EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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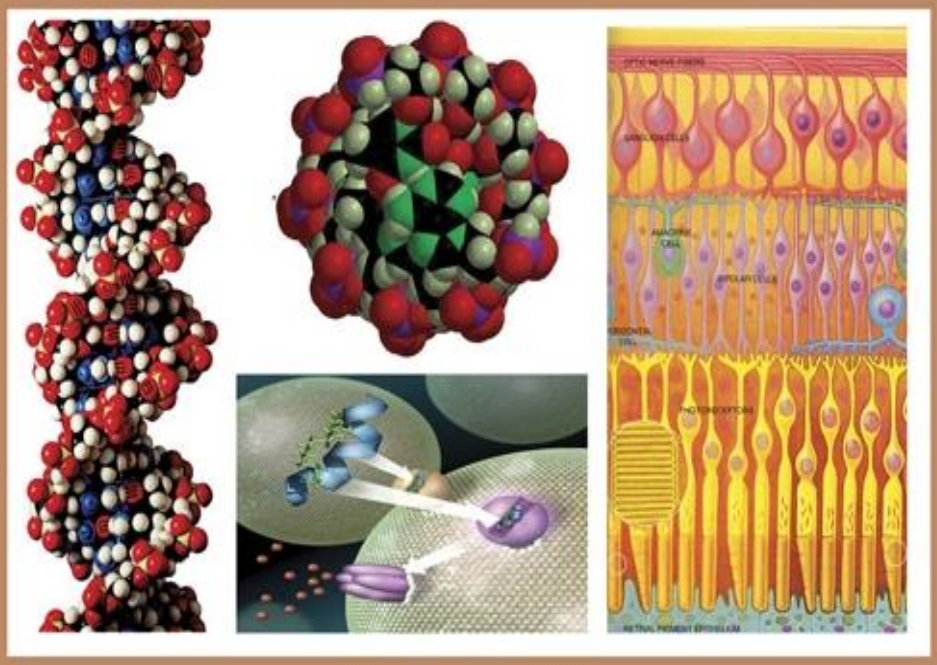

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 15 No. 2 (2023)

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Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(2) pp11-23 (2023)*
DOI: 10.21608/EAJBSC.2023.307820

Egypt. Acad. J. Biolog. Sci., 15(2):11-23 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg

Synthesis and Biological Activity of Barbituric acid- linked Isatin Derivatives

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ARTICLE INFO
Article History
Received:1/6/2023
Accepted:15/7/2023
Available:19/7/2023

Keywords:
Barbituric acid,
Biological Activity,
derivatives of Isatin.

ABSTRACT

The study focuses on the synthesis of isatin- barbituric acid compounds through a two-step process. In the first step, using mannich reaction, new derivatives of Isatin are synthesized by reacting Isatin with either barbituric acid, along with formaldehyde. This reaction results in the formation of Isatin-barbituric acid derivative. In the second step, these Isatin derivatives are further reacted with various aniline derivatives to generate new compounds containing Isatin. Spectroscopic methods including FTIR, ^{1}H NMR, and ^{13}C NMR are used to characterize the produced molecules. The study investigates the biological effects of several of the synthetic chemicals on certain bacterial strains, including Klebsiella pneumoniae and Staphylococcus aureus. The potential antibacterial activities of these substances are evaluated.

INTRODUCTION

Isatin, also known as indenedione and indolequinone, is a chemical compound that is widely present in mammalian tissues and bodily fluids. It is a biologically active heterocyclic moiety with vivid biological and pharmacological properties. Isatis plants are found all over the world at different latitudes, and they contain indolic compounds that have demonstrated therapeutic effects (Zhou.e *et al.*,2011 and Amr *et al.*, 2006).

It has a nitrogen atom at position 1, two carbonyl groups at positions 2 and 3, and two carbonyl groups. In (Fig. 1) Two cyclic rings-one with six members and the other with five—make up its structure. Both rings have a planar shape. The six-membered ring is aromatic, in contrast to the five-membered ring, which is anti-aromatic. Using nitric and chromic acids, Erdmann and Laurent first isolated isatin as an indigo oxidation byproduct. Isatin and its various derivatives, derived from natural products, have been extensively employed in medicine for many centuries due to its substantial chemical and biological properties (Xiao. *et al.*, 2002 and Hoessel, 1999).

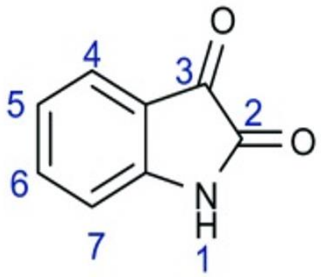


Fig. 1: Structure of isatin (1*H*-indole-2,3-dione).

German chemist Adolph von Baeyer initially created the class of aromatic hydrocarbons known as barbituric acids in 1864. (Baeyer, 1864). Fischer and von Mering discovered the ability of the first active barbituric acid compounds to put people to sleep in 1903. (Fischer *et al.,* 1924). In biology and medicine, barbituric acid derivatives have a significant impact. Barbiturates, which are derivatives of barbituric acid, are therefore employed as anticonvulsants, sedatives, and therapies for the central nervous system (Knabe *et al.,1982* and Archana *et al.,*2003). Many biological properties, such as antioxidant, antiurease, and antibacterial ones, are present in barbituric acid derivatives (Sokmen, *et al.,* 2013). Moreover, the derivatives of 5-benzylidene barbiturate exhibited inhibitory effects on both bacterial and mushroom tyrosinase (Yan *et al.,*2009). Barbituric acid derivatives had antibacterial, antifungal, and antidiabetic effects (Faidallah *et al.,*2012).

Barbituric acid that was recently produced showed immunomodulatory and anti-HIV properties (Naguib *et al.,*1993). Barbiturates also exhibit anticancer properties in melanoma (Dhorajiya *et al.,* 2014 and Ramisetti *et al.,*2018). Recent research showed that brand-new barbituric acid compounds prevented male Wistar rats from developing NAFLD (Ma *et al.,*2011 and Li *et al.,* 2011).

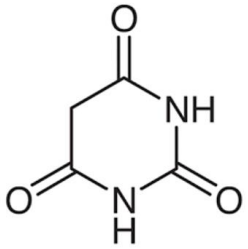


Fig. 2: Structure of Barbituric Acid.

A three-component condensation known as the Mannich reaction involves substrates with an active hydrogen atom (XeH), an aldehyde component (usually R1 - CHO), and an amine reagent. These aminoalkylation reactions yield Mannich bases, which are the substrate derivatives created through aminoalkyl moiety substitution. If formaldehyde is used in place of the aldehyde component, an aminomethylation procedure transforms the substrate into the correct Mannich base. In the Mannich reaction, secondary aliphatic amines (R2NH) are the most commonly employed amine reagents. However, primary amines and even ammonia (as an ammonium salt) can be utilized for aminomethylations or aminoalkylations. In 1912 (Mannich *et al.,* 1912), Carl U. F. Mannich first reported on the reaction that occurs between in situ-produced imines and enols, which produces - amino carbonyl compounds.

In 1864, the world of science was introduced to Schiff's base, thanks to the efforts of German researcher Hugo Schiff. His pioneering work on the byproducts created when primary amines reacted with carbonyl compounds earned him this distinction (Chiff *et al.,* 1864). Nowadays, the term "Schiff's base" is used to describe imines that have a hydrocarbyl group on the nitrogen atom (R2C = NR′) and are also known as Schiff bases. Although they're often thought of as just another type of azomethine, their unique composition sets them apart (Moss *et al.,* 1997). Schiff bases can combine with the ions of transition metals to produce complexes. They perform as L-type ligands in these complexes, which are ligands with two electron donors that do not undergo electron modifications on their valence shells, similar to how amines, amides, and phosphines do (Lundgren *et al.,* 2016). These Schiff base derivatives can form mono-, di-, and polynuclear metal complexes with various metal ions.

Depending on the locations and quantity of electron-donating groups, these complexes act as monodentate, bidentate, or tridentate ligands (Naz *et al.,* 2011 and Bukhari *et al.,*2005).

MATERIALS AND METHODS

The Department of Chemistry at the University of Kufa utilized a Fourier transform infrared Alpha-Bruker infrared spectrophotometer from Germany to capture FTIR spectra. Meanwhile, the recording of nuclear magnetic resonance spectra was conducted with a Bruker ARX400 (FT, 400 MHz for 1H; 100 MHz for 13C) by the Department of Chemistry at Basra University. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent resonance of DMSO-d6. Finally, the melting point was measured using an Electrothermal made in the United Kingdom.

Synthesis of Compound B (Zhao *et al.,*2015):

Absolute ethanol (30 mL) was used to reflux a mixture of Barbituric acid (0.64g, 0.005 mol), formaldehyde (37%, 5 ml, 0.06 mol), and Isatin (0.735g, 0.005 mol) for 6 hours. Once cooled slightly, an additional 5 ml of formaldehyde (37%, 0.06 mol) was introduced to the mixture and then refluxed again for another 6 hours before being left overnight to stand. Throughout the reaction, TLC was utilized to monitor progress. Once the reaction reached completion, an ice bath was used to cool the resulting red solution, causing the formation of the brown precipitate. Using the smallest amount of absolute ethanol possible, we recrystallized the product resulting in a satisfying reddish orange product with a yield of 90.5%, totaling 1.3 g. Its melting point was between 193-195 degrees Celsius. The FT-IR cm^{-1} spectrum exhibited important peaks at 3361 (N-H), 2966 (C-H aromatic), 2908 (C-H aliph), 1699 (C=O), and 1610 (C=C).

Synthesis of Compound D1:

Adding p-nitro aniline (0.057g, 0.0005 mol) to compound B (0.143g, 0.0005 mol) in a solution of 15 mL absolute ethanol (Scheme 2-4) proved effective. To the blend, a few drops of glacial acetic acid were incorporated, followed by reflux for 9 hours. Cooling this mixture prompted a precipitate, which was filtered and rinsed with ethanol. The resulting product was then recrystallized with ethanol, resulting in a dark yellow powder (yield: 88.3%, weight: 0.18g). Its M.P. was recorded at 258-260℃. Characterization was confirmed by FT-IR cm^{-1}: with 3364 (N-H), 3087(C-H aromatic), 2973 (C-H aliph), 1732 (C=O), 1683(C=N), 1595(C=C) peak values.

Synthesis of Compound D5:

Using 15 mL of absolute ethanol, 0.063g (0.0005 mol) of p-chloro aniline was mixed with 0.143g (0.0005 mol) of compound B already dissolved in absolute ethanol. The solution was acidified with a few drops of glacial acetic acid and then heated under reflux for 7 hours. Upon cooling, a precipitate was filtered and rinsed with ethanol. The yield of brown powder obtained through recrystallization with ethanol was 85.6% and measured at 0.17g. This product's melting point was between 200-202℃, and its FT-IR cm^{-1} peaks for specific functional groups were as follows: 3740 (N-H), 2972 (C-H aromatic), 2360 (C-H aliph), 1701 (C=O), 1607 (C=N),1501 (C=C).

Study of Biological Activity*:*

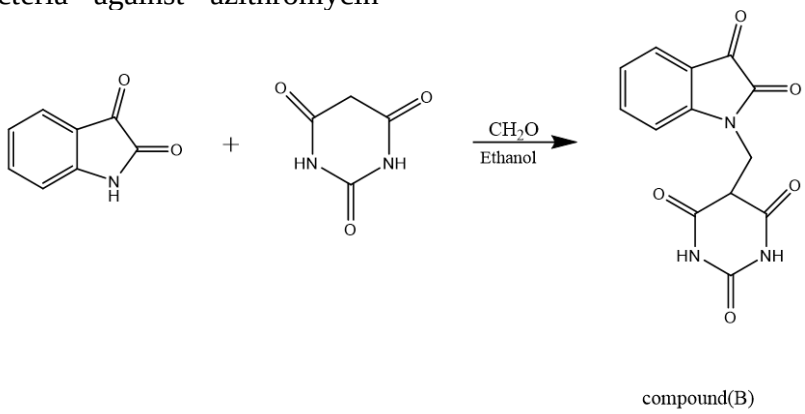
Using the agar well diffusion method, the antibacterial properties of various compounds were tested against drug-resistant strains. The prepared solutions D1 and D5 were specifically screened for their effectiveness against Staphylococcus aureus (a 0.12g Gram-positive bacteria) and Klebsiela pneumonia (a 0.12gGram-negative bacteria). To generate the culture, four fresh colonies were suspended in 5mL of BHIB and then placed in a 37°C incubator for 3 hours. Following incubation, the turbidity of the resulting culture was matched to the 0.5 McFarland standard - equivalent to a concentration of 1.5 X 108 cells/ml. Finally, sterile cotton swabs were dipped into the solution for testing. Using a Mueller Hinton Agar tray, cotton swabs were dipped and then streaked across its surface.

The following step involved creating six holes, each with a diameter of 6 mm, using sterile cork Pore, Pores (Hudzicki *et al.*, 2009and C. *et al.*,2000). Subsequently, each compound was diluted to a concentration of 100 and 200 µg mL-¹, at physiological pH (7). The prepared concentrated solutions of the aforementioned chemical compounds were then placed in the holes to determine their biological activity. The paten dish was thereafter incubated for 24 hours at 37 oC. For measurement of the effectiveness of each compound, the diameter of the inhibition zone was assessed using a ruler. The standard limits of sensitivity of the same species of bacteria against azithromycin were utilized for comparison purposes. Finally, this method was implemented to determine the success of each compound.

RESULTS AND DISCUSSION

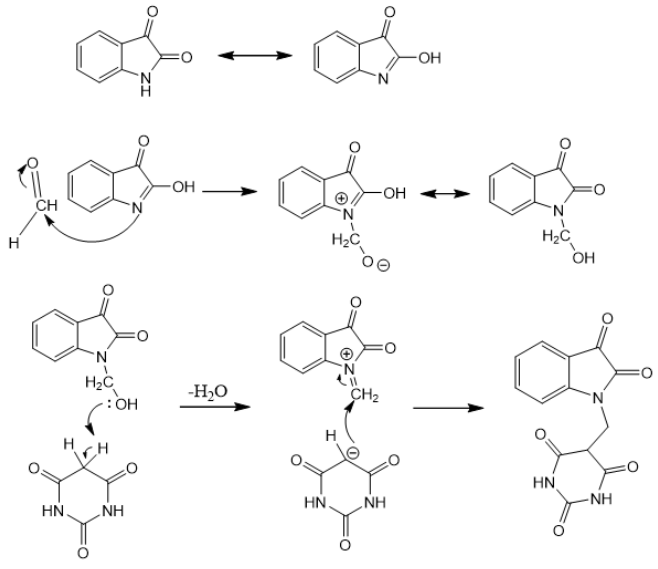
Synthesis Of Isatin Linked-Barbituric Acid Compound (Mannich Reaction):

Isatin linked-barbituric acid compound (B) was synthesized by reacting isatin, formaldehyde and barbituric acid in absolute ethanol (Scheme 1). The resulting product was recrystallized from a minimum amount of absolute ethanol to yield orange powder with a yield of 90.5% yield. The melting point of the compound was found to be within the range of (193-195)℃.



Scheme 1: Synthesis of compound B.

According to a Mannich reaction, Scheme 2 depicts the reaction's mechanism. An intermediate iminium ion was created in the first step using substituted isatin and formaldehyde. Barbituric acid then binds to the intermediate to produce the end result.



Scheme 2: mechanism of synthesis of compound B.

Identification of Compound B:

Various wavenumbers were detected in the FT-IR spectrum of compound (B). The N-H stretching vibrations peaked at 3361 cm^{-1} while C-H aromatic stretching vibrations appeared at 2966 cm^{-1}. Additionally, C-H aliphatic stretching vibrations were registered at 2908 cm^{-1}, and C=O stretching vibrations were measured at 1699 cm^{-1}. To round out the spectrum, C=C stretching vibrations were highlighted at 1610 cm^{-1} as evidenced in Figure 3.

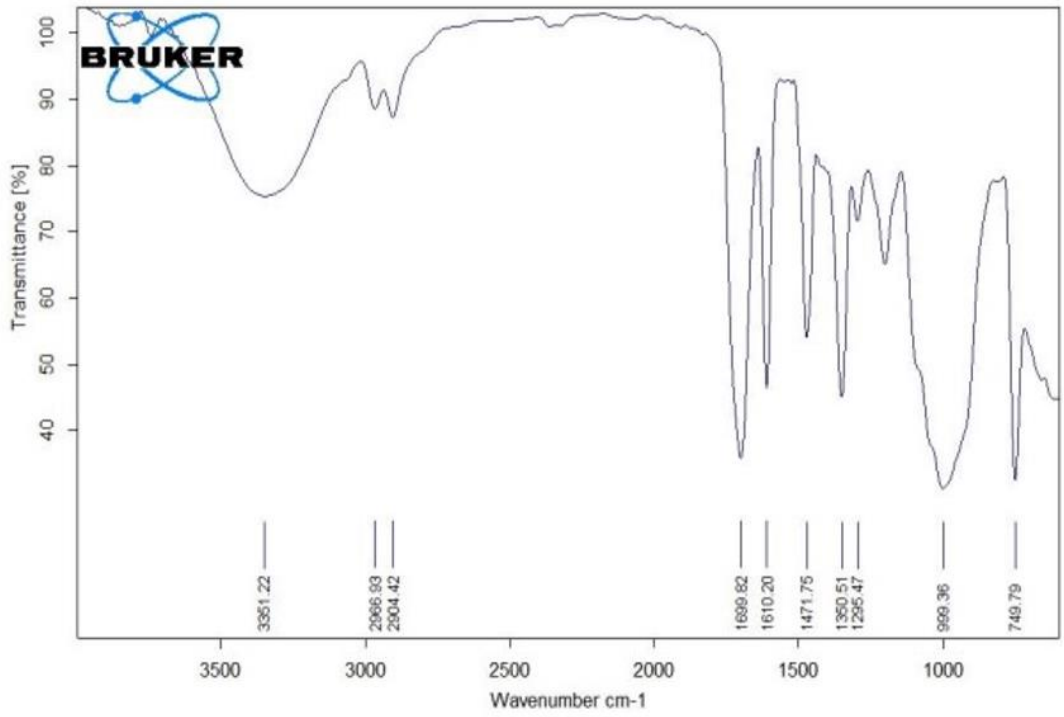


Fig. 3: FT-IR spectrum of compound B.

Obtaining various signals confirmed the compound's identity. In Figure 4, the 1H NMR spectrum showed a singlet signal corresponding to (N-H) at (11.72) ppm in DMSO-d6. An assigned (CH2) doublet signal appeared at (4.72-4.59) ppm, while a broad peak (CH) group signal was detected at (5.09) ppm. Multiple hydrogen atoms of the aromatic ring range expressed in (7.15-7.67) ppm.

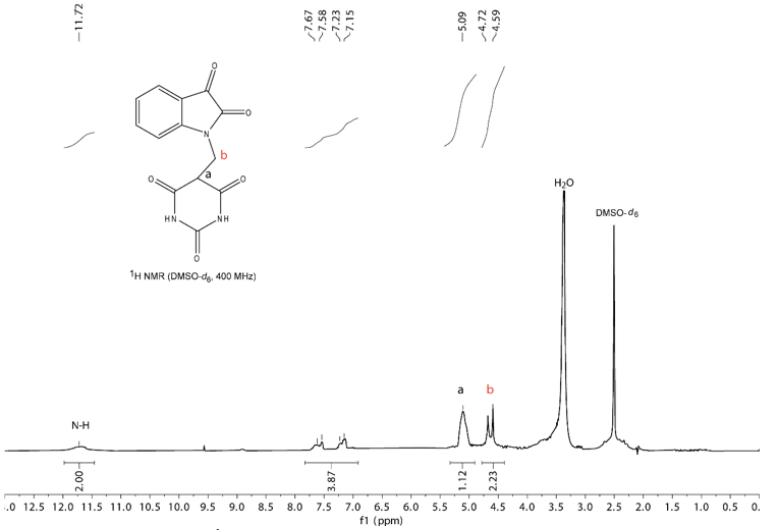
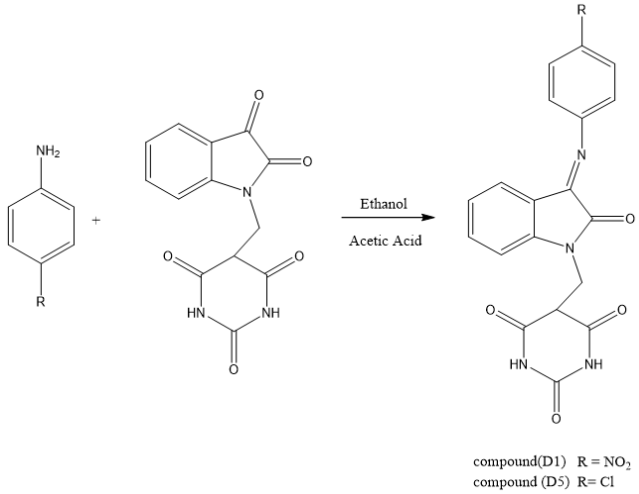


Fig. 4: ^{1}HNMR spectrum of compound B.

Synthesis of Isatin Linked-Barbituric Acid Derivatives (Schiff bases) Compound (D1 and D5):

Compounds (D1 and D5) were synthesized by reacting substituted aniline and compound (B) in absolute ethanol and a few drop from acetic acid (Scheme 3). The resulting product was recrystallized from a minimum amount of absolute ethanol to yield dark yellow powder with a yield of 88.3% for compound (D1), while compound (D5) obtained brown powder with a yield of 85.6%. The melting point of the compound (D1) was found to be within the range of (258-261)℃, and (200-202) ℃ for compound (D5).



Scheme 3: Synthesis of compounds D1 and D5.

3-4 Identification of Compounds (D1 and D5):

Displayed in Figure 5 are the characteristic peaks of the compound (D1) found at various wavenumbers: 1732 cm^{-1} for C=O stretching vibrations, 3364cm^{-1} for N-H stretching vibrations, 2973 cm^{-1} for C-H aliphatic stretching vibrations, 3087 cm^{-1} for C-H aromatic stretching vibrations, 1683 cm^{-1} for C=N stretching vibrations, and 1595 cm^{-1} for C=C stretching vibrations. The FT-IR spectrum of compound (D5) revealed a set of peaks that characterized this compound at different wavenumbers. Among these were the C=C stretching vibrations at 1501cm^{-1}, N-H stretching vibrations at 3740cm^{-1}, and C-H aliphatic stretching vibrations at 2360 cm^{-1}. Additionally, there were C-H aromatic stretching vibrations at 2972 cm^{-1}, C=O stretching vibrations at 1701 cm^{-1}, and C=N stretching vibrations at 1607 cm^{-1} (Fig. 6**).**

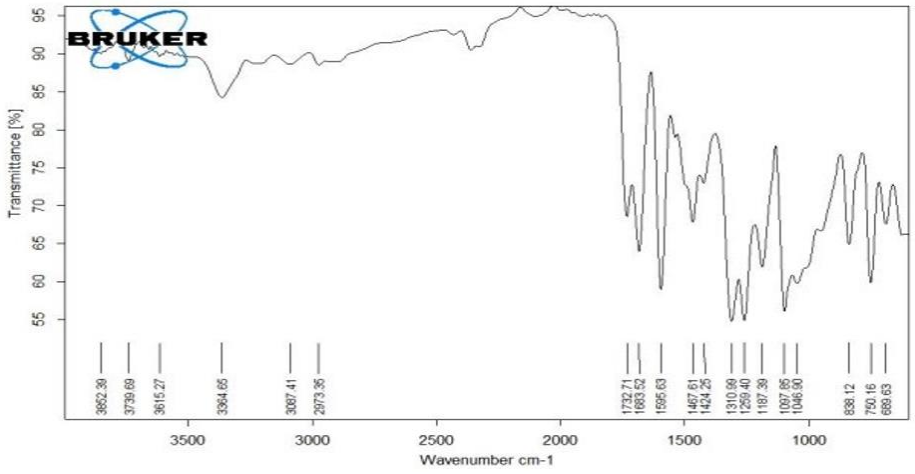


Fig. 5: FTIR spectrum of compound D1.

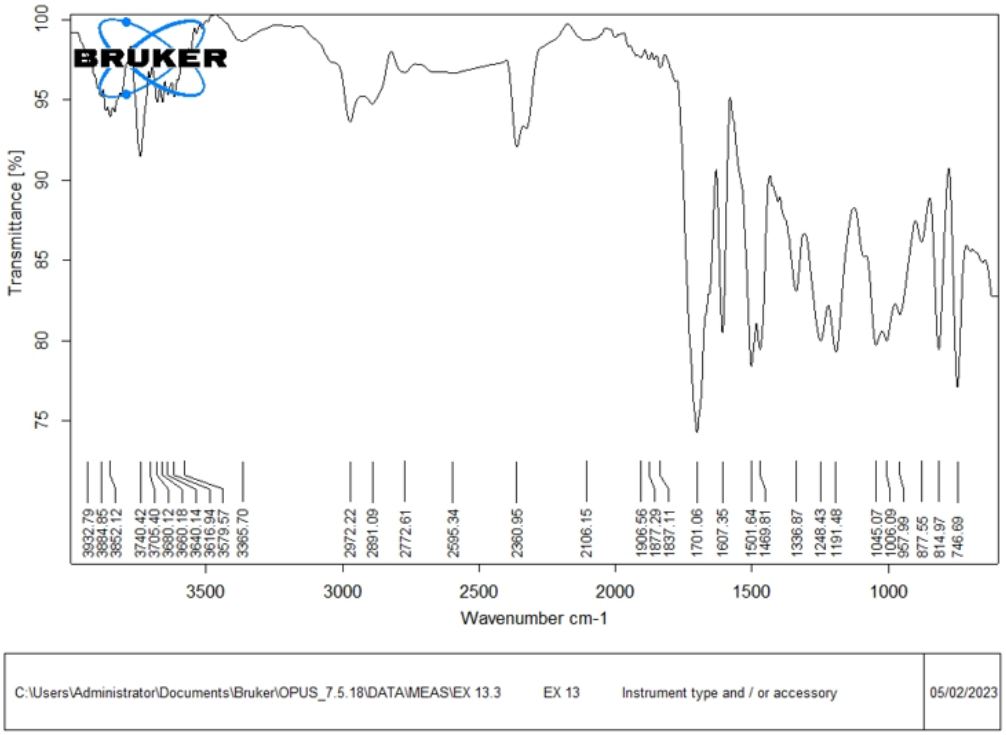


Fig. 6: FTIR spectrum of compound D5.

The study of compounds (D1 and D5) with ^{1}H NMR is consistent with its identity. The ^{1}H NMR spectrum in DMSO-d_6 of compound (D1) (Fig. 7) showed a singlet signal appeared at (12.05) ppm expressed (N-H). Another singlet signal appeared at (8.05-6.79) ppm expressing the aromatic (C-H). Triplet signal appeared at (5.12-5.09) ppm for (C-H). A doublet signal appeared at (4.39-4.36) ppm for (CH_2). For the compound (D5), The ^{1}H NMR spectrum in DMSO-d_6 showed a singlet signal assigned to (N-H) at (11.67)ppm, while signals assigned for (CH) of the aromatic ring at (7.69-6.72) ppm, triplet signal appeared at (5.09) ppm for (CH), while a doublet signal appeared and assigned for (CH_2) group at (4.37-4.40) ppm (Fig. 8).

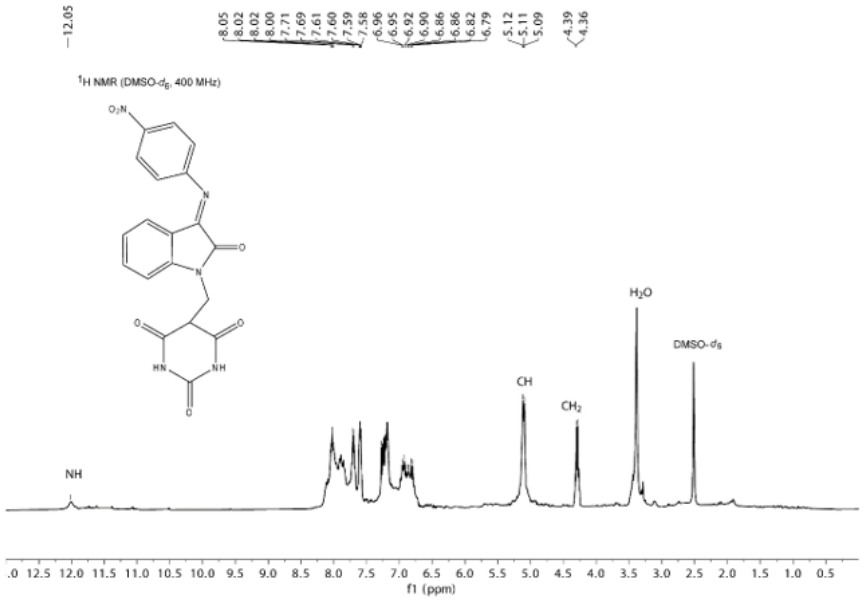


Fig. 7: ^{1}H NMR spectrum of compound D1.

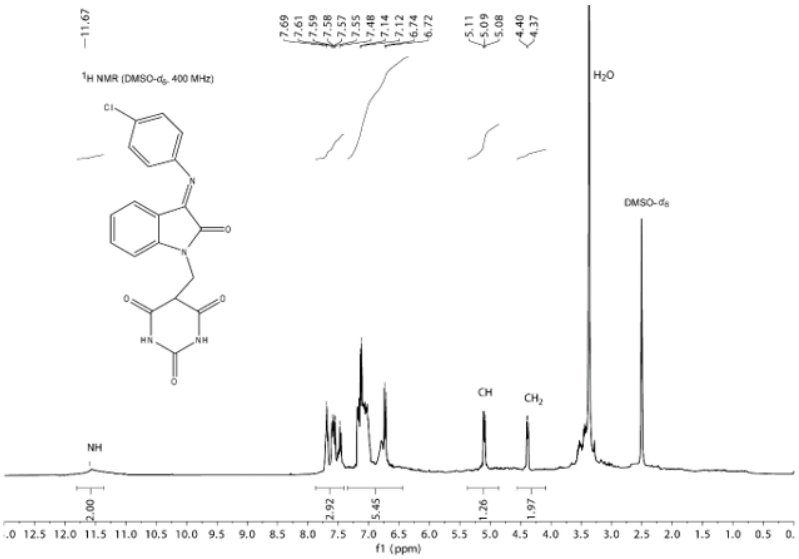


Fig. 8: ^{1}H NMR spectrum of compound D5.

In the ^{13}C-NMR spectrum of compound (D1) obtained in DMSO-d_6, several signals were observed at characteristic chemical shifts. The spectrum showed at (183.99-150.57) ppm a signal appeared assigned for (C=O) of barbituric acid. At (172.81) ppm another signal was assigned for (C=O) of isatin. The assigned signal for (C=N) appeared at (158.91)ppm. A signal noticed at (158.09-111.94)ppm was assigned for carbons of the aromatic ring. A signal appeared at (64.10) ppm assigned for (CH_2). A signal assigned for (CH) appeared at (69.90) ppm (Fig. 9). For compound (D5), the spectrum showed at (184.43-150.76) ppm a signal appeared assigned for (C=O) of barbituric acid, while (C=O) of isatin ring appeared at (172.83)ppm. The assigned signal for (C=N) appeared at (158.93) ppm. At (158.03- 111.50) ppm a multiple signal is assigned for (C-H) of the aromatic ring. A signal was assigned for (CH) at (69.50) ppm, while a signal appeared at (64.10) ppm assigned for (CH_2) (Fig. 10).

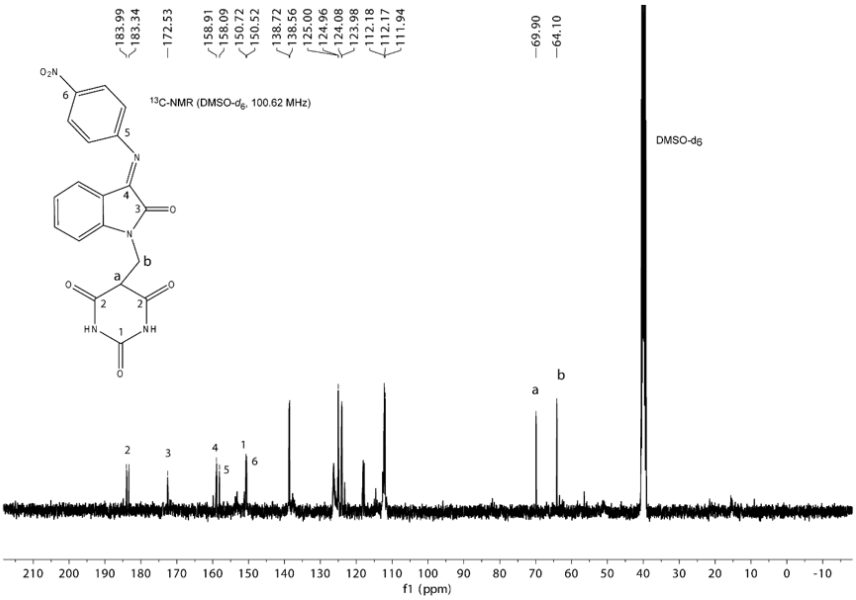


Fig. 9: ^{13}C NMR spectrum of Compound D1.

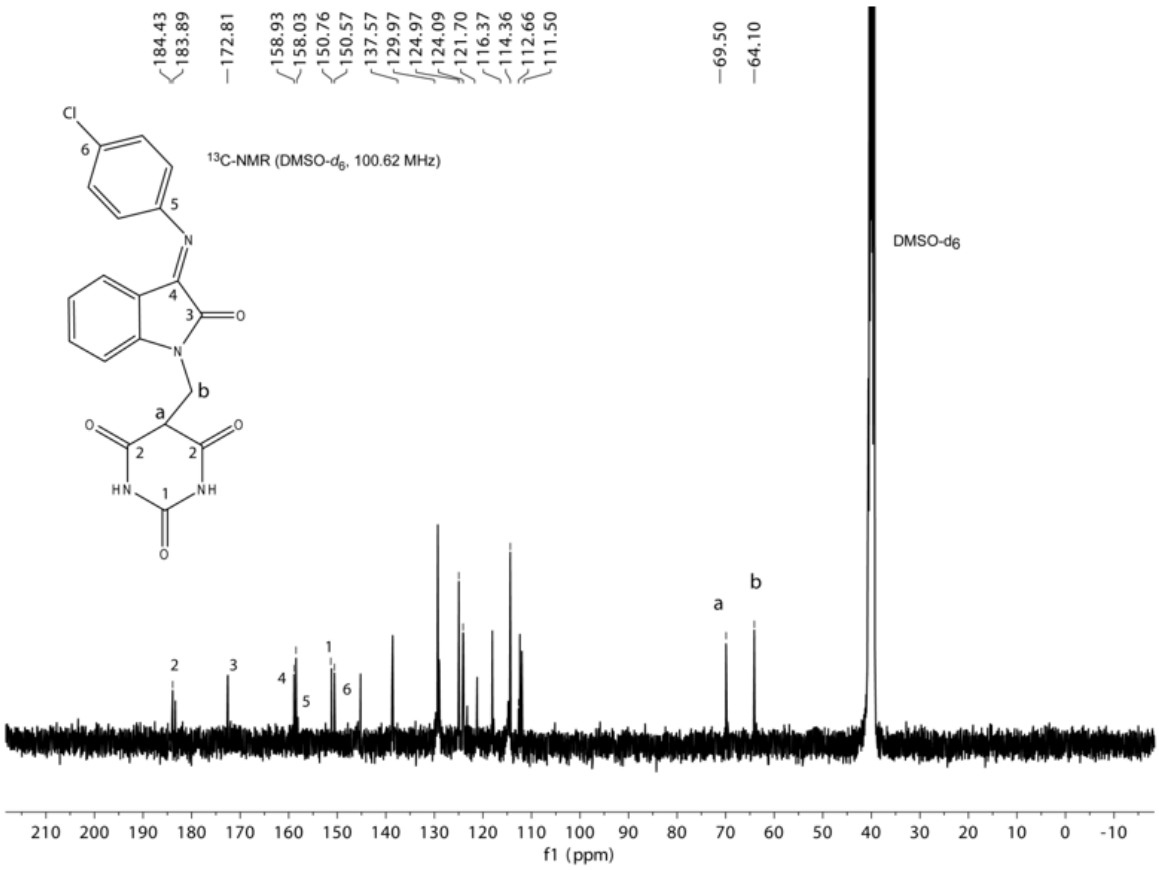


Fig. 10: ^{13}C NMR spectrum of Compound D5.

3.5 Biological Activity of prepared compounds:

Using the modified Kirby Bauer disc diffusion method on Muller Hinton agar, we examined how susceptible pathogenic bacteria were to antimicrobial agents. Our approach involved measuring the diameter of inhibition zones around antibiotic discs in line with CLSI standards (2018). As part of our study, we subjected both (0.12) Gram-positive (Staphylococcus aureus) and (0.12g)Gram-negative (Klebsiela pneumonia) bacteria to two compounds we prepared (D1, D5) (Fig. 11).

As shown in Table 1, the diameters of the inhibition zone for gram-positive bacteria highly sensitive to chemical compounds, namely D1 and D5, were 32 and 23 mm respectively. As for gram-negative bacteria, they displayed sensitivity to all chemical compounds.

Table 1. Antibiotic susceptibility of the Gram-positive and negative isolates 0.12g/mol.

Tested bacteria	Inhibitions zone (mm)	
	D1	D5
S. aureus	32	23
Klebsiela pneumonia	29	17

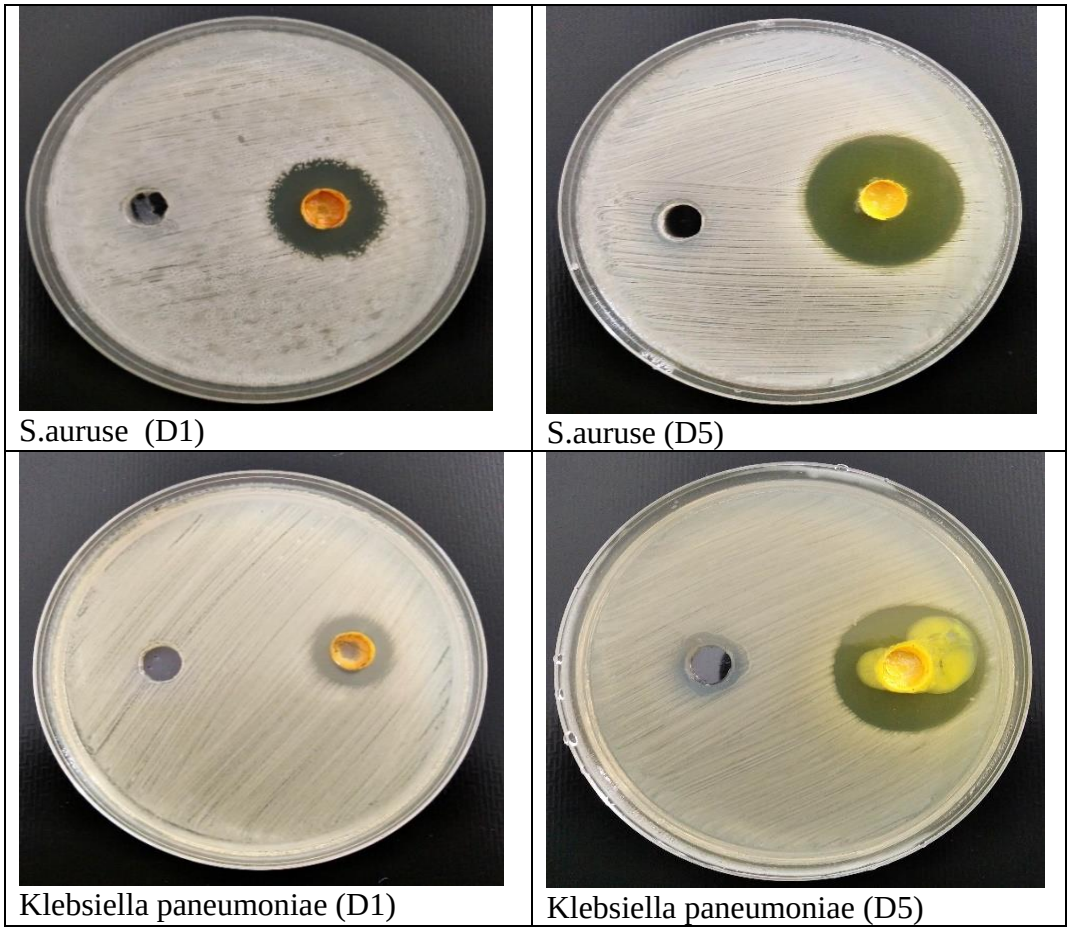


Fig. 11: Antibacterial activity of prepared compounds against(*Staph aureus and Klebsiella pneumoniae).*

Studied in a variety of bacteria is the bacterial degradation of various compounds, with these materials being used as the sole source of carbon and energy. Proposed degradation pathways have also been characterized along with the corresponding enzymes and genes (Chengbin, *et al.*,2009).

Diverse isatin derivatives have undergone screening for potential as anti-bacterial agents, with some exhibiting significant in vitro and in vivo efficacy. The treatment of bacterial infections presents a challenge, as germs continuously evolve new mechanisms of resistance; this has created demand for the development of new antibacterial methods (Liu, *et al.*, 2022). The compounds developed in this study include a naturally occurring isatin moiety (D1 and D5), which may possess antibacterial properties due to its effects on a variety of enzymes, proteins, and receptors.

The incubation time, pH, composition of the culture medium, and growth in light or darkness affect the morphology and particle size, based on various chemical and physical parameters. Joerger, *et al.*, 2000).

Because the cell walls of gram-negative bacteria are more complex than those of gram-positive bacteria, Klebsiela pneumonia is less sensitive than s. aureus. The structure of gram-negative microorganisms cells is both structurally and chemically intricate, containing two layers outside the cytoplasmic membrane. This represents a greater physical barrier to overcome, giving it a tougher defense against invaders with a ranking of (Kong, *et al.*,2010). Lipopolysaccharides found in the outer membrane of Gram-negative bacteria create a hydrophilic surface for the bacterium. The anionic groups present within the lipid components of lipopolysaccharide molecules, such as phosphate and carboxyl,

aid in the stability of the layer. This stability is upheld through electrostatic interactions with divalent cations (Helander, *et al.,* 1997).

CONCLUSION

It has been successfully proven by this research that Isatin-barbituric acid compounds can be synthesized using a two-step process. During the first step, barbituric acid and formaldehyde were used together with the Mannich reaction to react with Isatin, resulting in the production of derivatives that contained both Isatin and barbituric acid. In the following step, a variety of aniline derivatives were used to react with the aforementioned derivatives, creating new compounds with Isatin included. The compounds that were synthesized were examined using spectroscopic strategies such as FTIR, ^{1}H NMR, and ^{13}C NMR, which provided crucial information about the compounds' connectivity and functional groups. The information gathered from these techniques enabled the identification and structural analysis of the compounds created. Some of the Isatin-based compounds synthesized in the study proved to have great promise as antibacterial agents. The evaluation of the potential antimicrobial properties of these compounds was carried out against specific bacteria strains such as Klebsiella pneumoniae and Staphylococcus aureus. The goal was to identify possible candidates for further development into effective antimicrobial agents. The biological activity assessment revealed that some of the synthesized compounds exhibited significant antimicrobial activity against the tested bacteria strains. Hence, the findings suggest that these Isatin-based compounds have the potential to be developed into highly effective antibacterial agents.

Acknowledgments

The author gratefully acknowledges the financial assistance of Kufa University's Supplementary Educational Fund, Faculty of Science, Department of Chemistry, Iraq.

REFERENCES

Amr A. E. G. E., Abdel-Latif N. A., Abdalla M. M.(2006). ,Synthesis and antiandrogenic activity of some new 3-substituted androstano [17, 16-c]-5′-aryl-pyrazoline and their derivatives . *Bioorganic & medicinal chemistry,* 14:(2),373–384.

Archana Rani P., Bajaj K., Srivastava V.K., Chandra R., Kumar A. (2003) ,Synthesis of newer indolyl/ phenothiazinyl substituted 2-oxo/ thiobarbituric acid derivatives as potent anticonvulsant agents. *Arzneimittelforschung* . ;53(5):301–306.

Baeyer A. (1864). Mittheilungen aus dem organischenLaboratorium des Gewerbe institutes in Berlin: Untersuchungen über die Harnsäure gruppe. *Justus Liebigs Annalen der Chemistry,* 130(2), 129-175

Bukhari, I. H., Sher, M., & Arif, M. (2005). Synthesis, characterization and biological evaluation of Schiff base transition metal complexes with ampicillin, *Science International (Lahore)*, 17(3) 223-226

Chengbin, X., *et al.,* (2009). Biodegradation of aniline by a newly isolated Delftisp. XYJ6. *Chinese Journal of Chemical Engineering,* 17(3), 500-505

Chiff H. (1864) Mittheilungen aus dem Universitäts-laboratorium in Pisa: 2. Eineneue Reiheor ganischer Basen [Communications from the university laboratory in Pisa: 2. A new series of organic bases]. *Justus Liebigs Annalen der Chemie,* 131(1), 118-119.

Dhorajiya B.D., Dholakiya B.Z., Mohareb R.M. *C.* (2014).Hybrid probes of aromatic amine and barbituric acid: Highly promising leads for anti-bacterial, anti-fungal and anti-cancer activities. *Medicinal Chemistry Research ;*23:3941–3952

Dhorajiya B.D., Ibrahim A.S., Badria F.A., Dholakiya B.Z. .*(* 2014). Design and synthesis of novel nucleobase-based barbiturate derivatives as potential

anticancer agents. *Medicinal Chemistry Research,* 23: 839-847.

Faidallah H.M., Khan K.A.(2012),Synthesis and biological evaluation of new barbituric an thiobarbituric acid fluoro analogs of benzenesulfonamides as antidiabetic and antibacterial, agents.*Journal of Fluorine Chemistry,* 142,p 96-104.

Fischer, E., & von Mering, (1924). Über eine neueKlasse von schlafmitteln, vorträge und abhandlungen allgemeinen inhalts,671-679

Helander, M.G. and L.J.E. Zhang,(1997). Studies of comfort and discomfort in sitting. *Ergonomics,* 40.9: 895-915.

Hoessel, R.; Leclerc, S.; Endicott, J.A.; Nobel, M.E.M.; Lawrie, A. Tunnah, P.; Leost, M.; Damiens, E.; Marie, D.; Marko, D.; *et al.* .(1999) Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nature cell biology,* 1.1: 60-67.

Hudzicki, J.,(2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology,* 15: 55-63.

Joerger, R., Klaus, T. and Granqvist, C.G.(2000). Biologically produced silver–carbon composite materials for optically functional thin-film coatings. *Advanced Materials,* 12.6: 407-409

Knabe J., Büch H., Reinhardt J. Pharm. (1982);Derivatives of barbituric acid, 32. Central nervous activity of racemic and optically active barbituric acids with basic substituents. *Archiv der Pharmazie*, 315.10: 832-839

Kong, M., *et al.* (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. *International journal of food microbiology,* 144.1: 51-63

Liu, B., Jiang, D. and Hu, G.,(2022),The antibacterial activity of isatin hybrids. *Current Topics in Medicinal Chemistry,* 22.1: 25-40

Lundgren R.L., Stradiotto M. (2016), Key concepts in ligand design: an introduction, Wiley: West Sussex, England,(pp. 1-13).

Ma L., Li S., Zheng H., Chen J., Lin L., Ye X., Chen Z., Xu Q., Chen T., Yang J., *et al*.(2011). Synthesis and biological activity of novel barbituric and thiobarbituric acid derivatives against non-alcoholic fatty liver disease, *European journal of medicinal chemistry*, 46.6: 2003-2010

Mageed, A. H., & Al-Ameed, K. A. (2013). Synthesis and theoretical investigation of 5-(4-dimethylaminobenzylidene) thiobarbituric acid. *Asian Journal of Chemistry*, 25(6), 2953.

Mannich C; Krösche W. (1912). UebereinKondensationsproduktaus Formaldehyd, Ammoniak und Antipyrin. *Archives of Pharmacy* , 250.1:647-667.

Moss G.P., Smith P.A.S., Tavernier D. (1997) Glossary of class names of organic compounds and reactivity intermediates based on structure (IUPAC Recommendations) Pure, *Applied Chemistry*, 67. 1307-1375

Naguib F.N., Levesque D.L., Wang E.-C., Panzica R.P., El Kouni M.H. (1993), 5-Benzylbarbituric acid derivatives, potent and specific inhibitors of uridinephosphorylase. *Biochemical pharmacology,* 46 (7) : 1273–1283.

Naz, N. and Iqbal, M.Z. (2011) Synthesis, Spectroscopic and Biological Studies of Transition Metal Complexes of Novel Schiff Bases Derived from Cephradine and Sugars. *Science International (Lahore)*, 23, 27-31.

Ramisetti S.R., Pandey M.K., Lee S.Y., Karelia D., Narayan S., Amin S., Sharma A.K. (2018). Design and synthesis of novel thiobarbituric

acid derivatives targeting both wild-type and BRAF-mutated melanoma cells. *European journal of medicinal chemistry*, 143: 1919-1930

Sokmen B.B., Ugras S., Sarikaya H.Y., Ugras H.I., Yanardag R. (2013). Antibacterial, Antiurease, and Antioxidant Activities of Some Arylidene Barbiturates. *Applied biochemistry and biotechnology*, 171:2030–2039.

Xiao, Z.; Hao, Y.; Liu, B.; Qian,L. (2002), Indirubin and Meisoindigo in the Treatment of Chronic Myelogenous Leukemia in China. *Leukemia lymphoma,* 43,(9) 1763–1768.

Yan Q., Cao R., Yi W., Chen Z., Wen H., Ma L., .(2009); Song H. Inhibitory effects of 5-benzylidene barbiturate derivatives on mushroom tyrosinase and their antibacterial activities. *European journal of medicinal chemistry,* 44:4235–4243, doi: 10. 1016/ j.ejmech.05.023.

Zhao, J., *et al*.(2015), Enantioselective Construction of Vicinal Tetrasubstituted Stereocenters by the Mannich Reaction of Silyl Ketene Imine with Isatin -Derived Ketimines .*Angewandte Chemie International Edition*, 54(1): p. 241-244

Zheng H., Li S., Ma L., Cheng L., Deng C., Chen Z., Xie C., Xiang M., Jiang W., Chen L. (2011);A novel agonist of PPAR-γ based on barbituric acid alleviates the development of non-alcoholic fatty liver disease by regulating adipocytokine expression and preventing insulin resistance. *European journal of pharmacology* ;659:244–251.

Zhou, J.; Qu, F.(2011), .Analysis of the Extracts of Isatistinctoria by New Analytical Approaches of HPLC, MS and NMR. African Journal of Traditional, *Complementary and Alternative Medicines,*. 8, 33–45