doi.org/10.36721/PJPS.2020.33.2.REG.675-683.1

Synthesis and characterization of Schiff base of nicotinic hydrazide as antibacterial agent along with *in vivo* wound healing activities and atomic force microscopic study of bacterial cell wall affected by synthesized compound

Samiullah Burki[1], Zeba Gul Burki[2], Shazia Haider[3*], Mehjabeen[1] and Ijaz Ahmed[3]

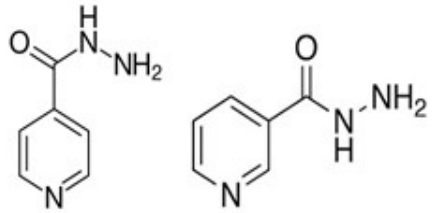
[1]Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University of arts, science and technology, Karachi, Pakistan
[2]HEJ research institute of chemistry, International Center for Chemical and Biological Sciences, Karachi University, Karachi Pakistan
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan

Abstract: The present work reports the synthesis of Schiff base series of nicotinic hydrazide (C-1-C-5) and it's antibacterial and wound healing evaluation. The synthetic molecules were characterized with different spectroscopic techniques and explored for their antibacterial potential. The objective of this work was to explore antimicrobial agent using two types of microorganisms, one Gram-positive (*S. aureus* ATCC 9144) and one Gram-negative (*E. coli* ATCC 10536). C-2, C-4 and C-5 potentially inhibit bacterial growth ($p<0.001$). Atomic force microscopy (AFM) imaging was obtained to get high-resolution images of the effect of treated drugs on the bacterial morphology. The images obtained also revealed the antibacterial effects of potent molecule. The magnified pictures captured under AFM suggest significantly damaged cell surface and disturbed morphology. The compounds were further analyzed for *in vivo* wound healing potential on mice. The compound C-2, C-4 and C-5 heal the wounds comparatively in less time duration as compared to control group ($p<0.001$). Compound C-1 and C-3 took more time to heal the wound as compare to compound C-2, C-4 and C-5. The re-epithelialization process of wound in animals group treated with potent compound was highly significant ($p<0.001$) and faster than control. Results of this study suggest that the compounds C-2, C-4 andC-5 possess pronounced antibacterial and wound healing potential and need to be further evaluated for mechanism of action.

Keywords: Nicotinic hydrazide, antibacterial, AFM, Schiff base, wound healing.

INTRODUCTION

Antibiotic discovery especially Schiff bases are a significant class of compounds exhibiting tremendous biological significance and have been vastly employed to treat infectious disease after the discovery of isoniazid and its derivatives. The emergence of antibiotics is defiantly a greater blessing of human history that has protected millions of people. Conversely, microorganisms develop resistance against the existing antibiotics. Nonetheless, current approach to overcome this issue is the alteration of existing antibiotics to fight against emerging resistant pathogens (Zaman *et al*., 2017). Among different approaches for the modification of existing antimicrobials to overcome bacterial resistance, Schiff base compounds hold an exclusive prestige in this field. Schiff base compound are synthesized when ketone or an aldehyde is treated with primary amines under suitable conditions. These Schiff-base organic compounds are largely used in various fields for a number of applications. The biological actives of Schiff-base compounds comprised of antimalarial, anti-proliferative, antibacterial, antifungal, anti-inflammatory, antipyretic, and antiviral activity (Przybylski *et al*., 2009 and Drabent *et al*., 2004). These compounds contain imine groups in their structure, which is a demanding functional group for various biological and pharmacological activities (Guo *et al*., 2007 and Xia *et al*., 2003). Hydrazide-hydrazone derivatives are documented for promising antimicrobial potential (Bayrak *et al*., 2009 and da Silva *et al*., 2011). Different derivatives of nicotinic acid hydrazine have been reported for significant antimicrobial activities (Narang *et al*., 2012 and Cacic *et al*., 2006).



Isostere structures of isoniazid and pyridine-3-carbohydrazide

Schiff bases are considered as promising antibacterial agents. Isoniazid (Pyridine -4-carbohydrazide) is an active anti-mycobacterium tuberculosis agent and very similar to nicotinic hydrazide (Pyridine-3-carbohydrazide). The derivative of isoniazid obtained via Schiff base reaction was more potent than its parent Isoniazid and less toxic (Hearn and Cynamon, 2004). On the other hand, pyridine-

**Corresponding author:* e-mail: samiullahburki83@yahoo.com

3-carbohydrazide is biologically active aromatic hydrazide and structural isomer of isoniazid bearing potent peroxidase-activated inhibitor of the POX activity of PGHS-2 (Ouellet *et al*., 2004 and Zhao *et al*., 2006). Considering the importance of isoniazid Schiff base derivatives This work has focused on the development of the easy one pot synthesis of Schiff bases of nicotinic acid hydrazide (Scheme 1) and assessment of their wound healing activity and antibacterial activity against human pathogenic bacteria.

MATERIALS AND METHODS

Experimental

The solvents used in current study were of Analytical grade. 4-hydroxy benzaldehyde, 2,6 - dichlorobenzaldehyde, 4-(dimethylamino) benzaldehyde, terepthalaldehyde and nicotinic acid hydrazide were purchased from Sigma- Aldrich, Germany through local supplier. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Polylysine (PLL) were procured from BDH, UK, and Merck, Germany, respectively. Infra-Red (IR) spectra were analyzed on a Jasco 302, FTIR spectrophotometer using KBr disc. Varian Mass spectrometer MAT 311A spectrometer, Varian Mass spectrometer MAT 312 used for Mass spectrometry. Nuclear magnetic resonance (^{1}HNMR) spectral analysis was carried out at Bruker AM 400 spectrophotometer.

General scheme for synthesis of compounds

In 50 mL round bottom flask were added HPLC grade ethanol 20 mL and 68.5 mg (0.5 mmol) of nicotinic acid hydrazine followed by addition of 1mL catalyst (glacial acetic acid) and dissolved. To the mixture one equivalent weight of aldehyde (1:1 mole ratio) was added and the reaction was refluxed at 80ºC for 12 hrs. TLC plates and iodine vapors are used to monitor the reaction. The reaction was stopped on completion, followed by cooling at room temperature. On cold water addition the precipitates obtained of required product. Precipitates were filtered and washed with water and ethanol. The pure product was dried in desiccators with the help of anhydrous calcium sulphate. Melting points of products were noted on melting point apparatus on Buchi 434.The required obtained products were in good yields ranging from 90-95 %.

Antibacterial Assay

Two ATCC strains of test microorganism were selected for antibacterial assay, in which one is from gram-positive bacteria, *S. aureus* ATCC 9144 and another one from gram-negative bacteria, *E. coli* ATCC 10536. Bacterial stock cultures were prepared in sterile Mueller Hinton broth, with final of 5×10^5 cfu/ml.

Tetrazolium microplate assay

A unique colorimetric method tetrazolium microplate assay was used to perform antibacterial activity and minimum inhibitory concentration determination(Piaru *et al*., 2012). The previously prepared culture of *S. aureus,* and *E. coli* was seeded in 96-well clear microtiter plate at 5×10^5cfu/ml in each well. The synthesized compounds 1-5 at concentrations of 160 to 5µg/mL were prepared by serial dilution in Muller Hinton broth. From these dilutions about 200µl of each concentration was added to the microtiter plate in triplicate and incubate at 37ºC for 18-24 hrs. About 50µl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide MTT having a concentration of 0.2mg/ml was added to each well of microtiter plate and the plate was subjected to reincubation of plate for 30 minutes at 37ºC. For this assay ciprofloxacin was used as standard. The bacterial growth inhibition was determined by taking absorbance of dye at 570nm as previously reported by (Sarkar *et al*., 2007). The IC-5_0 was calculated via formula:

$$IC_{50} = \left[\frac{\text{O.D. in control} - \text{O.D.of test}}{\text{O.D. in control}}\right]\times 100$$

Atomic force microscopic study (AFM) for morphological changes in bacterial cells

For the purpose of morphological change in bacterial cell i.e. *E. Coli S. and aureus,* the diluted culture of bacterial strains of density 10^5cfu were applied polylysine on mica slides 10µL. Briefly the slides were dried at ambient temperature. After drying the slides were subjected to AFM evaluation.

2D and 3D topography images drug untreated and drug treated gram-positive *S. aureus,* and gram-negative *E. coli* were obtained. The Pico View 1.2 imaging analysis software was used for image processing as discussed by (Allison *et al*., 2011)

Wound healing activity

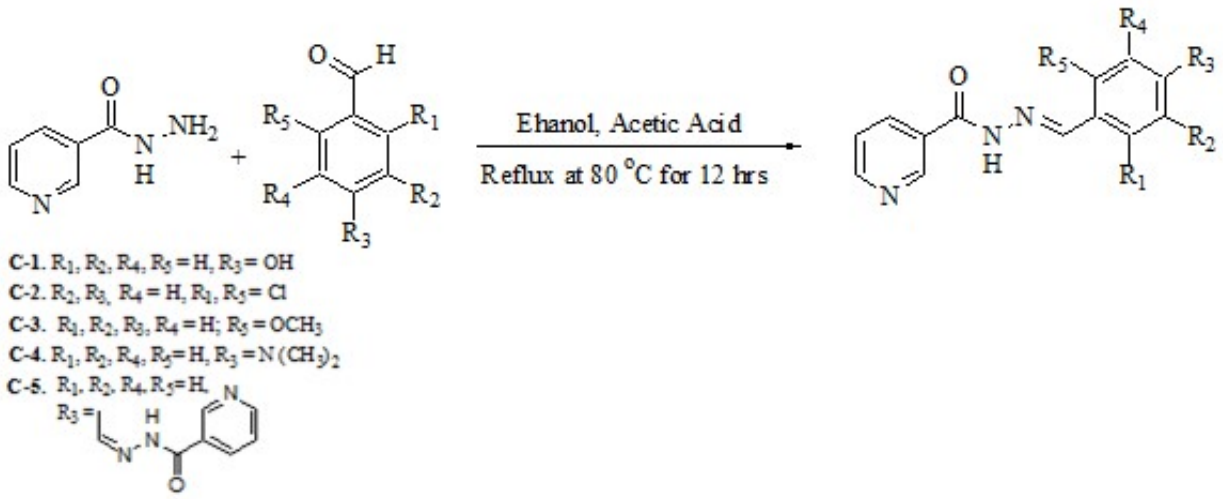
Animals

Swiss albino mice (20-25 g) were taken for *in vivo* pharmacological wound healing activity. Animals were kept under standard environmental and nutritional and conditions as reported by (Burki *et al*., 2018). Animal ethical committee clearance was obtained from FUUAST (Protocol # NH-MS-18A)

Excision wound model

Animals were divided for experiment into three groups. Six animals were kept in each group. Group I animals were given normal saline (control), group II animals were treated with povidone iodine ointment; 5.0% (standard), and group III (test compounds).

Prior excision the animals were anesthetized by administering 1 ml ketamine hydrochloride 10 mg/kg of intravenously. The dorsal skin of animal was cleared by shaving the fur of the animal and sterilized with 70% ethanol solution. The excision wound of 1 cm, 0.1 cm in width and depth was created by using surgical blades and scissors respectively. The whole wound was left open. The experiment was conducted under sterile condition.



Scheme 1: Synthetic approach towards Nicotin hydrazide Schiff bases (C-1-C-5)

Table 1: Minimum inhibitory concentration (MIC_{50}) of compound (C-1– C-5)

Microorganism *Compounds*	MIC_{50} concentration of compounds (µg/mL)					
	C-1	C-2	C-3	C-4	C-5	Cip
S. aureus	80^{**}	40^{***}	80^{**}	40^{***}	40^{***}	40^{***}
E. coli	120^{*}	120^{*}	120^{*}	80^{***}	80^{***}	40^{***}

Cip= ciprofloxacin, statistically *p<0.05, **p<0.01 and ***p<0.001

Table 2: Wound surface area (cm^2) in mice treated with compounds (1-5)

Group		% wound healing						Period of epithelialization (days)
		Days						
		1	4	7	10	14	17	
Group I		0.0	14.4±1.1	28.2±1.8	42.5±1.7	57.3±2.2	67.2±1.8	24.6±1.3
Group II		0.0	21.8±5.4	47.5±5.3	58.7±2.6	71±2.6	$84.5±3.3^{*}$	21.4±2.9
Group III	C-1	0.0	24.7±2.4	44.7±2.5	58.8±2.6	$72.2±1.8^{N}$	$81.7±2.2^{N}$	$23.8±1.6^{N}$
	C- 2	0.0	28.7±3.3	49.5±1.2	61.8±2.7	$77. 1±2.4^{\ddagger}$	$91.6±1.9^{\ddagger}$	$19.2±1.9^{\ddagger}$
	C -3	0.0	25.6±2.5	38.9±1.5	62.4±1.9	$78.1±1.4^{N}$	$83.6±1.6^{N}$	$20.6±2.5^{N}$
	C- 4	0.0	42.6±3.7	58.2±2.1	67.5±2.9	$86.4±1.5^{\ddagger}$	$94.8±2.3^{\ddagger}$	$17.5±2.7^{\ddagger}$
	C- 5	0.0	38.6±2.7	56.2±2.0	68.5±2.9	$82.4±3.5^{\ddagger}$	$97.8±1.3^{\ddagger}$	$16±1.7^{\ddagger}$

Values represented as mean ± SD of all groups on different days, group I= control, group II= (standard) 5.0% povidone iodine ointment, group III= C-1, C-2, C-3, C-4, C-5, Statistically $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ SD = Standard Deviation

After creation of wound, the animals in group I were treated with normal saline, animals in group II were treated by applying topically povidone iodine ointment (5 %), while group III animals (each sub group); were treated with compounds C-1-C-5, 15 mg/kg body weight (topically) for once daily for seventeen days.

The wound margin measurements were traced on 1st day and then on each 4th day till the last visit on 17st day with the help of transparent graph paper and a marker. The gradual reduction in wound area was monitored periodically. Wound closing and time required for epithelialization were studied. The epithelialization time was calculated as the time required for healing of wound as explained by (Krishna *et al*., 2017) with slight modification. Wound contraction was expressed as percentage of wound area that had healed. Using below formula:

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{Specific wound size}}{\text{Initial wound size}} \times 100$$

The time of re-epithelization was calculated by recording the days required for complete healing of wound leaving no scare of wound.

STATISTICAL ANALYSIS

For statistical investigation one-way ANOVA was performed at *p<0.05, **p<0.01 and ***p<0.001. All the

results are expressed as mean ± SD. GraphPad prism for graphical analysis having specifications; version 5.01 (La Jolla) Software Inc., was used.

RESULTS

Synthesis and structural studies of compounds (1-5)

Compound C-2, C-3 and C-5 were synthesized, while compound C-1, C-4were resynthesized from nicotinic acid hydrazide by condensing with various aldehydes. The reactions were carried out in ethanol and few drops of acetic acid. The analytical techniques like melting point, FTIR, MS, and ^{1}H NMR were used for confirmation of structures of synthesized compounds.

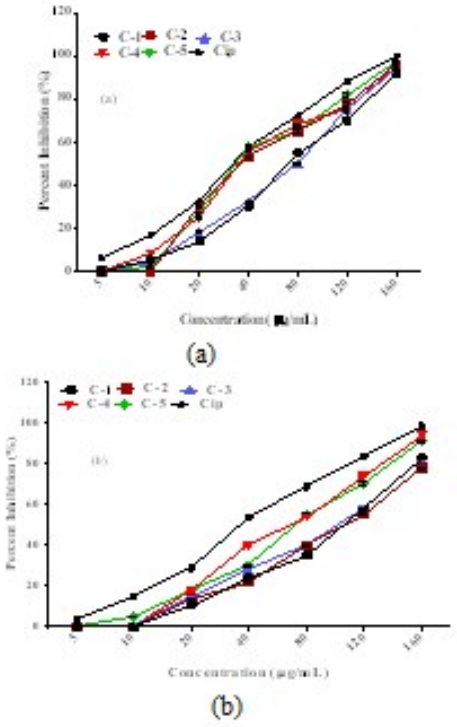


Fig. 1: Anti-bacterial activity of Schiff base compounds C-1-C-5 against (a) *S. aureus* and (b) *E. coli.* Results are presented as mean ±SD. The assay was performed in triplicate. Cip (ciprofloxacin)

Compound C-1:(Z)-N'-(4-hydroxybenzylidene)nicotinohydrazide

Yield: 220 mg, 91.28%, m.p 243.4-246.8ºC, FT-IR (KBr): 3442.9cm^{-1} (OH), 1650.1 cm^{-1} (C=O), 1604.0cm^{-1} (aromatic CH), 1515.6 cm^{-1} (-C=N-), EI-MS: mass 241.2 m/z and the value calculated was 241.09 m/z, ^{1}HNMR (400 MHz, MeOD) ***δ***: 6.82 (d, 2H, CH, J=8.4 Hz), 7.53 (t, 3H, CH, J= 9.6 Hz), 8.22 (d, 1H, CH, J= 8.0 Hz), 8.34 (s, 1H, -N=CH-), 8.73 (d, 1H, CH, J=4 Hz), 9.04 (s, 1H, CH), 9.95 (s, 1H, OH), 11.81 (s, 1H, O=CH) (fig. S1, S2).

Compound C-2

(Z)-N'-(2,6-dichlorobenzylidene) nicotinohydrazide

Yield: 275 mg, 93.85%, m.p 207.4-210.5ºC, FT-IR (KBr): 3189.1 cm^{-1}(NH), 1652.8 cm^{-1} (C=O), 1586.6 cm^{-1} (C=C aromatic), 1549.8 cm^{-1} (C=N-), 708.6 cm^{-1} (Cl), EI-MS: mass 293.1 m/z, and the value calculated was 293.01m/z, ^{1}HNMR (400 MHz, DMSO) ***δ***: 7.43 (d, 2H, CH, *J* = 8 Hz), 7.56 (d, 2H, CH, *J* = 8 Hz), 8.26 (d, 1H, CH, *J* = 7.6 Hz), 8.65 (s, 1H, CH), 8.76 (d, 1H, CH, *J* = 3.6 Hz), 9.07 (s, 1H, CH), 12.27 (s, 1H, NH) (fig. S3, S4).

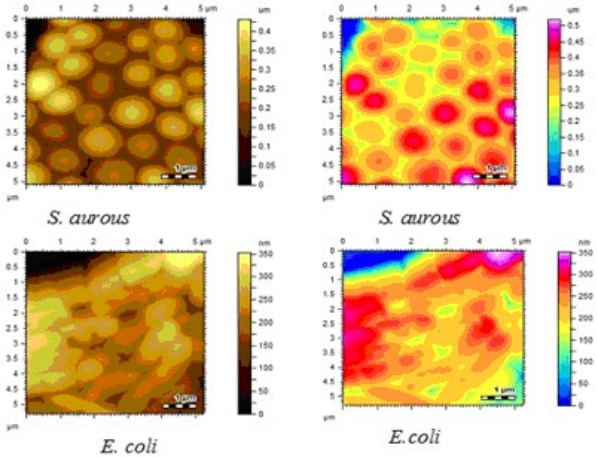


Fig. 2: AFM images of *S. aurous* and *E. coli* (untreated with compound). The rainbow colored images of *S. aurous* and *E. coli* (untreated compound)

Compound C-3

(Z)-N'-((E)-3-(2-methoxyphenyl)allylidene)nicotinohydrazide

Yield: 285 mg, 91.25 %, m.p 109. -111 ºC, FT-IR (KBr): 3220.3 cm^{-1}(NH), 1683.2 cm^{-1} (C=O), 1629.7 cm^{-1} (C=C aromatic), 1546.5 cm^{-1} (C=N-), EI-MS: detected mass 281.2 m/z and the value calculated was 281.1m/z, ^{1}HNMR (400 MHz, MeOD) ***δ***: 3.86 (s, 3H, CH_3), 7.19 (m, 4H), 7.31 (d, 1H, CH, *J* = 8 Hz), 7.55 (d, 1H, CH, *J* = 7.6 Hz), 8.21 (m, 2H, CH), 8.75 (d, 1H, CH, *J* = 3.6 Hz), 9.03 (s, 1H, CH), 11.83 (s, 1H, NH) (fig. S5, S6).

Compound C-4

(Z)-N'-(4-(dimethylamino)benzylidene)nicotinohydrazide:

Yield: 290 mg, 96.12%, m.p 136-138ºC, FT-IR (KBr): 3440.8 cm^{-1}(NH), 1661.4 cm^{-1} (C=O), 1602.1cm^{-1} (C=C aromatic), 1524.0 cm^{-1} (C=N-), EI-MS: mass 268.0 m/z and the value calculated was 268.1m/z, ^{1}HNMR (400 MHz, MeOD) ***δ***: 2.97 (s, 6H, $N(CH_3)_2$), 6.75 (d, 2H, CH, *J* = 8.8 Hz), 7.53 (m, 3H,), 8.22 (m, 2H, CH), 8.73 (m, 1H, CH), 9.02 (m, 1H, CH), 11.69 (s, 1H, NH) (fig. S7, S8).

Compound C-5

(N',N'''Z,N',N'''E)-N',N'''-(1,4-phenylenebis(methanylylidene))di(nicotinohydrazide):

Yield: 387 mg, 91.35 %, m.p 209-211 ºC, FT-IR (KBr): 3201.2 cm^{-1}(NH), 1655.3 cm^{-1} (C=O), 1522.3 cm^{-1} (C=C aromatic), 1503.6 cm^{-1} (C=N-), EI-MS: mass 372.1 m/z and the value calculated was 372.13 m/z, ^{1}HNMR (400 MHz, MeOD) ***δ***: 7.57 (m, 3H, CH), 7.84 (s, 3H, CH), 8.26 (d, 2H, CH, *J*=7.6 Hz), 8.47 (s, 2H, CH), 8.76 (d, 2H, CH, *J*=4 Hz), 9.07 (s, 2H, CH), 12.08 (s, 2H, NH) (fig. S9, S10).

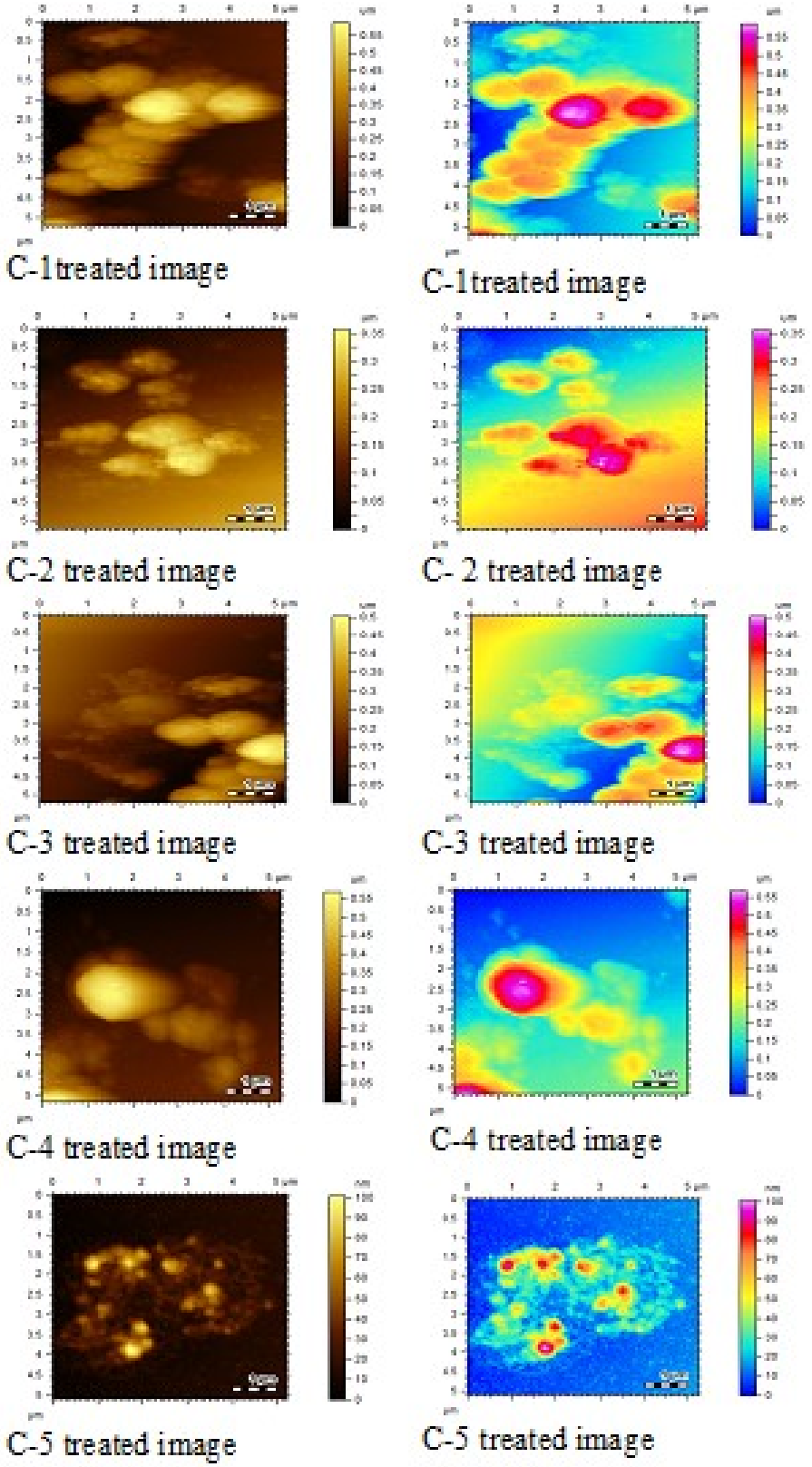


Fig. 3: AFM images of *S. aurous* (C-1-C-5 treated) The rainbow colored images of *S. aurous* treated with each compound

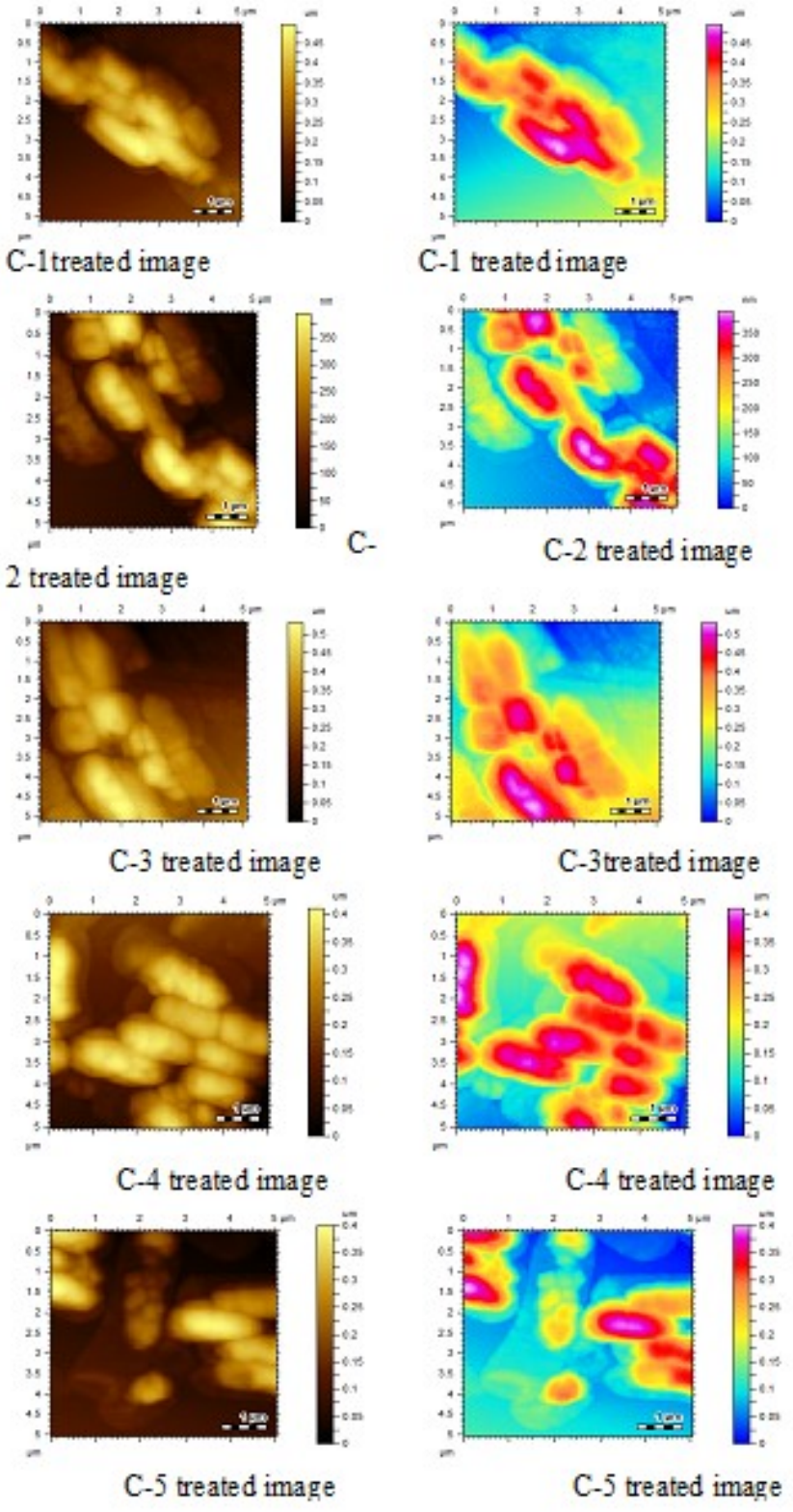


Fig. 4: AFM images of compound (C-1-C-5) treated *E. coli*. The rainbow colored images of *E. coli* treated with each compound

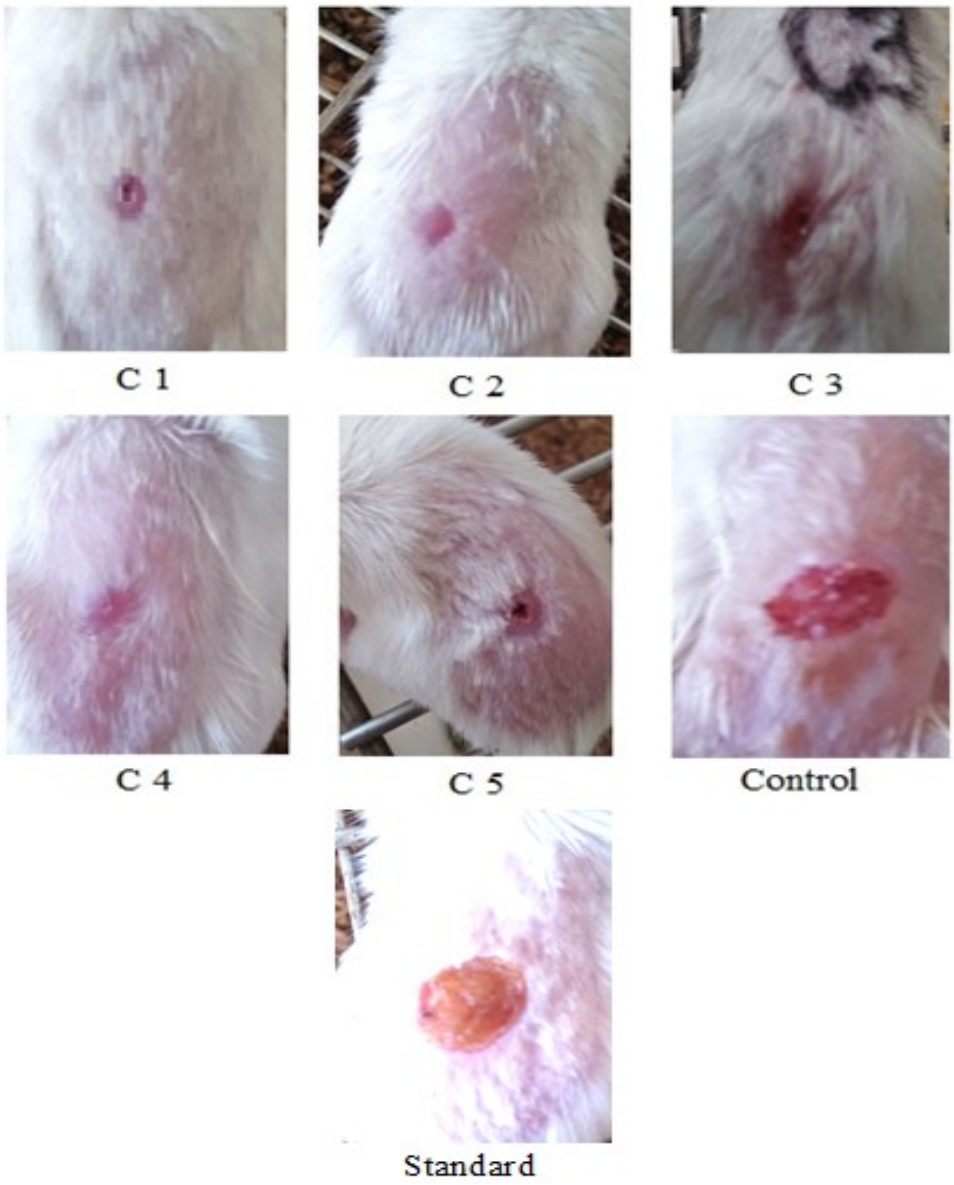


Fig. 5: Excision wound model in mice, Group III (each sub group) treated with compound C-1-C-5, 15mg/kg w/w body weight (topically). Standard= Povidone iodine ointment 5%, (topically) was used as a standard.

Antibacterial assay

The antibacterial activity was carried out through tetrazolium microplate assay technique for (*S. Aureus*, ATCC 9144) and (*E. Coli*, ATCC 10536). The percent inhibition of all synthesized compound against tasted pathogens were expressed in (fig. 1a and 1b). MIC_{50} of compound C-2, C-4, and C-5 against *S. aurous* was 40 µg/ml at ($p<0.001$), while C-1 and C-3 acquired $MIC\text{-}5_0$ at 80µg/ml ($p<0.01$) against *S. aurous*. Compound C-4 and C-5 achieved 50% inhibition against *E. coli* at 80 µg/ml ($p<0.001$), while C-1-C-3 accomplished this target at 120 µg/ml (table 1).

Furthermore, the compounds treated *S. aureus* ATCC 9144 and *E. coli* ATCC 10536 were observed under AFM to see the effect of compounds on bacterial cell wall and bacterial cell morphology. Untreated bacterial cells (control) (fig. 2) were in normal shape with smooth surface and there was no any sign of damage.

The compound treated *S. aureus* cells were magnified after scanning at 20 × 20µm as shown in (fig. 3). The captured images of bacterial cell in the fig. 3 shows damaged surface, particularly bacterial cells treated with compound C-2, C-4 and C-5. Image of *S. aureus* treated with compound C-2 also show significant cell damaged.

Compound C-4 and C-5 treated *E. coli* were remarkably damaged as shown in (fig. 4). The colored images are authentication of original images captured in AFM

trapped mood. The cell surfaces of *E. coli* and their morphology treated with compound C-1-C-3 were also rough and damaged. These results could be linked with the noticeable antimicrobial activity.

Wound healing effect of compounds

The animal skins of different groups were carefully examined daily and the cure rate of wound was recorded. No mortality was noticed during the study. The recovered wound area in all animals at the end of study is shown in (fig.5), the wound size was significantly reduced and the re-epithelialization can be clearly seen. On day 4, there was a significant improvement in the percent wound contraction in all treated animal groups when compared with control (table. 2). On day 17^{th} animals treated with compound C-2, C-4 and C-5 showed significant 91.6±1.9, 94.8±2.3, and 97.8±1.3% ($p<0.001$) wound healing, while in negative control (normal saline) and positive control (Povidone iodine ointment 5.0%) it was 67.2±1.8 and 84.5±3.3. Compound C-1 and C-3, 81.7±2.2 and 83.6±1.6 % ($p<0.01$) heal the wound. Period of epithelialization was also related to their respective wound healing rate. With compound C-2, C-4, and C-5 epithelization complete in 19.2±1.9, 17.5±2.7 and 16±1.7 days ($p<0.001$), while, compound C-1 and C-3 took 23.8 and 20.6 days ($p<0.01$).

DISCUSSION

The architecture of two smaller molecules in order to produce potentially new bioactive molecule is considered as highly successful for the effective treatment of infectious diseases. Due emergence of resistance by pathogens to the drugs available in the market provoke researchers to investigate new molecules of natural and synthetics origin (Tsemeugne *et al*., 2018). Among different synthetic molecule Schiff bases have remarkable medicinal characteristic in the field of pharmaceutical sciences (Naganagowda *et al*., 2014). Compound C-1 and C-4 has been previously synthesized by (Zafar *et al*., 2017) and reported for xanthine oxidase inhibitory activity. To the best of our knowledge no *in vitro* antibacterial, atomic force microscopic study of compounds treated bacterial cells and *in vivo* wound healing activity reported. The antimicrobial activity of these particular Schiff bases along with atomic force and wound healing study has been carried out for the first time. The antimicrobial activity of compound C-2, C-4 and C-5 was comparatively significant. The MIC-$5_0$of C-2, C-4 and C-5 achieved at 40µg/mL against*S. Aureus* while MIC-5_0 against *E. coli* was achieved at 80µg/mL. The antibacterial activity results were comparable with standard ciprofloxacin. The AFM images also showed remarkable damage of the cell wall of gram-positive *S. aureus* and gram-negative bacteria *E. coli* treated with compound C-2, C-4 and C-5. The images of the bacteria treated with compound C-2, C-4 and C-5 trapped in the rainbow mode showed damaged biofilm around gram positive *S. aureus* and gram negative *E. coli* bacteria. The wound healing activity performed on mice was also significant with compound C-2, C-4 and C-5 and the results were comparable with positive control Povidone iodine (5.0%). The epithelialization process by compound C2, C-4 and C-5 was comparatively completed in less time than C-1 and C-3. Regarding SAR, all the synthesized compounds have similar structure except changes at R3 position in aromatic ring. Presence of hydroxy and methoxy group decrease antimicrobial and healing effect while substitution of chlorine and nitrogen containing substituent increased the activity. In the future these compounds will be mechanistically studied for their bactericidal activity on bacterial cell wall and bacterial DNA.

CONCLUSION

In conclusion, the synthesized derivatives of nicotinic hydrazide were tested via*in vitro* antibacterial potential against *S. aureus* and *E. coli*. The antibacterial activity was further authenticated via observing the compound treated bacterial cells under atomic force microscope. The bacterial cells morphology demonstrates that the synthesized compounds efficiently damaged bacterial cell wall. The aforementioned activity was also conformed via *in vivo* excision wound healing model. The excision wound after surgery was treated with these synthesized Schiff base compounds. The results of wound healing showed improved cure rate and organized re-epithelialization. This study suggest that the synthesized Schiff base compounds possess pronounced antibacterial and wound healing effects and need to be further studied at molecular levels.

REFERENCES

Allison DP, Sullivan CJ, Mortensen NP, Retterer ST and Doktycz M (2011). Bacterial immobilization for imaging by atomic force microscopy. *J. Vis. Exp.*, **54**: e2880.

Bayrak H, Demirbas A, Demirbas N and Karaoglu S A (2009). Synthesis of some new 1, 2, 4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities. *Eur. J. Med. Chem.,* **44**(11): 4362-4366.

Burki S, Burki Z G, Mehjabeen, Shah Z A, Imran M and Khan M (2018). Phytochemical screening, antioxidant and in vivo neuropharmacological effect of *Monotheca buxifolia* (Falc.) barks extract. *Pak. J. Pharm. Sci.,* **31**(4): 1519-1528.

Cacic M, Trkovnik M, Cacicv F and Has-Schon E (2006). Synthesis and Antimicrobial Activity of Some Derivatives on the Basis (7-hydroxy-2-oxo-2H-chromen-4-yl)-acetic Acid Hydrazide. *Molecules.,* **11**(2): 134-147.

da Silva C M, da Silva D L, Modolo L V, Alves R B, De Resende M A, Martins C V and De Fatima A (2011). Schiff bases: A short review of their antimicrobial activities. *J. Adv. Res.,***2**(1): 1-8.

Drabent K, Białońska A and Ciunik Z (2004). New porous crystals of Cu (I) complexes with Schiff-base-containing triazole ligands. *Inorg. Chem. Commun.,***7**(2): 224-227.

Guo Z, Xing R, Liu S, Zhong Z, Ji, X, Wang L and Li P (2007). Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydr. Res.,* **342**(10): 1329-1332.

Hearn MJ and Cynamon MH (2004). Design and synthesis of antituberculars: Preparation and evaluation against Mycobacterium tuberculosis of an isoniazid Schiff base. *J. Antimicrob. Chemothery,* **53**(2): 185-191.

Krishna PS, Sudha S, Reddy KA, Al-dhabaan FA, Prakasham R and Charya MS (2017). Studies on wound healing potential of red pigment isolated from marine Bacterium vibrio sp. *Saudi. J. Biol. Sci*., **26**: 723-729.

Naganagowda G, Meijboom R and Petsom A (2014). Synthesis and antimicrobial activity of new schiff base compounds containing 2-hydroxy-4-pentadecyl-benzaldehyde moiety. *Dv. Chem.,* **2014**: 1-9.

Narang R, Narasimhan B, Sharma S, Sriram D, Yogeeswari P, De Clercq E, Pannecouque C and Balzarini J (2012). Synthesis, antimycobacterial, antiviral, antimicrobial activities and QSAR studies of nicotinic acid benzylidene hydrazide derivatives. *J. Med. Chem.,* **21**: 1557-1576.

Ouellet M, Aitken S M, English A M and Percival M D (2004). Aromatic hydroxamic acids and hydrazides as inhibitors of the peroxidase activity of prostaglandin H2 synthase-2. *Arch. Biochem. Biophys.,* **431**: 107-118.

Piaru SP, Mahmud R and Perumal S (2012). Determination of antibacterial activity of essential oil of *Myristica fragrans* Houtt. using tetrazolium microplate assay and its cytotoxic activity against vero cell line. *Int. J. Pharmacol.,* **8**(6): 572-576.

Przybylski P, Huczynski A, Pyta K, Brzezinski B and Bartl F (2009). Biological properties of Schiff bases and azo derivatives of phenols. *Curr. Org. Chem.,* **13**(2): 124-148.

Sarkar A, Parikh N, Hearn SA, Fuller MT, Tazuke S I and Schulz C (2007). Antagonistic roles of Rac and Rho in organizing the germ cell microenvironment. *Curr. Biol.,***17**(14): 1253-1258.

Tsemeugne J, Sopbue fondjo E, Tamokou JDD, Rohand T, Ngongang A D, Kuiate J R and Sondengam B L (2018). Synthesis, characterization and antimicrobial activity of a novel trisazo dye from 3-amino-4H-thieno [3, 4-c][1] benzopyran-4-one. *Int. J. Med. Chem.,* 2014: 1-9.

Xia Y, Yang P, Sun Y, Wu Y, Mayers B, Gates B, Yin Y, Kim F and Yan H (2003). One dimensional nanostructures: Synthesis, characterization and applications. *Adv. Mater.,* **15**(5): 353-389.

Zafar H, Hayat M, Saied S, Khan M, Salar U, Malik R, Choudhary MI and Khan KM (2017). Xanthine oxidase inhibitory activity of nicotino/isonicotinohydrazides: A systematic approach from *in vitro*, in silico to *in vivo* studies. *Bioorganic & Medicinal Chemistry,* **25**(8): 2351-2371.

Zaman S B, Hussain M A, Nye R, Mehta V, Mamun K T and Hossain N (2017). A review on antibiotic resistance: Alarm bells are ringing. *Cureus.,* **9**(6): e1403.

Zhao X, Yu H, Yu S, Wang F, Sacchettini JC and Magliozzo RS (2006). Hydrogen peroxide-mediated isoniazid activation catalyzed by mycobacterium tuberculosis catalase peroxidase (KatG) and its S315T Mutant. *Biochemistry,* **45**(13): 4131-4140.