1H-pyrazolo[3,4- b]quinoline-3-amine (6) with three different acid anhydrides. Structures of these amides were confirmed based on spectral data and elemental analyses. The IR spectra of these compounds showed the characteristic C,O stretching bands at the range of 1666–1669 cm_1. In

addition, the 1H NMR spectra of the same derivatives showed singlet signals corresponding to NH protons at d 12.8–13.8 ppm.

The synthetic pathway for compounds under consideration is illustrated. The present scaffold 3a is a part of synthesis of new chemical entities in form of antimicrobial agents. Compound 4-((2-chloroquinolin-3-yl)methylene)-2- phenyloxazol-5(4H)-one (2) is prepared in an excellent yield in one step by Perkin condensation of hippuric acid, 2-chloroquinoline- 3-carbaldehyde (1) and acetic anhydride in the presence of anhydrous sodium acetate. The condensation reaction is carried out in microwave irradiation at 300W intermittently at 30 s intervals for 3 min. In the final step, intermediate (2) is reacted with N-amino aryl carbo xamide in pyridine and refluxed for a specified time to furnish the final product N-(4-((2-chloroquinolin-3-yl)methylene)-5-oxo-2-phenyl- 4,5-dihydro-1H-imidazol-1-yl)(aryl)amides (3a–l), while the same product is obtained in microwave irradiation at 400 W intermittently at 30 s intervals for a specified time . Classical method for the synthesis of (2) and (3a–l) required 4 and 6–9 h, respectively, for the completion of reaction. But in the paper, we have received very good results from MWI method for the synthesis of (2) and (3a–l). The same compounds are synthesized in 3–6 min. These results clearly reveal the superiority of MWI method over the classical procedure.

The structures of all the synthesized intermediates and final compounds were established by various spectral techniques viz. 1H NMR, 13C NMR, mass spectrometry and elemental data. Intermediates 6a/6b were confirmed by the formation of broad peaks in the range of d 12.4–12.1 ppm in their 1H NMR spectra which corresponds to the –OH proton. In the 13C NMR spectrum, the AC,O carbon of –COOH groupin 6a and 6b resonates at d 163.5 and 162.8 ppm, respectively. The amide –NH in intermediates 7a/7b appears as a singlet at around d 8.47 ppm in the 1H NMR spectrum. Further, the four aliphatic protons of the cyclopropyl ring appear as a multiplet whereas the junction –CH of the cyclopropyl ring gives a multiplet due to coupling with the neighbour protons. In the 13C NMR spectra of 7a and 7b, the methylene carbon of the cyclopropyl ring appears at around d 6.2 ppm whereas the junction carbon resonates at around d 23.1 ppm. The formylation at position-3 of the indole ring was clearly established by the disappearance of an aromatic proton and the appearance of a new sharp singlet at around d 9.92 ppm (due to –CHO group) in the 1H NMR spectra of 8a and 8b. The presence of –CHO group was further confirmed by the appearance of a peak at around d 184.0 ppm in their 13C NMR spectra. Subsequent conversion of these formylated intermediates into different chalcones 9a–v was also confirmed by NMR spectral studies. The olefinic protons in these chalcones resonate as two distinct doublets with the coupling constant (J) value in the range of 15.4–15.8 Hz. Also, these alkenyl protons appear in the downfield region (d 7.9–7.5 ppm) of the spectrum. Both of these evidences

strongly indicate that the hydrogens of the olefin have attained trans (E) configuration. In addition, the absence of a proton peak corresponding to the aldehydic group further supports the formation of respective chalcones. The cyclization of these pre-final intermediates (9a–v) to their corresponding target compounds 10a–v was confirmed in the 1H NMR spectrum by the formation of a new singlet peak in the range of d 6.64–6.80 ppm owing to the presence of an amino group. Moreover, the complete disappearance of peaks due to olefinic protons also supports the proposed final structure of these molecules. The chemical structure of all the final molecules was further established by their 13C NMR spectra. Additionally, the mass spectra are also in analogy with the structural molecular mass giving M+H peak viz. in the positive mode. The complete analytical and spectral data of all the intermediates and title compounds are discussed in the experimental section and their physical data are presented.

**Conclusion**

The newly synthesized amides of 5- sulphosalicyclic acid have great potential as antibacterial agents, and therefore can be explore further for the development of new drugs for treatment of bacterial diseases.

In conclusion, we successfully expunged the cytotoxic effects of MP through the design of [I5, R8] MP. Such modifications seem not to alter the α-helical conformation, but rather enhance the antimicrobial activity without destroying eukaryotic cells.

Novel Schiff bases and 4-thiazolidinones of pyridine and benzothiazole derivatives were synthesized; the results of the antimicrobial activity of the synthesized compounds revealed that compounds containing –Cl, –NO2 group and furan nucleus are more active than the remaining compounds.

synthesis of 1,4-dihydropyridine was reported in one-pot condensation reaction of aldehydes, ammonium acetate, dimedone or ethyl acetoacetate in the presence of catalytic amounts of aminated multi-walled carbon nanotubes (0.001 g) under thermal condition. The antimicrobial activity against gram positive bacteria except 3-ethoxy carbonyl -4-(4-methyl) phenyl-2,7,7-trimethyl-5-oxo-1,4-dihydropyridine (6e) also 4g is effective against fungi in addition to gram positive bacteria.

Almost all of synthesized compounds gave moderate to low efficacy against B. Subtilis compounds which gave good activity with 72% and 76% relative to standard respectively. On the other hand, no activity was found against either P. Aeruginosa or C. albicans by any of the tested compounds.

Demands for new bio-active molecules in the field of healthcare, combined with the pressure to produce these substances expeditiously and in an environmentally benign fashion, pose significant challenges to the synthetic chemical community. We have successfully synthesized heterocyclic compounds by using green techniques, such as selective MW heating of neat reactants under solvent-free conditions. In general, improvements in rate and yield of reactions are observed in MWI.

The result of synthesised a new library of indole-3-pyrimidine hybrids consisting of an amide linkage at position-2 of the indole ring and a diversely substituted phenyl ring on the pyrimidine moiety. The structural identification of all the intermediates and target compounds was carried out by spectroscopic techniques namely 1H NMR, 13C NMR, ESI–MS and elemental analyses. The target molecules were investigated for their in vitro anti-proliferative and antimicrobial properties by MTT assay and disc diffusion methods respectively. The most susceptible cell lines comprised of HepG2 and MCF-7 with the majority of compounds showing higher inhibition against these cell lines. Compounds 10f, 10o and 10v inhibit significantly the growth of all three cancerous cells indicating their broad spectrum anti-tumour activity. On the other hand, in the case of anti-microbial studies, compounds 10b, 10c, 10g, 10h, 10m, 10n, 10r and 10s which contain a chloro or fluoro substitution (R2) at either position-3 or 4 of the phenyl ring displayed higher potency than the rest of the molecules. Moreover, low toxicity on the benign Vero cells and higher efficacy of the active molecules may provide a potential lead for the development of novel therapeutic agents in future.

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