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Synthesis, Anti-*Staphylococcal* Evaluation, and Hepatic Histopathological Safety of a Novel Thiazolo[3,2-a]benzimidazole-4-Aminoantipyrine Hybrid for Topical Dermatitis Therapy

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Abstract: The clinical utility of topical antibiotics for *Staphylococcus aureus*-induced dermatitis is often hampered by a critical translational gap: the frequent discrepancy between potent in vitro bactericidal activity and the unforeseen systemic toxicities that occur following dermal absorption. In order to overcome this, a novel thiazolo[3,2-a]benzimidazole-4-aminoantipyrine hybrid, compound **I** ([2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene][1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one) was synthesized in good yield via the reaction of the substrate TBIO with 4-Aminoantipyrine and investigated thoroughly, and most importantly, its preclinical hepatic safety. The FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra were used for structural confirmation. Antibacterial assays against *S. aureus* showed a potent inhibitory effect comparable to the reference standard, streptomycin. However, because the liver is the primary sentinel for xenobiotic metabolism and the main site of drug- induced injury after systemic absorption. Choosing a detailed histopathological examination of albino mice liver tissue as the ultimate endpoint for safety assessment. Histological analysis of liver cytoarchitecture was performed in detail in albino mice, with emphasis on the maintenance of hepatocyte cords, sinusoidal openness, nuclear shape, and absence of inflammatory infiltrates after dermal application. Interestingly, liver sections of compound **I**-treated animals showed completely preserved parenchymal architecture without any evidence of steatosis, hydropic degeneration, necrosis, or granulomatous lesions, as confirmed by a high acute LD_{50} and negligible in vitro cytotoxicity. The histological confirmation of this non-hepatotoxic profile, coupled with its strong anti-*staphylococcal* efficacy, indisputably shows the local therapeutic effect of compound **I** without jeopardizing hepatic integrity, making it a highly promising and safe lead candidate for the topical treatment of *staphylococcal* dermatitis.

Keywords: benzimidazole, hepatic histopathology, LD_{50}, *Staphylococcus aureus* dermatitis, topical antibiotic.

INTRODUCTION

THE rising incidence of *Staphylococcus aureus* as the pathogen in superficial skin and soft tissue infections, especially recurrent dermatitis, highlights an urgent and unmet clinical need for new topical therapeutic approaches.[1,2] Systemic antibiotherapy is the standard of care for disseminated disease, but its long-term use is increasingly limited by the appearance of multidrug-resistant strains, e.g., MRSA, and a variety of off-target toxicities, most notably hepatotoxicity and nephrotoxicity.[3,4] Topical delivery is a rational alternative for localized skin diseases, in theory limiting high drug concentrations to the epidermis and dermis.[5] However, the design of a successful topical antibiotic is a delicate balancing act. The molecule must be strongly bactericidal to *S. aureus*, but it must also have an excellent safety margin if absorbed through the skin.[6]

The molecular hybridization of privileged heterocycles has become a powerful paradigm in the discovery of novel antimicrobial scaffolds. The [1,3]thiazolo[3,2-a]benzimidazole scaffold is known for its broad spectrum of bioactivity, including its strong antibacterial activity, which is attributed to its interaction with bacterial DNA gyrase.[7,8] Similarly, the 4-aminoantipyrine (pyrazolone) moiety is a

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versatile pharmacophore with documented antimicrobial and anti-inflammatory activities and often acts as an effective chelating agent for essential bacterial metalloenzymes.[9–11] These independent virtues notwithstanding, the synthesis and preclinical validation of their direct hybrid through a hydrazinylidene linker is unexplored. Here, we report a new synthetic compound (I) prepared by the condensation of TBIO with 4-aminoantipyrine, with the assumption that this structural integration may result in a synergistic antibacterial efficacy.

The critical scientific bottleneck, however, that this investigation aims to address is not merely the production of yet another in vitro-active heterocycle, but rather the stringent confirmation of its hepatic safety. The liver is the principal site for first-pass metabolism and detoxification and is highly susceptible to drug-induced injury.[12,13] Even topically applied xenobiotics can reach sufficient systemic concentrations to cause subclinical hepatocellular damage. This is usually not detected in standard in vitro cytotoxicity assays, but is evident at the histological level.[14] Routine serum biochemical markers (e.g., ALT, AST) may be transient and lack spatial anatomical context to identify early-stage injury, such as perivenular hepatocyte ballooning, cholestatic plugging, or incipient fibrotic changes.[15,16] Therefore, a thorough histomorphometric assessment of hepatic tissue is not just an ancillary toxicological endpoint, but the ultimate gold standard for determining the non-toxic translational viability of any new dermal therapeutic. The integrity of the hepatic cytoarchitecture (regular hepatocyte plates, normochromic nuclei, clear sinusoidal spaces, and no inflammatory foci) is a clear indication of systemic safety, regardless of local potency.[17,18]

Hence, the present study (Figure 1) was conceptualized with an integrated dual objective: (1) to achieve the efficient synthesis and complete spectroscopic characterization of compound I and benchmark its in vitro anti-staphylococcal activity against the standard drug streptomycin, and (2) to move beyond traditional screening paradigms through rigorous, histology-centric safety workup of I. This includes in vitro cytotoxicity profiling, acute LD_{50} determination, and most importantly, a comprehensive qualitative and quantitative histopathological interrogation of albino mouse liver tissues following topical application. In addition, the potent antibacterial action of compound I is unequivocally paralleled by complete preservation of normal hepatic parenchyma, providing strong pre-clinical evidence to advance the compound as a safe, effective, and clinically viable topical antibiotic for the treatment of *S. aureus*-associated dermatitis.

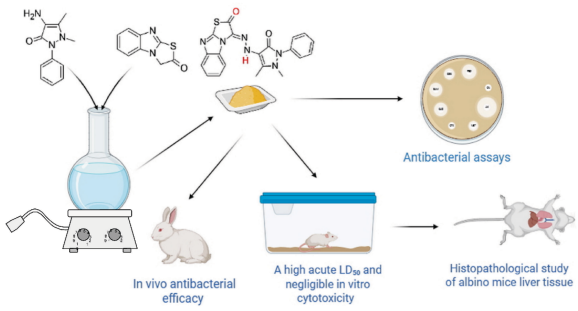


Figure 1. Schematic illustration of the synthesis and biological evaluation of the proposed antibiotic (I). The proposed synthesized antibiotic (I) was evaluated for in vitro antibacterial activity, in vivo antibacterial effectiveness, acute toxicity (LD_{50}), in vitro cytotoxicity, and histopathological impacts on the liver tissue of albino mice.

EXPERIMENTAL

Materials and Methods

All chemicals used for the preparation of the novel compound were purchased from Sigma-Aldrich and used without further purification. The ^{1}H NMR spectra were obtained on a Bruker AM-300 spectrometer (300 MHz and 100.6 MHz for ^{13}C NMR) using DMSO-d_6 as solvent and tetramethylsilane as the internal reference. Melting points were determined using a Stuart digital SMP30. The mass spectrum was recorded on a Shimadzu GCMSQP 1000 EX. Progress and purity were monitored with TLC using *n*-hexane-ethyl acetate (9 : 1) as the eluent, and the spots were visualized under UV light. Elemental analysis for CHNS/O was performed on a Thermo Scientific Flash*Smart* Elemental Analyzer. Infrared spectrum was registered with a SHIMADZU FT-IR 8400S spectrometer over the coverage of 4000–600 cm^{-1}. In vivo- in vitro experiments were conducted at the Iraqi Center for Genetics and Cancer Research. The histological study was examined under a LABOMED optical microscope. Albino rabbits and mice were housed in environmentally controlled animal facilities. All testing processes were completed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, under the sponsorship, supervision, and permission of the Iraqi Center for Genetics and Cancer Research. Animals were daily observed systematically to ensure the optimal health and availability of food and water during the study period.

The Compound [2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene][1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (I)

The compound [1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (TBIO) was prepared according to reference,[19] which was exactly the same as the data mentioned in the reference.

Method for the synthesis of compound (**I**): A solution of 4-Aminoantipyrine (0.01 mol, 2.02 g) was diazotized with HCl / H_2O in a ratio of 1.05 : 1.0, and 0.01 mol of $NaNO_2$ was added and stirred vigorously at 0–5 °C for 5 min. This was gradually added to a solution (0.01 mol, 1.9 g) of TBIO in 25 mL of Pyridine, and the mixture was stirred for 30 min at the same temperature. The reaction mixture is stirred for 5 h, then stored in a refrigerator for 10 h. The cold mixture was added to water, and the precipitate was filtered and repeatedly rinsed with water; the precipitate was recrystallized using ethanol. Yield 89 %, melting point 272–274 °C. IR ν, cm^{-1}: 3171 (NH), 3024 (CH aromatic), 2943 (CH aliphatic), 1710 (C=O thiazol), 1698 (C=O pyrazol), 1608 (C=N); ^{1}H NMR spectrum (DMSO-d_6): δ, ppm: 11.49 (s, 1H, NH), 8.23–7.37 (m, 9H, Ar), 4.58 (s, 3H, CH_3–N), 1.35 (s, 3H, CH_3). The ^{13}C NMR spectrum shows the carbon signals δ_C, ppm: 183.9 (thiazole ring carbonyl C=O), 168.3 (pyrazole ring carbonyl C=O), 150.5 (carbon of the C=N bond in the hydrazone moiety), 141.8, 140.8, 134.0, 132.7, 131.5, 129.2, 128.6, 126.7, 125.1, 123.4, 121.3, 116.0, 113.6, 111.1, 34.0 (methyl carbon bonded to nitrogen N–CH_3), 13.1 (methyl carbon bonded to a pyrazole ring C–CH_3). Found, %: C 59.34; H 3.96; N 20.73; O 7.87; S 7.89. $C_{20}H_{16}N_6O_2S$. Calculated, %: C 59.39; H 3.99; N 20.78; O 7.91; S 7.93. Mass spectrum (ESI), m / z (I_{rel}, %): 404 (95).

Assessment of Antibacterial Activity *in vitro*

The Bacteriology Laboratory of Al Zahraa Hospital was used as a source for pathogenic bacteria in this work, Al-Kut, Wasit, Iraq. The synthesized drug was investigated for sensitivity against the *Staphylococcus Aureus* strain using the agar diffusion method at 62.5, 125, 250, and 500 μg cm^{-3} concentrations, with Dimethyl sulfoxide as a (–) control and Streptomycin as a (+) control.[20] Evenly inoculated colonies of Gram-positive bacteria were inoculated in each Petri plate on the Nutrient Agar Medium. The antibacterial activity of the investigated chemical was determined by the disc diffusion method. A disc (5 mm) of filter paper was impregnated using 50 μL of the test solutions. During a 24-hour period, it was incubated at a temperature of 37 ± 2 °C, after which the inhibition zone was measured.

Determination of the LD_{50}

The synthesized [2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene][1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (**I**) was tested for acute toxicity (median lethal dose, LD_{50})[21] by using 8-week-old, medium-weight 20-gram, female albino mice. The laboratory albino mice were divided into 5 groups, with 6 in each group, to get the ideal outcome. Albino mice were injected intraperitoneally with 0.5 mL of synthesized (**I**) in doses of 500, 600, 700, 800, 900, and 1000 mg mL^{-1} b.wt. The solvent DMSO was assessed by administration of 0.5 mL intraperitoneal (i.p.) injection to a cohort of albino mice. Acute toxicity, survival, and any observable behavioral abnormalities were continuously observed for 24 h after intraperitoneal administration of compound **I** to mice. No mortality was recorded in the control and 500 and 600 mg kg^{-1} body weight treated groups, indicating that these doses were well tolerated under the experimental conditions. The first mortality was observed at 700 mg kg^{-1} body weight, with 1 of 6 mice (16.7 %) dying within the 24-h observation period. The doses of 800 and 900 mg kg^{-1} body weight caused mortality of 3 out of 6 mice (50.0 %) in each group. The highest dose (1000 mg kg^{-1} body weight) caused the greatest mortality of 4 out of 6 mice (66.7 %) within 24 h. The LD_{50} value of compound (**I**) was evaluated in female albino mice from the above experiment.

Histological Impact

The livers of the group 600 mg kg^{-1} body weight, and the control group were studied microscopically (400×) for the histological state of the liver and the physiological consequences of the proposed antibiotic (**I**). After administration of the proposed antibiotic (**I**), the mice in all groups were sacrificed and scrutinized. Histological sections were prepared in accordance with the technique.[22] Histological changes were studied by dissection of the excised liver tissues from both groups. The livers were placed (90 mL NaCl 0.9 % and 10 mL formalin), and then exposed to various doses of ethanol (50 %, 70 %, 80 %, 90 % and 100 %) for a few hours. The liver was treated with xylene and then fixed with paraffin for twenty minutes at R.T. The liver dried in air at 37 °C after being sliced into 5mm pieces, counterstained using glass slides and Meyer's albumin. The pigments were employed to make slips for microscopic examination, with hematoxylin being applied foremost for ten minutes. For less than one minute, the eosin was used like a counterstain. The slides were rinsed in distilled water and then in a sequence of different concentrations of alcohol (from 70 % to 100 %). Then the slides were examined under an optical microscope.

In vivo Assessment of Antibiotic efficacy

The Iraqi Center for Genetics and Cancer Research was the place of this portion of the study, following the method described in reference.[23] The bacterial culture was prepared by inoculating the saturated liquid medium of tryptic soy broth (Nine milliliters) with one colony over 18 hours. The resultant culture was centrifuged at 3000 rpm for 10 min. The benefit of 0.9 % sodium chloride as a suspending factor results in the bacterial debris being transformed into a suspension in a volume identical to the dumped supernatant. The inoculums were diluted 1 : 10 in normal saline solution. Bacteria were grown for 24 h, and a predetermined volume

of cell suspension containing an inoculum of 30 × 10^{10} CFU was used to contaminate the albino rabbits. A pathological examination was carried out on four male albino rabbits with an average body weight of 1.5 kg. The male albino rabbits were divided into 2 groups: the treated and the control groups. With 0.5 mL, all the albino rabbits were subcutaneously inoculated with the inoculum (*S. aureus*), were relatively injured, and set in separate places with availability of food and water during the 3 days. This was continuously observed and monitored (behavior and clinical signs). The wounds (in all groups) after three days showed signs of contamination with *S. aureus* bacteria through the spread of Ulcers and abscesses. Also reported were apathy, moderate fever, and decreased appetite.

RESULTS AND DISCUSSION

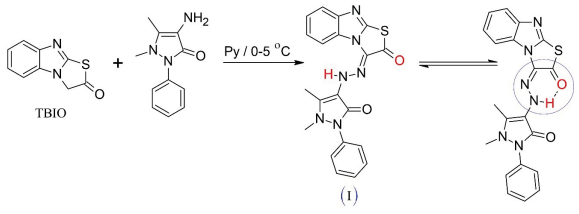
The proposed synthesized antibiotic (**I**) was prepared by reaction between diazonium salts of 4-aminoantipyrine and substrate TBIO to give a good yield 89 % (Scheme 1). Using pyridine as a solvent and a catalyst raises the purity and yield by reducing the use of strong bases and acids, such as sodium hydroxide and hydrochloric acid. The formation of the target compound in an ionic form is thus reduced.

The structure of the proposed antibiotic (**I**) was evaluated by using different spectroscopic techniques. The spectroscopic data of the synthesized compound (**I**) yield adequate results, guaranteeing the success of the reaction and the requisite configurations. IR spectrum of compound **I** exhibits an absorption at 3171 cm^{-1} regarding the NH stretching group. The weak absorption band at 3171 cm^{-1} of the NH group in FT-IR is due to intramolecular HB, forming between the O atom of the C=O group and the NH group of hydrazinylidene[19] (Scheme 1). On the other hand, absorption bands at 1710 cm^{-1} and 1697 cm^{-1} are related to (C=O thiazole) and (C=O pyrazole), respectively. The ^{1}H-NMR of compound **I** gives signals at δ, ppm: 11.49 (singlet, 1H, due to NH),[24] 4.58 (singlet, 3H, due to CH_3-N), and 1.35 (singlet, 3H, due to CH_3). The mass spectrum and elemental analysis of compound (**I**) provide the molecular ion and fragments that correspond to the molecular weight and molecular formula of compound **I**. In addition, they measure the percentage of C, H, N, S, and O in compound **I**. The ^{13}C NMR spectrum shows all the unique carbon atoms in compound **I**. The ^{13}C NMR spectrum shows the carbon signals δ_C, ppm: 183.9 (thiazole ring carbonyl C=O), 168.3 (pyrazole ring carbonyl C=O), 150.5 (carbon of the C=N bond in the hydrazone moiety), 141.8, 140.8 (ring carbons bonded to N or S atoms), 134.0, 132.7, 131.5, 129.2, 128.6, and 126.7 (carbons of a benzene ring fused to a benzimidazole), 125.1, 123.4, 121.3, 116.0, 113.6, and 111.1 (carbons of a phenyl ring bonded to nitrogen), 34.0 (methyl carbon bonded to nitrogen N–CH_3), 13.1 (methyl carbon bonded to a pyrazole ring C–CH_3).

The proposed structure of compound I incorporates a hydrazinylidene moiety, whose assignment was evaluated using complementary spectroscopic and analytical data. The FT-IR spectrum exhibits an absorption band at 3171 cm^{-1}, assigned to N–H stretching, while the ^{1}H NMR spectrum displays a singlet at δ 11.49 ppm attributable to the N–H proton. The relatively weak N–H stretching band may be associated with intramolecular hydrogen bonding between the N–H group and the carbonyl oxygen, potentially leading to a six-membered ring arrangement. Similar hydrazinylidene-containing heterocyclic structures and related hydrogen-bonding interactions have been reported previously.[19,25,26] These statements reinforce the proposed structural assignment but are not conclusive on their own. Accordingly, the structure of compound I was assessed by considering the NMR data together with the FT-IR spectrum, mass spectrometry, and CHN elemental analysis.

Biology

The antibacterial activity of the proposed antibiotic (**I**) against *Staphylococcus aureus* was determined by measuring the inhibition zone diameters (μg cm^{-3} = μg mL^{-1}) at a four-concentration serial dilution (62.5, 125, 250, and



Scheme 1. Synthesized compound (I) and intramolecular HB.

Figure 2. The inhibition concentration μg mL^{-1} values of compound (I) against the Gram-positive *Staphylococcus aureus* strain.

500 μg mL^{-1}). In addition, streptomycin was used for the positive control (see Figure 2 and S5). The compound (**I**) has different groups that can increase the antibacterial activity, such as six groups of secondary and tertiary amines and two carbonyl groups of thioester and amide.[27]

Increase in activity dose-dependently: The zones of inhibition for compound **I** increased gradually with increasing concentrations, giving values of 2 mm (at 62.5 μg mL^{-1}), 5 mm (at 125 μg mL^{-1}), 8.5 mm (at 250 μg mL^{-1}), and 16 mm (at 500 μg mL^{-1}). This is an 8-fold increase in zone diameter (from 2 to 16 mm) with an 8-fold increase in concentration (from 62.5 to 500 μg mL^{-1}), indicating a directly proportional dose-response in this range. Comparison with streptomycin (9 mm): Compound **I** (2 mm) had only 22.2 % of the activity of streptomycin at 62.5 μg mL^{-1}. Its efficacy was 55.6 % of that of streptomycin at 125 μg mL^{-1} (5 mm). At 250 μg mL^{-1} (8.5 mm), the activity was almost similar, being 94.4 % of the standard inhibition (only 0.5 mm less than streptomycin). At 500 μg mL^{-1} (16 mm), compound **I** was 1.78 times (77.8 % higher) than the reference drug. It was significantly higher than streptomycin. Potency threshold: The concentration of compound **I** required to obtain the exact 9 mm activity of streptomycin was interpolated to be ~267 μg mL^{-1}, a value much higher than the effective dose of the standard antibiotic; however, the higher activity of the compound at 500 μg mL^{-1} (16 mm) demonstrates a strong intrinsic antibacterial potential at higher loading.

LD_{50} is the median lethal dose. It is the quantity of the dose (liquid or solid) that has been demonstrated to be lethal (kill) fifty percent of animals, such as rats or mice, within a limited time. The LD_{50} depends on the route of administration: intraperitoneal, subcutaneous, oral, and intravenous. The route of administration can also influence absorption and availability in the systemic circulation. For example, intraperitoneal administration may provide rapid absorption into the systemic circulation due to the extensive peritoneal surface area and rich blood and lymphatic supply. Dermal absorption is governed by the skin barrier, and, to a certain degree, systemic absorption is determined by the compound's physicochemical properties and formulation, dose, dose frequency, surface area, skin integrity, and duration of exposure. Systemic exposure after topical application can therefore differ by a small percentage from exposure after intraperitoneal administration. Accordingly, the median lethal dose (LD_{50}) value of 550 mg kg^{-1} - estimated in this study -reflects acute toxicity following intraperitoneal administration and may differ to some extent from the acute toxicity observed following dermal administration. The cytotoxicity (LD_{50}) of the proposed antibiotic (**I**) was also assessed in this study. Compound (I) was injected intraperitoneally (i.p.) in mice at various doses of 500, 600, 700, 800, 900, and 1000 mg kg^{-1} body weight. Mortality was recorded in a separate group,

Table 1. Karber's arithmetic method for determining the median lethal dose (LD_{50}) $^{n=6}$.

Group	mg kg^{-1}	Number of dead	$a^{(a)}$	$b^{(b)}$	Probity ($a \times b$)
1	control	0	-	-	-
2	500	0	0	0	0
3	600	0	100	0	0
4	700	1	100	0.5	50
5	800	3	100	2	200
6	900	3	100	3	300
7	1000	4	100	3.5	350

LD_{50} = Least lethal dose - sum ($a \times b$) / n.

(a) a = dose difference between the two groups.

(b) b = mortality in the 2nd group + 1st group ÷ 2. The LD_{50} = 700 – (900 ÷ 6) = 550 mg kg^{-1} body weight (i.p.)

and the LD_{50} of compound (**I**) in female albino mice was found to be 550 mg kg^{-1} body weight (i.p.).[28,29] Karber's arithmetic method was used to analyze the median lethal dose Table 1.

The acute toxicity of the test chemical was determined by Karber's mathematical method for the determination of the median lethal dose (LD_{50}) using cumulative mortality at increasing dose levels. Mortality data are presented in Table 1. No mortality was observed in the control group and in subjects administered 500 and 600 mg kg^{-1}, indicating the good tolerance of these concentrations in the experimental condition. The toxic effects showed a dose-dependent pattern beginning at 700 mg kg^{-1}, with 1 / 6 animals (16.7 %) deceased. An increase in the dose increased the death rate. Administration of 800 and 900 mg kg^{-1} caused the death of 3 / 6 animals (50 %), and 1000 mg kg^{-1} increased the rate of mortality to 4 / 6 animals (66.7 %). The progressive increase in mortality with increasing dose clearly shows the dose-response relationship and thus lends credibility to the toxicity assessment.

The calculated LD_{50} indicates that the test substance has a moderate level of acute toxicity under the conditions of this study. Also, the continuous increase in death rate with increasing dose corroborates that toxic effects are concentration dependent and that levels below the LD_{50} determined have much lower acute lethality. These results give an important basis for safe dose limits in future pharmacological or toxicological investigations.

Albino mice were used for the acute toxicity (LD_{50}) test by intraperitoneal injection of the proposed antibiotic (I) and for histopathological study of the livers. The in vivo topical efficacy of the proposed antibiotic **I** in the *Staphylococcus aureus* skin-infection model was performed in rabbits. We selected rabbits due to their larger skin area, which is perfect for setting up and controlling the infection sites and topical treatment. We also chose this model because

of a previously published experimental protocol.[19] Future studies might suffice with one animal species.

The Histological Impact

The liver was removed from mice that escaped death, preserved with formalin, and then all the ingredients given in the experimental section were used. To analyze the histological state of the liver and the physiological consequences of the proposed antibiotic (**I**), the examination of the slides of group three (Figure 3) and group one (Figure 4) illustrates a nourishing liver form without any pathological alterations, showing an increase in Kupffer cells, orderly arrangement of hepatic cells, and a well-defined central vein.[30]

No histopathological changes were detected in the sections examined. Histopathological examination of liver sections from mice treated intraperitoneally with compound **I** (600 mg kg^{-1} body weight) showed preservation of the normal hepatic architecture similar to the solvent-treated control group (Figure 4). Hepatocytes maintained their typical polygonal shape and were organized in well-defined hepatic cords with mainly central nuclei. The hepatic sinusoids were patent, and the vascular formations were morphologically normal. Histopathological examination of the treated group showed no significant changes, including hepatocellular degeneration, necrosis, inflammatory cell infiltration, vascular congestion, hemorrhage, or disruption of hepatic architecture. These results suggest that administration of compound **I** at 600 mg kg^{-1} body weight did not exert any observable acute hepatotoxic effects under the conditions of the present study.

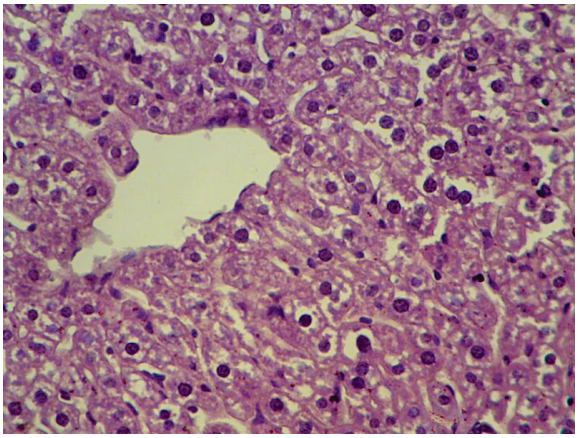


Figure 3. Liver cross-section representing normal form in the group (600 mg kg^{-1} body weight) for the proposed antibiotic (**I**).

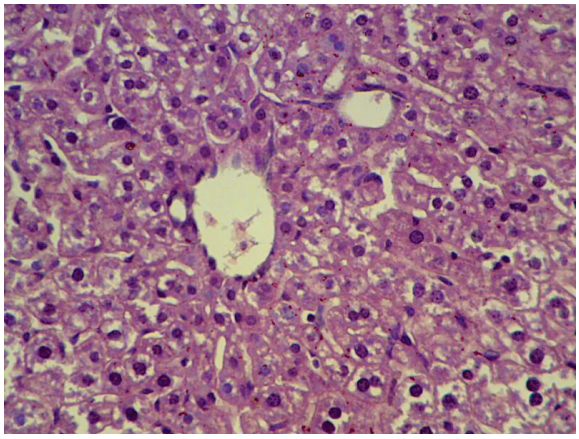


Figure 4. Liver cross-section representing the expected form in group one.

In vivo Antibacterial Efficacy

Staphylococcus aureus strains may yield a wide range of diseases and are thought to be the major agent of skin abscesses and suppurative dermatitis.[31] *In-vivo* antibacterial efficacy was evaluated using *S. aureus* strains (pathogenic bacteria). The male albino rabbits in these pathological tests were split into two groups: treatment and control. Infection of the two groups was performed with the given volume of suspended cells at a dosage of 30×10^{10} CFU. The injuries of the treatment group were treated with an ointment treatment approach by applying compound (I) (250 mg kg^{-1} body weight) with Vaseline to the wounds thrice daily. The injured area began to recover gradually over 5 days and completely healed 10 days later. Clinical symptoms improved, and the animal's appetite increased. However, the control group showed persistent infection in the infected area. This proves the efficacy of compound (**I**) as an antibacterial agent against *S. aureus* strains.

CONCLUSION

This study describes the synthesis, characterization, and histological properties of a new compound (I). The results revealed that compound (I), bearing six groups of secondary and tertiary amines and two carbonyl groups of thioester and amide, possessed excellent antibacterial activity in comparison to streptomycin. Further, the proposed antibiotic has non-toxic properties with an LD_{50} value of 550 mg kg^{-1} body weight (i.p.). The liver of albino mice with compound (**I**) does not show any histological effects, which is a positive aspect from the therapeutic point of view. In vivo study on the antibacterial activity of compound (**I**) showed a significant efficacy on *S. aureus* strains. The preparation of compound (**I**) is easy and straightforward. The yields are good. The in vivo toxicity is acceptable, and the antibacterial activity is good. Compound (I) is a candidate for a topical antibiotic agent.

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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca4431.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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