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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL HYDANTOIN DERIVATIVES

[1]Yashraj Joshi, [2]Mehta Parulben D [3] Dr. Kaushelendra Mishra, [4]Dr. Govind Nayak

[1]Research Scholar, [2] Director & Professor, [3] Professor, [4]Professor
[1]Pharmaceutical Chemistry,
[1]Lakshmi Narain College of Pharmacy, Bhopal, India

Abstract : New compounds were synthesized containing novel hydantoin derivatives and tested for their antimicrobial activity against gram positive and gram negative strains. The scheme was adopted for the synthesis of hydantoin derivatives was Bucherer-Bergs method. In this method, firstly benzoin was prepared by the pinacol-pinacolone rearrangement reaction of benzaldehyde with thiamine which was then reacted with concentrated HNO_3 to form benzil and then it was further reacted with urea. Total five compounds were synthesized and characterization was done using various physical and spectral techniques such as Thin Layer Chromatography, Melting point ,Solubility determination, UV spectroscopy and FTIR spectroscopy and ^{1}HNMR spectroscopy. All the synthesized compounds were biologically evaluated for in-vitro antimicrobial activity against gram positive and gram negative bacteria by well diffusion method. The bacterias chosen was *Bacillus subtilis* for gram positive and *Proteus vulgaris* for gram-negative. The antibacterial assay was performed for all the synthesized compounds at different concentrations and compared with standard with single concentration ranging 100µg/ml against microorganism. The antibacterial activity of hydantoin derivatives showed the significant zone of inhibition against, *Bacillus subtilis* and *Proteus vulgaris*. Thus from the study it is concluded that these new hydration derivatives could serve as effective antimicrobial agents.

IndexTerms **– hydantoin, benzaldehyde , antimicrobial activity, antibacterial activity.**

INTRODUCTION

Hydantoin was discovered in 1861 Baeyer, who isolated it as one of the reduction products of allantoin during the course of his now classic study of uric acid. A hydantoin (IUPAC "imidazolidine-2,4-dione") is a 5-membered ring with two nitrogens in the ring. There are two carbonyls in the ring, one of them between the two nitrogens. The hydantoin moiety can be found widely in alkaloids isolated from marine organisms (and to a lesser extent bacteria) and many hydantoin containing alkaloids have been shown to have interesting biological profiles for the treatment of various disorders. The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens. During the last few decades, it has been possible to combat the bacterial and fungal infections through major improvement in early detection techniques, and newly developed antibiotics. Nevertheless, many of the currently used antimicrobials often lead to toxicity and undesirable side effects, or drug-drug interactions. The problem of antibiotic resistance has become particularly alarming due to the emergence of multi-drug resistant strains exhibiting simultaneous resistance to two or more classes of antibiotics.

Despite the many antimicrobial and chemotherapeutic agents are available in market, the emergence of old and new antibiotic resistant by bacterial species in the last decade lead to a substantial need for the discovery of new classes of antimicrobial compounds.[1] In recent years, hydantoin derivatives have become a subject of interest due to their antibacterial, antifungal, and antimycobacterial activities.[2] Here, we present results on a new series of hydantoin derivatives being synthesized and evaluated for antimicrobial activity.

NEED OF THE STUDY

Infectious diseases are still a major cause of deaths, especially in developing countries, as new infectious diseases arise and an increasing number of multi-drug resistant strains of microbial pathogens emerge. Nowadays antibiotic resistance has become a serious public health problem. As a result of the rapid development of resistance to the existing portfolio of antimicrobial drugs, there is an increasing needto design new antibacterial and antifungal agents with better activity profiles and lower toxicity[3].

Many antimicrobial compounds used for the prevention or treatment of infections have been rendered less effective through evolved bacterial drug resistance.[3] This has engendered an urgent need for new antimicrobial compounds which display both broad-spectrum activity and reduced potential for development of antibiotic resistance. The discovery and development of efficient antibacterial and antifungal medicines with novel mechanisms of action have thus become critical goals for infectious disease research programmers due to the major medical problem of bacterial and fungal resistance and the rapid rate at which it develops.[4,5] The antimicrobial agents available now have various drawbacks such as toxicity, drug resistance to microbes, and narrow spectrum of activity. The research on the development of new antimicrobial agents has mainly focused on two aspects like increasing potent bacterial antigen drugs and the appearance of new bacteria pathogen. The main aspect of synthesizing effective drugs is their structural characteristic and the rate of activity. Initially, there has been consideration of a heterocyclic compound as a parent compound to synthesize the effective antibacterial drug. The assessment of those vast antimicrobial literature data admits that heterocyclic compound has played a vital role in the clinical field. Therefore, in the field of pharmacology, heterocycles are very popular for their unique controlling properties within a drug, such as a solubility, lipophilicity, and polarity, and are also being investigated several times for the discovery of desired active drugs. The fascinating biological significance of antibacterial pathogens has led to the urgency for drug discovery and the synthesis of new antimicrobial compounds. Hence the design of new compounds to deal with these problems has become one of the most challenging targets in antibacterial and antifungal research today.[6]

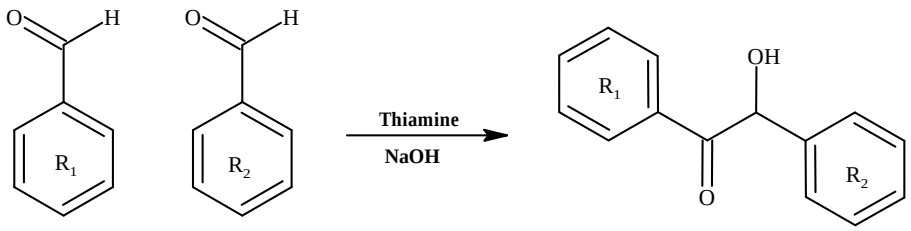
RESEARCH ENVISAGED

In the clinical world, consideration of retardation of pathogenic bacteria towards the available antibiotic was becoming a major worldwide problem as many bacterial pathogens have already established resistance against them. Therefore, there could be the appearance of increasing in the mortality rate for gram-positive and/or gram-negative bacterial diseases. It is mainly due to the over-growth in population and extreme modernization for comfort. Hence, the rapid development of microbial resistance leads to discovering a new effective antimicrobial drug agent, which can reduce the bacterial mortality rate. To set up a revolutionary change in the failure of synthesizing selective antibacterial drugs, researchers have been endeavored by a lot of eminent expertise from last few decades. To achieve a new effective scaffold for an efficient fight against bacterial viruses is required. The research on the development of new antimicrobial agents has mainly focused on two aspects like increasing potent bacterial antigen drugs and the appearance of new bacteria pathogen. The main aspect of synthesizing effective drugs is their structural characteristic and the rate of activity. Initially, there has been consideration of a heterocyclic compound as a parent compound to synthesize the effective antibacterial drug. The assessment of those vast antimicrobial literature data admits that heterocyclic compound has played a vital role in the clinical field. Therefore, in the field of pharmacology, heterocycles are very popular for their unique controlling properties within a drug, such as a solubility, lipophilicity, and polarity, and are also being investigated several times for the discovery of desired active drugs. The fascinating biological significance of antibacterial pathogens has led to the urgency for drug discovery and the synthesis of new antimicrobial compounds. In recent years, hydantoin derivatives have become a subject of interest due to their antibacterial, antifungal, and antimycobacterial activities. The development of hydantoins, the derivatives of 2,4- imidazolidinedione, for antibacterial applications has been taking place for a long time[7,8]. The mechanism of action of hydantoin derivatives is complex and not well understood, possibly due toa combination of various modes including damage to bacterial DNA, binding to bacterial ribosomesto inhibit synthesis of critical bacterial enzymes, and so on.
This study was designed to develop some novel hydantoin derivatives for the purpose of screening its antimicrobial activity against some gram positive and gram negative bacteria.

RESEARCH METHODOLOGY

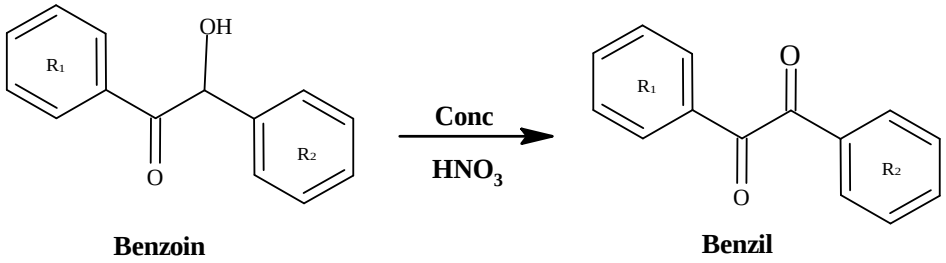
Materials And Methods: Chemicals used in the synthesis of title compound described were purchased from SD fine chem limited 71-A, M I G Colony, Indore, Madhya Pradesh. All the other chemical and distilled water were used of laboratory grade. The melting point of all the synthesized compounds was carried out via Thiel's tube method and was uncorrected. For structural characterization of synthesized compound, the IR spectra will recorded in Perkin Spectrum BX spectrophotometer. The confirmation of reaction at every step were observe by TLC, using silica gel plates (silica gel 60 F254) in a developing solvent system of n-hexane:ethyl acetate (9:1) and the spots were visualized with the help of UV chamber and iodine vapors or sulfuric acid (30%v/v) and Rf value were determined. 1H NMR of the synthesized compound showing best antimicrobial activity was performed by the Fourier Transform Nuclear Magnetic Resonance spectroscopy, Model AVNACENEO500 Ascend Bruker BioSpin International AG[58].

Step 01) Preparation of benzoin from benzaldehyde: In a 100 ml round-bottomed flask, ethanol (20 ml 95%), benzaldehyde (4.7 ml, 0.047 mol) and a solution of thiamine (1.5 g) in water were placed and refluxed for 3 hrs. Then, the contents of the flask were cooled in an ice bath. Benzoin was precipitated as a solid material and collected over a Buchner funnel. It was washed several times withcold distilled water to remove the excess thiamine. Pure benzoin was obtained as a white crystalline material.



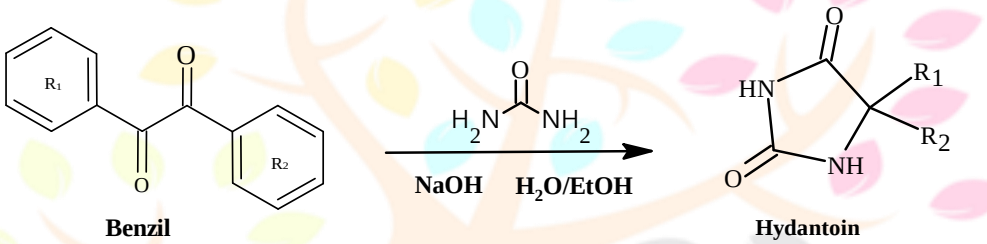
2 moles of Benzaldehyde **Benzoin**

Step 02) Preparation of benzil from benzoin: Benzoin (5g, 0.235 mol) was placed in a 100 ml Erlenmeyer flask and concentrated nitric acid (25 ml) was added into it in a fume cupboard. The mixture was heated on a hot plate with occasional shaking until all the red-colored nitrogen oxide gas was evolved (NO). The mixture was transferred to another 200 ml Erlenmeyer flask which contained 100 ml distilled water and stirred vigorously until the oil solidified as a yellow crystalline material. It was filtered over a Buchner funnel and washed with a liberal quantity of cold water until all the excess HNO_3 was removed.



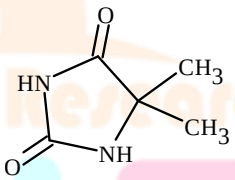
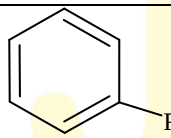
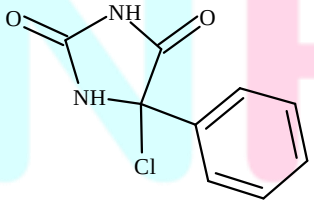
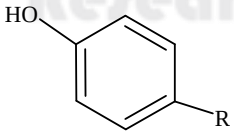
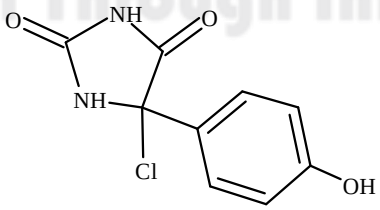
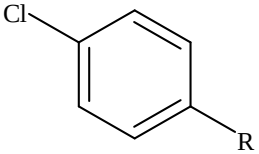
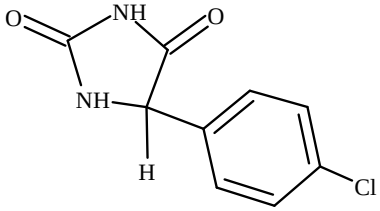
Benzoin **Benzil**

Step 03) Preparation of phenytoin: In a 100 ml round-bottomed flask which contained 20 ml absolute ethanol and equipped with a reflux condenser, benzil (5 g, 0.023 mol), urea (5 g, 0.083 mol) and sodium hydroxide pellets (5 g, 0.125 mol) were placed and refluxed for 8 h. Then, the mixture was poured in an ice bath which resulted in the formation of a solid material which was filtered over a Buchner funnel. To the filtrate, concentrated sulphuric acid was added until the pH became 5.5-6.0 and a white solid material was obtained. It was collected over a Buchner funnel and a white crystalline material was obtained.



Benzil **Hydantoin**

Table 01. Proposed structure of hydantoin derivatives.

S. No.	R_1	R_2	Proposed Structure	Final Hydantoin Derivatives IUPAC Name
1	R-CH_3	R-CH_3		5,5-dimethyl imidazolidine-2,4-dione
2	R-Cl			5-chloro-5-phenyl imidazolidine-2,4-dione
3	R-Cl			5-chloro-5-(4-hydroxy phenyl) imidazolidine-2,4-dione
4	R-H			5-(4-chlorophenyl) imidazolidine- 2,4-dione

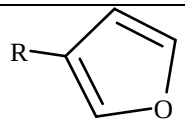
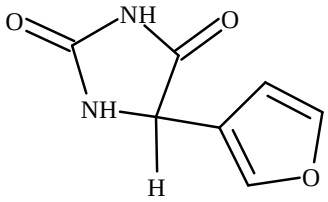
5	R-H			**5-(furyl) imidazolidine-2,4-dione**

Table 02. Physicochemical data of the compounds.

Compound Code	Yield (%)	Melting Point	R_f value
H1	54%	225-230°C	0.52
H2	43%	325-330°C	0.73
H3	28%	440-445°C	0.62
H4	35%	315-320°C	0.56
H5	25%	280-285°C	0.51

H1: 5,5-dimethyl imidazolidine-2,4-dione
UV-Visible : λ_{max} is found to be 303.45nm.

FT-IR (KBr cm^{-1}): 3436.64 cm^{-1} (N-H stretching), 3058.23 cm^{-1} (N–H stretching), 1700.96 cm^{-1} (C=O stretching), 1267.25 cm^{-1} (C–N stretching), 1458.78 cm^{-1} (C-H bending), 2870.34 cm^{-1} (H-C=O: C-H stretching).

H2: 5-chloro-5-phenyl imidazolidine-2,4-dione
UV-Visible : λ_{max} is found to be 230.64 nm.

FT-IR (KBr cm^{-1}): 3392.34 cm^{-1} (N-H stretching), 2938.87 cm^{-1} (N–H stretching), 1609.73 cm^{-1} (C=O stretching), 1283.18 cm^{-1} (C–N stretching), 1448.00 cm^{-1} (C-H bending), 2370.08 cm^{-1} (H-C=O: C-H stretching), 1517.46 cm^{-1}(C-C stretching), 625.90 cm^{-1} (C-Cl stretching).

H3: 5-chloro-5-(4-hydroxy phenyl) imidazolidine-2,4-dione
UV-Visible : λ_{max} is found to be 295.78 nm.

FT-IR (KBr cm^{-1}): 3457.71 cm^{-1} (N-H stretching), 2918.27 cm^{-1} (C–H stretching), 3754.29 cm^{-1} (O-H stretching), 1296.66 cm^{-1} (C–N stretching), 1703.69 cm^{-1} (C=O stretching), 1458.47 cm^{-1} (C-C stretching), 547.77 cm^{-1} (C-Cl stretching).

H4: 5-(4-chlorophenyl) imidazolidine- 2,4-dione
UV-Visible : λ_{max} is found to be 251.37 nm.

FT-IR (KBr cm^{-1}): 3476.05 cm^{-1} (N-H stretching), 2925.35 cm^{-1} (C–H stretching), 3652.11 cm^{-1} (O-H stretching), 1262.29 cm^{-1} (C–N stretching), 1734.10 cm^{-1} (C=O stretching), 1460.11 cm^{-1} (C-C stretching), 729.64 cm^{-1} (C-Cl stretching).

^{1}H NMR (500MHz, $CDCL_3$): δ 7.59 (s, 1H of imidazolidine), 7.51 (s, 1H of amine group), 7.16 (s, 1H of amine group), 6.8 (s, 1H of phenol), 4.62 (s, 3H of chlorophenyl).

H5: 5-(furyl) imidazolidine-2,4-dione
UV-Visible : λ_{max} is found to be 273.57 nm.

FT-IR (KBr cm^{-1}): 3392.56 cm^{-1} (N-H stretching), 3231.28 cm^{-1} (N–H stretching), 2900.97 cm^{-1} (C-H stretching), 1298.15 cm^{-1} (C–N stretching), 1635.69 cm^{-1} (C=O stretching), 1534.23 cm^{-1} (C-C stretching), 108.72 cm^{-1} (C-O stretching).

BIOLOGICAL EVALUATION

Antibacterial Activity:
The antimicrobial assay of all the synthesized compound was performed by Well Diffusion method. To evaluate the effect of synthesized compound against both gram+ve and gram-ve organism strains representing of each taken. These are *Bacillus Subtilis* and *Proteus Vulgaris.* Nutrient media used for bacterial strains, stock culture of the microbial strains were prepared from original lyophilized strains using standard method. The nutrient agar media was prepared separately by standard method, and inoculum of 1 gram+ve and 1 gram -ve was prepared, test organism were incubated in 10 ml Nutrient broth. Then, 10 mg of standard (Ofloxacin) was taken with 1 ml solvent (distilled water) to make 10mg/10ml solution. The bacterial suspension was standardized to 108 CFU/ml of bacteria and kept into the shaker. Then, 50µl of the inoculum from the broth (containing 108 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface. Four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different concentration (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) of sample and another plate well was filled with 50µl of standard drug respectively. It was allowed to

diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37ºC. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well and compared to that of conventional compound. All the compounds showed comparable activities as that of Standard Ofloxacin (mm) against *Proteus Vulgaris.* H4 [5-(3chlorophenyl)imidazolidine-2,4-dione] showed best ZOI (Zone of Inhibition) of 32.5mm and 34.8mm in diameter at 100 µg/ml concentration against gram positive bacteria (*Bacillus Subtilis*) and gram negative bacteria (*Proteus Vulgaris*) which is found to be more prominent to that of standard _____(gram +ve) _____ mm and Ofloxacin (gram -ve) _____mm.

Antimicrobial activity of all compounds against *Baccilus subtilis*

Compound code	Different concentrations			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
H1	6mm	6.2mm	6.6mm	8.0mm
H2	6.1mm	6.2mm	6.7mm	7.5mm
H3	6.6mm	6.9mm	7.5mm	8.3mm
H4	7.6mm	7.9mm	10.5mm	12.2mm
H5	6.4mm	6.5mm	7.2mm	7.7mm

Table 03: Compound code, Diameter in mm Inhibition.

Antimicrobial activity of all compounds against *Proteus vulgaris*

Compound Code	Different concentrations			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
H1	6mm	6.2mm	6.6mm	7mm
H2	6.4mm	6.5mm	7.2mm	8.4mm
H3	7mm	8mm	10.2mm	12.5mm
H4	7.7mm	9.4mm	10.8mm	12.9mm
H5	7mm	8mm	10.2mm	11mm

Table 04: Compound code, Diameter in mm Inhibition.

IV. RESULTS AND DISCUSSION

The present study was aimed to synthesized, and characterized some novel Hydantoin derivatives as an anti-microbial agent. The scheme was adopted for the synthesis of hydantoin derivatives is Bucherrer- Berg reaction. Total five compounds were synthesized and percentage yield was calculated. Characterization was done using various physical and spectral techniques such as Thin Layer Chromatography, Melting point, Solubility determination, UV-Visible Spectroscopy and Fourier Transform Infrared Spectroscopy. Thin Layer Chromatography of all the synthesized compounds was performed on silica gel plate and n-hexane: ethyl acetate was used as mobile phase, R_f values of all the synthesized compounds were calculated. The compounds were synthesized successfully and their structure were confirmed using FT-IR and ^{1}HNMR. In FT-IR spectra of compounds, the characteristic peak of N-H stretching, C=O stretching, C–N stretching, C–H stretching, O-H stretching, C-C stretching and C-Cl stretching were observed at 3476.05 cm^{-1}, 1734.10 cm^{-1}, 2925.35 cm^{-1}, 1262.29 cm^{-1}, 3652.11 cm^{-1}, 1460.11 cm^{-1}, 729.64 cm^{-1} respectively confirmed the structure of title compounds. Compounds (H1-H5) were screened for antibacterial activity against one-gram positive bacteria *Bacillus Subtilis* and one gram negative bacteria *Proteus Vulgaris.* The antibacterial activity of all derivatives showed the significant zone of inhibition against *Bacillus Subtilis*, and *Proteus Vulgaris*. Thus from the study it is concluded that these new derivatives could serve as effective antimicrobial agents, where H4 [5-(4-chlorophenyl) imidazolidine-2,4-dione] have shown their antimicrobial activity less then 12mm against microbial strain. The activity of H4 was found to be good activity.

CONCLUSION

The present study was to synthesized, and characterized some novel Hydantoin derivatives as an anti-microbial agents. Among all the hydration derivatives synthesized, compound H4 showed greater zone of inhibition of 12.2 mm and 12.9 mm against *Baccilus subtilis* and *Proteus vulgaris*. From this study, it is concluded that hydantoin derivatives could serve as effective antimicrobial agent and further studies can be performed to evaluate the efficacy and safety of these derivatives.

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REFERENCES

[1] Inca, S. Z,; Selma, S.; Semra, C.; Kevser, E. Bioorg. Med. Chem. 2006, 14, 8582.

[2] Woo, G. L. Y.; Mittelman, M. W.; Santerre, J. P. Synthesis and characterization of a novel biodegradable antimicrobial polymer. Biomaterials 2000, 21, (12), 1235- 1246.

[3] Brown, M. L.; Aldrich, H. C.; Gauthier, J. J. Appl. Environ. Microbiol. 1995, 61, 187–193;

[4] Dever, L. A., & Dermody, T. S. (1991). Mechanisms of bacterial resistance to antibiotics. *Archives of internal medicine*, *151*(5), 886-895.

[5] Spratt, B. G. (1994). Resistance to antibiotics mediated by target alterations. *Science*, *264*(5157), 388-393.

[6] Russell, A. D. (2004), Types of antibiotics and synthetic antimicrobial agents. *Hugo and Russell's Pharmaceutical Microbiology*, 152-18

[7] Zilberberg, M. D., Shorr, A. F., Micek, S. T., Vazquez-Guillamet, C., & Kollef, M. H. (2014). Multi-drug resistance, inappropriate initial antibiotic therapy and mortality in Gram-negative severe sepsis and septic shock: a retrospective cohort study. *Critical care*, *18*(6), 1-13.

[8] Morar, M., & Wright, G. D. (2010). The genomic enzymology of antibiotic resistance. *Annual review of genetics*, *44*, 25-51.

[9] Biltz, H. (1908). Über die Konstitution der Einwirkungsprodukte von substituierten Harnstoffen auf Benzil und über einige neue Methoden zur Darstellung der 5.5 Diphenyl hydantoine. *Berichte der deutschen chemischen Gesellschaft*, *41*(1), 1379- 1393.

[10] Raj, R., Mehra, V., Gut, J., Rosenthal, P. J., Wicht, K. J., Egan, T. J., ... & Kumar, V. (2014). Discovery of highly selective 7-chloroquinoline-thiohydantoins with potent antimalarial activity. *European Journal of Medicinal Chemistry*, *84*, 425-432.

[11] Kalchayanand, N., Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Guerini, M. N., Shackelford, S. D., ... & Koohmaraie, M. (2009). Effectiveness of 1, 3-Dibromo-5, 5 Dimethylhydantoin on Reduction of Escherichia coli O157: H7–and Salmonella-Inoculated Fresh Meat. *Journal of food protection*, *72*(1), 151-156.

[12] Rao, Z., Zhang, X., & Baeyens, W. R. (2002). Chemiluminescence flow injection analysis of 1, 3-dichloro-5, 5-dimethylhydantoin in swimming pool water. *Talanta*, *57*(5), 993-998.

[13] Erşen, D., Ülger, M., Mangelinckx, S., Gemili, M., Şahin, E., & Nural, Y. (2017). Synthesis and anti (myco) bacterial activity of novel 5, 5-diphenylpyrrolidine N-aroylthiourea derivatives and a functionalized hexahydro-1H-pyrrolo [1, 2-c] imidazole. *Medicinal Chemistry Research*, *26*(9), 2152-2160.

[14] Raj, R., Mehra, V., Gut, J., Rosenthal, P. J., Wicht, K. J., Egan, T. J., ... & Kumar, V. (2014). Discovery of highly selective 7-chloroquinoline-thiohydantoins with potent antimalarial activity. *European Journal of Medicinal Chemistry*, *84*, 425-432.

[15] Ali, O. M., El-Sayed, W. A., Eid, S. A., Abdelwahed, N. A., & Abdel-Rahman, A. A. (2012).**16]** Antimicrobial activity of new synthesized [(oxadiazolyl)methyl]phenytoin derivatives. *Acta poloniae pharmaceutica*, *69*(4), 657–667.