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Development, Comprehensive Characterization, and Antimicrobial Activity Evaluation of a Novel Class of Symmetrical 1,3-Benzoxazine Derivatives

Issam A.M.[1*], Huda Salah Kareem[2], Muhsin Jaber Jweeg[3], M.N. Mohammed[4], Thamer Adnan Abdullah[5], Oday I. Abdullah[6, 4, 7*], Chiya Othman Hassan[8]

[1]Department of Prosthodontics, Al-Nukhbah University College, Baghdad, Iraq; issam19641964@gmail.com
[2]Department of International Experiences and Educational Innovation, General Directorate of Curricula, Ministry of Education, Baghdad, Iraq; hudasalah71@gmail.com
[3]College of Technical Engineering, Al-Farahidi University, Baghdad 00965, Iraq; muhsin.jweeg@uoalfarahidi.edu.iq; orcid.org/0000-0001-7917-9952
[4]Mechanical Engineering Department, College of Engineering, Gulf University, Sanad 26489, Bahrain; dr.mohammed.alshekhly@gulfuniversity.edu.bh, orcid.org/0000-0002-1472-8401
[5]Applied Science Department, University of Technology, 10011 Baghdad, Iraq; 100249@uotechnology.edu.iq
[6]Department of Energy Engineering, College of Engineering, University of Baghdad, Baghdad 10071, Iraq; orcid.org/0000-0001-5450-021X
[7]College of Engineering, AlNaji University, Baghdad, Iraq
[8]Azmar College for Gifted Students/ Ministry of Education, Kurdistan, Iraq; Chiyaothman84@gmail.com
issam19641964@gmail.com, oday.abdullah@tuhh.de
[*]*Corresponding authors: e-mail:* issam19641964@gmail.com, oday.abdullah@tuhh.de

Completely symmetrical 6,6'-cyclohexane-1,1-diyl bis (3-substituted-3,4-dihydro-2H-1,3-benzoxazine) synthesized compounds have been able by a two-step process that yields a large amount of the compound. First, phenol and cyclohexanone were subjected to the Friedel-Craft process to produce 1,1'-bis(4-hydroxyphenyl) cyclohexane. The reaction between the bisphenol and formaldehyde and several primary amines produced unique symmetrical 1,3-benzoxazine compounds. Several spectroscopic methods, such as Fourier transform infrared spectroscopy, nuclear magnetic resonance (1H and 13C), GCMS spectrometry, and CHNS, were utilized in conjunction with tridimensional liquid chromatography (TLC) to investigate and validate the structures of the compounds created thoroughly. Two Gram-negative and two Gram-positive bacteria and a pathogenic fungus were tested for antimicrobial activity in contrast to the gold-standard medications streptomycin and nystatin. The antibacterial activity of some of these compounds was even better than that of the gold-standard medications, which is encouraging.

Keywords: Microorganisms, Antimicrobial activity, NMR, FTIR, 1,3-benzoxazine, Symmetrical.

INTRODUCTION

Natural heterocyclic compounds rely on various structural subunits, making them useful in many scenarios. Notable examples include vitamins, antibiotics, and hormones[1, 2]. Due to their biological activity, these compounds have attracted considerable attention[3]. Consequently, developing methods for obtaining these natural products has become a significant issue in organic chemistry. Among the various groups of heterocyclic compounds, 1,3-oxazines and their derivatives featuring the benzoxazine moiety hold considerable importance in the pharmacological and biological domains, offering a wide array of potential applications[4–6].

Several studies documented in the literature have highlighted the wide range of biological activities exhibited by 1,3-benzoxazine derivatives. These activities encompass antimicrobial[7–12], In addition to possessing antimicrobial, antitubercular, and fungicidal properties[13–19], analgesic, central nervous system (CNS) depression, and anti-inflammatory[20–25], anticoagulant[26], anticancer[27–29], antidiabetic, hypolipidemic[30], anti-proliferative[31], opening potassium channels[32, 33], inhibiting tumor growth[34, 35], lowering blood pressure[36], preventing angina pectoris[37], causing cytotoxicity[38], warding off rheumatism[19, 39], and preventing osteoporosis[40], as well as Mycobacterium tuberculosis[41, 42], and preventing cell proliferation in the MCF-7 cancerous human breast cell line[27]. Because these compounds have many biological applications, we are working on a new series of symmetrical 1,3-benzoxazine compounds that can be synthesized using Mannich-type condensation processes. Formaldehyde, in conjunction with various primary amines encompassing both aromatic and aliphatic variants, is employed for this purpose, with the selection based on the specific bisphenol-C compound under investigation. In addition, we compare the synthetic chemicals to the gold-standard chemical medications streptomycin (antibacterial) and nystatin (anti-fungal) to see how well they perform in vitro. The main objective of the study, is produce compounds that require fewer steps in the process that produce a large quantity, which have better antibacterial activity than even the gold standard drugs.

EXPERIMENTAL METHOD

Chemistry

Reagent-grade chemicals and solvents were used without any extra purification steps. Merck silica gel 60F254 plates with aluminum backings were always used in the thin-layer chromatography (TLC) procedures. Following development under UV illumination, the plates were examined using a variety of optimal mobile phases. An electrothermal melting point apparatus developed by Barnstead was used to ascertain the melting point. We recorded the samples' melting points in a Haematocrit tube from when they began

to melt to their final melting point. Using the Perkin-Elmer model spectrum 100 spectrometers, each molecule's Fourier Transform-Infrared (FTIR) spectra were verified to classify their functional groups. The spectra were taken at ambient temperature and covered an absorption band from 280 to 4000 cm^{-1}. All compounds were analyzed at room temperature on a JEOL ECA Nuclear Magnetic Resonance Spectrophotometer with 1H NMR (500 MHz) and 13C NMR (125 MHz) spectra. The solvent used was deuterated dimethyl sulfoxide (DMSO-d6 or dioxane-d8), and the internal standard was tetramethylsilane (TMS). Ionization is caused by electron impact at 70 Ev, and the mass spectrometry (MS) analysis is performed utilizing the equipped Shimadzu GCMS-QP5050 spectrometer. Direct Injection Mass Spectroscopy (DIMS) is the method applicable to solid substances. Carbon, hydrogen, nitrogen, and sulfur were tested using a LECO CHNS-932 apparatus. Within the range of ± 0.5% of the theoretical values, the samples were measured between 1.90 mg and 2.13 mg for every analysis.

The standard operating procedure for (bisphenol-C), 1,1'(4-hydroxy phenyl) cyclohexane

A Friedel-Craft catalyst consisting of a combination of hydrochloric acid and acetic acid (2:1 v/v, 10:5 ml) was used to treat cyclohexanone (0.05 mol, 4.9 g) with phenol (0.1 mol, 9.4 g) at 55 °C for 4 hours (Fig. 1). After filtering, the product was thoroughly rinsed with hot water and treated with an Aqueous solution of 2N NaOH. It then took on a pink hue. A cotton plug was used to filter and remove the resinous particles. The resulting yellowish solution was dried at 50 °C after being acidified with a diluted sulfuric acid, filtered, and washed with water. Repeated crystallization from benzene and methanol-water systems was performed on the product. Figure 2 Repeating the process yielded crystals that were pure white and shiny; the yield was 81%; the Rf was 0.71 (1:2 ethyl acetate: hexane); and the melting point was between 184 °C and 186 °C after being heated[40]. Infrared spectroscopy (FT-IR) measured values include 3483 and 3365 cm^{-1}, 3021 cm^{-1}, 2933 and 2858 cm^{-1}, and 1602, 1506, and 1438 cm^{-1} for aromatic C=C. 4H, br. s, cyclohexane-CH_2, 6H, m, cyclohexane-CH_2, 2H, s, 2 × phenol O-H, and 4H, d, J = 9.2, 4 × Ar-H, were observed in the ^{1}H NMR spectrum at 500 MHz in dioxane-d8. The ^{13}C nuclear magnetic resonance (NMR) spectroscopy, 3 × cyclohexane-CH_2, ipso C of cyclohexane, 2 × -CH, 2 × ipso C, MS: GCMS, Anal. Calcd. for $C_{18}H_{20}O_2$: H (7.51%) and C (80.56%), Found: H (7.22%) and C (80.23%).

Methodology of the Formation of 3-Substituted-3,4-Dihydro-2H-1,3-Benzoxazine compound

A template consisting of 1,1-bis (4-hydroxy phenyl) cyclohexane was utilized to synthesize 1.3- benzoxazine molecules, as shown in (Fig. 3). The condenser unit was placed in a 100 mL round-bottomed flask with three necks and a temperature gauge, and dropping funnel, an aqueous solution of formaldehyde that was 37% (0.04 mol) in dioxane of 15 mL was placed in an ice bath. The melting point of the mixture was 37%. After that, primary amines consisting of 0.02 mol in 10 mL of dioxane were added slowly dropwise while maintaining a temperature below 10 °C. Before adding the bisphenol-C solution (2.68, 0.01 mol) in 10 mL of dioxane, the mix stood agitated for approximately 1 hour. Next, the temperature was raised to 40 °C, and the reaction mix between 6–15 hr. Initial observations of the product were made with TLC, and a UV light source was used to see the product. A rotary evaporator was used to extract the solvent, and then 50 mL of ethyl ether was used to dissolve the viscous liquid in the container. After being washed with water on many

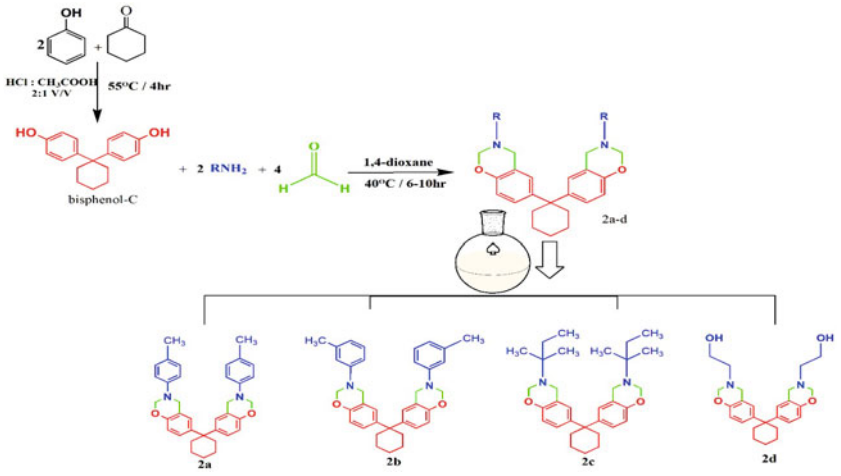

Figure 1. Flowchart of the main steps of work

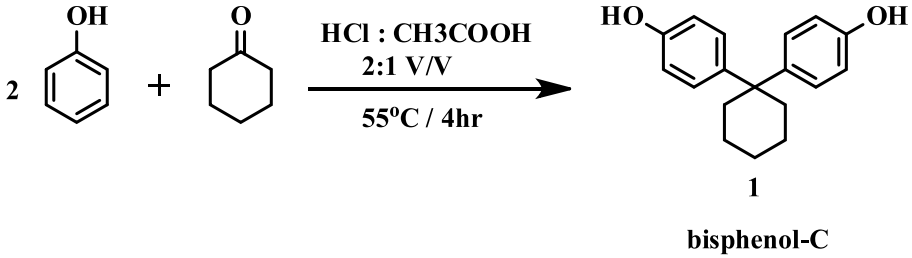

Figure 2. Creating 1,1-bis (4-hydroxy phenyl) cyclohexane

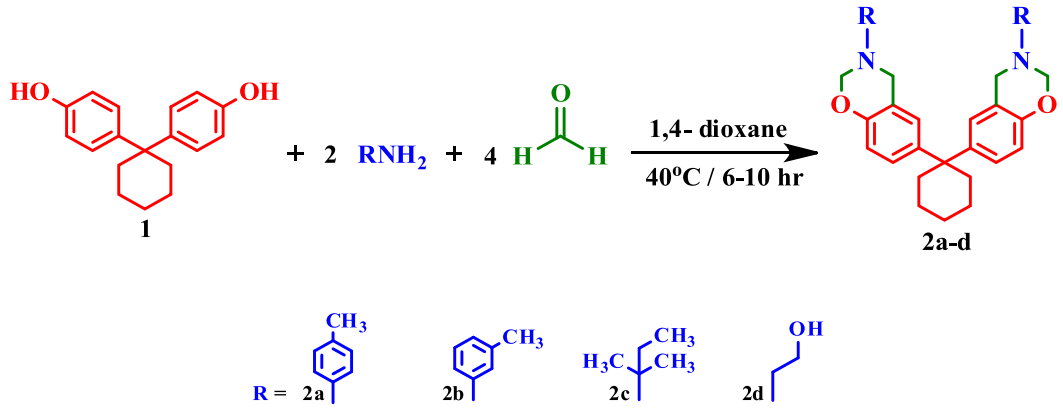

Figure 3. The creation of Symmetrical 1,3-Benzoxazines 2a–d

occasions, One last step was to dry the mixture using sodium sulfate. This was done to remove any formaldehyde or amine that may have been present. It was possible to remove the phenolic precursor by repeatedly rinsing the ether solution with 3N NaOH (aq). The pure compounds were obtained by recrystallization from the suitable solvent.

The compound 6,6'-(Cyclohexane-1,1-diyl) bis (3-(P-tolyl)-3,4-dihydro-2H-1,3-benzoxazine); 2a

To generate benzoxazine 2a, 2.143 grams of p-toluidine 0.02 mol were heated at 40 °C for 6 hr. Furthermore, this product was crystallized from ethyl acetate, and the compound resulted as a yellow solid (7.26 g) with 83% yield); Rf 0.76 (1:4) ethyl acetate: hexane; m.p. 153–155 °C; FT-IR (ν_{max} (film)/cm^{-1}); 3023 cm^{-1} (C-H) aromatic, 2929 cm^{-1}and 2857 cm^{-1} (C-H) aliphatic, 1610 cm^{-1}, 1503 cm^{-1} (C=C) aromatic, 1495 cm^{-1} ring of tri-substituted benzene, 1369 cm^{-1} (CH_2) ring of oxazine, 1239 cm^{-1} (Ar-O-C) unsymmetric, 1023 (C-O-C) symmetric, 1185 cm^{-1} and 807 cm^{-1} (C-N-C) unsymmetric and symmetric and 933 cm^{-1} (C-H) benzene stretch attached to ring of oxazine. Examinations can be noted for ^{1}H NMR, ^{13}C NMR, MS: GCMS (Figs. 4, 5, 6, 7), Anal. Calcd. for $C_{36}H_{38}N_2O_2$: H (7.22%) and C (81.47%) and N (5.28%); Found N (5.25%) and C (81.43%) and H (7.22%).

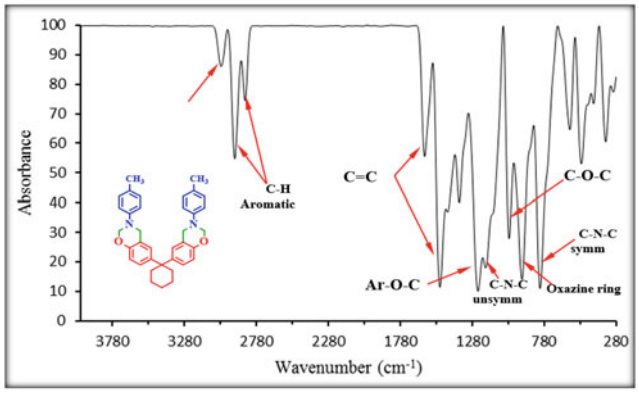

Figure 4. Spectrum of FTIR of innovative symmetrical 6,6'-(cyclohexane-1,1-diyl) bis(3-(p-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2a

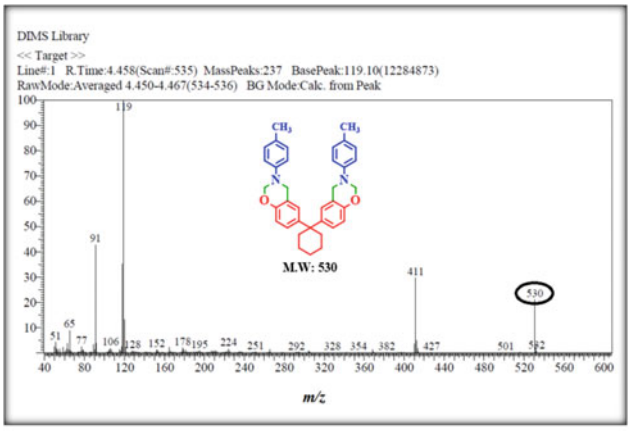

Figure 6. Spectrum of ^{13}C NMR of 6,6'-(cyclohexane-1,1-diyl) bis(3-(p-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2a

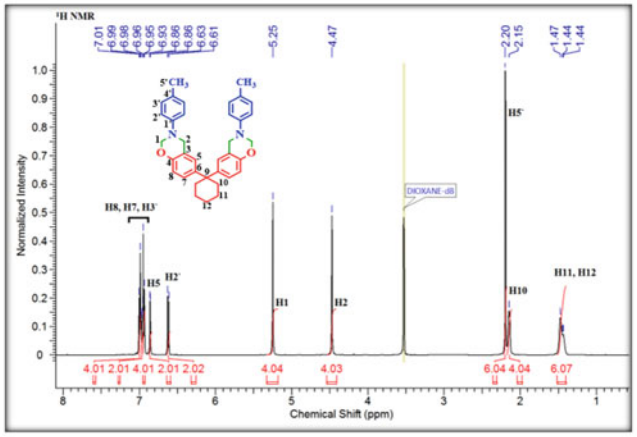

Figure 5. Spectrum of ^{1}H NMR of 6,6'-(cyclohexane-1,1-diyl) bis(3-(p-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2a

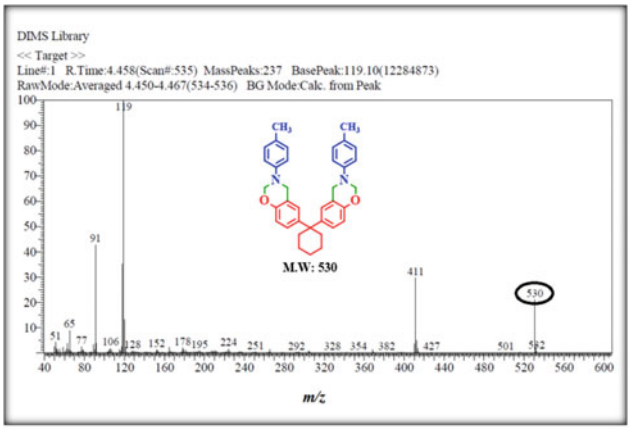

Figure 7. Spectrum of GCMS of 6,6'-(cyclohexane-1,1-diyl) bis (3-(p-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2a

The compound 6,6'-(Cyclohexane-1,1-Diyl) Bis (3-(M--Tolyl)-3,4-Dihydro-2H-1,3-Benzoxazine 2b

M-toluidine, which is 2.143 g and 0.02 mol, is heated at 40 °C for 6 hr to produce benzoxazine 2e**,** which was crystallized from ethyl acetate and titled compound was obtained as a brown solid (7.28 g) 81% yield; R_f 0.75 (1:4) ethyl acetate: hexane; m.p. 115–117 °C; FT-IR (ν_{max} (film)/cm^{-1}); 3023 cm^{-1} (C-H) aromatic, 2929 cm^{-1} and 2857 cm^{-1} (C-H) aliphatic, 1610 cm^{-1}, 1503 cm^{-1} (C=C) aromatic, 1495 cm^{-1} ring of tri-substituted benzene, 1369 cm^{-1} (CH_2) ring of oxazine, 1239 cm^{-1} (Ar-O-C) unsymmetric, 1023 cm^{-1} (C-O-C) symmetric, 1185 cm^{-1} and 807 cm^{-1} (C-N-C) unsymmetric and symmetric and 933 cm^{-1} (C-H) benzene stretch that attached to the ring of oxazine. Examinations can be noted for ^{1}H NMR, ^{13}C NMR, GCMS (Figs. 8, 9, 10, 11), Anal. Calcd. of

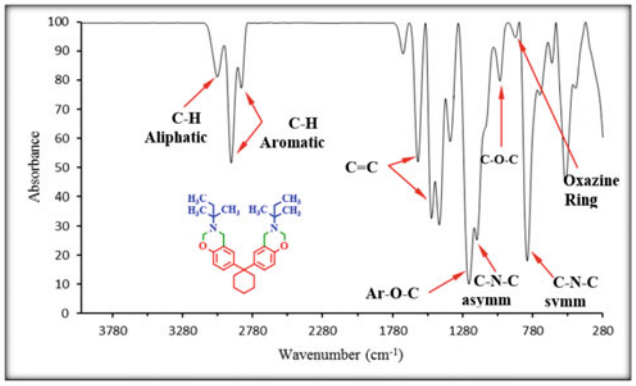

Figure 8. Spectrum of FTIR of 6,6'-(cyclohexane-1,1-diyl)bis(3--(*m*-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2b

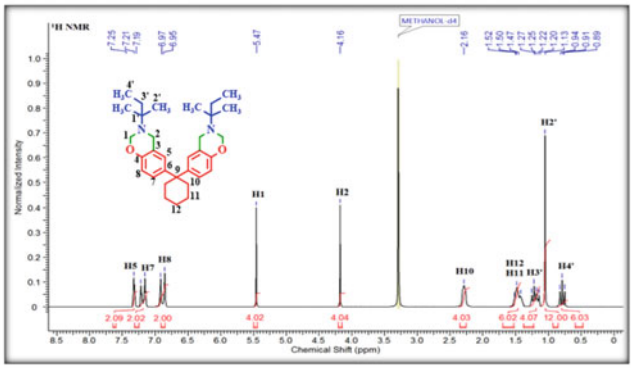

Figure 9. Spectrum of ^{1}H NMR of 6,6'-(cyclohexane-1,1-diyl) bis(3-(*m*-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2b

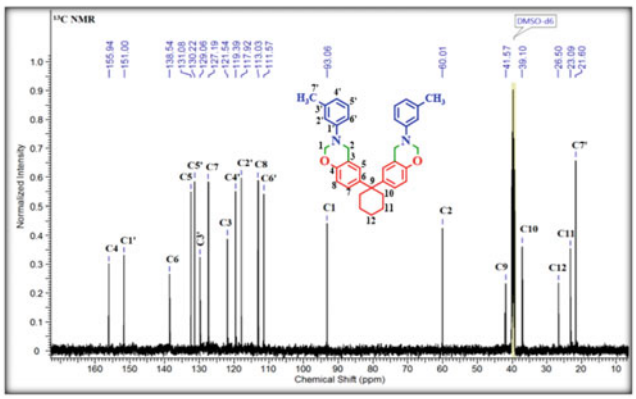

Figure 10. Spectrum of ^{13}C NMR of 6,6'-(cyclohexane-1,1-diyl) bis(3-(*m*-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2b

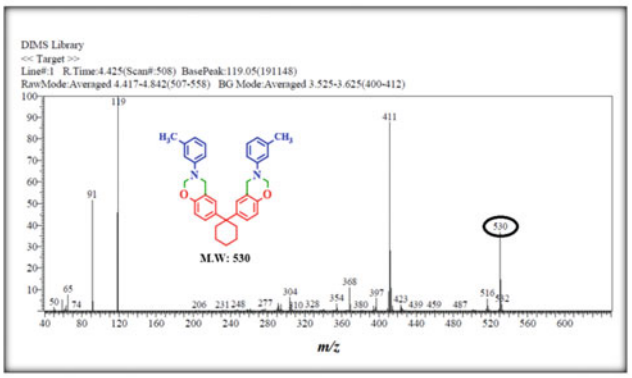

Figure 11. Spectrum of GCMS of 6,6'-(cyclohexane-1,1-diyl)bis(3--(*m*-tolyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2b

$C_{36}H_{38}N_2O_2$: H (7.22%) and C(81.47%) and N (5.26%); Found: H (7.22%) and C (81.44%) and N (5.29%).

The compound 6,6'-(Cyclohexane-1,1-Diyl)Bis(3-(Tertbutyl)-3,4-Dihydro-2H-1,3-Benzoxazine) 2c

Benzoxazine 2c can be produced by heating tert-amylamine (1.743 g, 0.02 mol) at 40 °C for 10 hr. This product was crystallized from a hexane-ethyl acetate system, and the named compound was made as a light yellow solid (6.68 g) 80% yield; R_f 0.54 (1:1) ethyl acetate: hexane; m.p. 191–193 °C; FT-IR (ν_{max} (film)/cm^{-1}); 3029 cm^{-1} (C-H) aromatic, 2932 cm^{-1}and 2859 cm^{-1} (C-H) aliphatic, 1599 cm^{-1}, 1502 cm^{-1} (C=C) aromatic, 1447 cm^{-1} ring of tri-substituted benzene, 1370 cm^{-1} (CH_2) wagging of oxazine ring, 1235 cm^{-1} (Ar-O-C) unsymmetric, 1014 cm^{-1} (C-O-C) symmetric, 1179 cm^{-1} and 807 cm^{-1} (C-N-C) unsymmetric and symmetric and 941 cm^{-1} (C-H) benzene stretch attached to ring of oxazine. Examinations can be

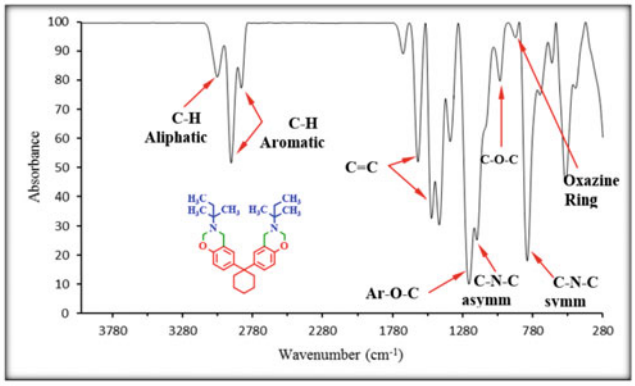

Figure 12. Spectrum of FTIR of 6,6'-(cyclohexane-1,1-diyl)bis(3--(*tert*-pentyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2c

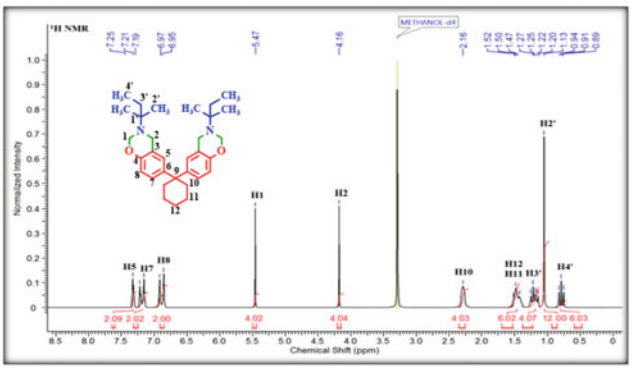

Figure 13. Spectrum of ^{1}H NMR of 6,6'-(cyclohexane-1,1-diyl) bis(3-(*tert*-pentyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2c

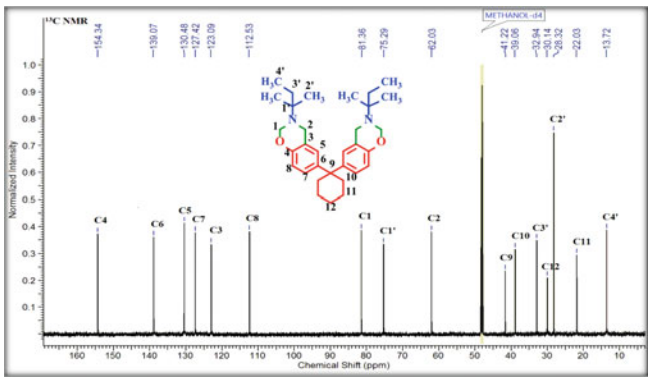

Figure 14. Spectrum of ^{13}C NMR of 6,6'-(cyclohexane-1,1-diyl) bis(3-(*tert*-pentyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2c

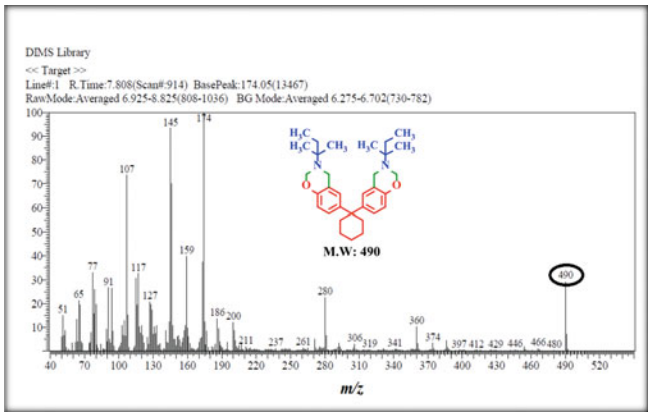

Figure 15. Spectrum of GCMS of 6,6'-(cyclohexane-1,1-diyl)bis(3--(*tert*-pentyl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2c

noted for ^{1}H NMR, ^{13}C NMR, and GCMS (Figures 12, 13, 14, 15). Anal. Calcd. of $C_{32}H_{46}N_2O_2$: H (9.15%) and C (77.88%) and N (6.05%), Found: H (9.12%) and C (77.84%) and N (6.00%).

The compound 6,6'-(Cyclohexane-1,1-Diyl)Bis(3-(Propa-non-L-Ol)-3,4-Dihydro-2H-1,3-Benzoxazine) 2d

To make benzoxazine 2d, 1.221 g of ethanolamine (0.02 mol) were heated at 40 °C for 10 hr, which was then crystallized from ethanol, and the named compound was made as a pale yellow solid (6.11 g) 78% yield; Rf 0.76 (1:1) ethyl acetate: hexane; m.p. 122–123 °C; FT-IR (ν_{max} (film)/cm^{-1}); 3274 cm^{-1} br. s (O-H), 2927 cm^{-1} and 2856 cm^{-1} (C-H) aliphatic, 1604 cm^{-1} (C=C) aromatic, 1492 cm^{-1} ring of tri-substituted benzene, 1360 cm^{-1} (CH_2) ring of oxazine, 1252 cm^{-1} (Ar-O-C) unsymmetric, 1035 cm^{-1} (C-O-C) symmetric, 1119 cm^{-1} and 815 cm^{-1}

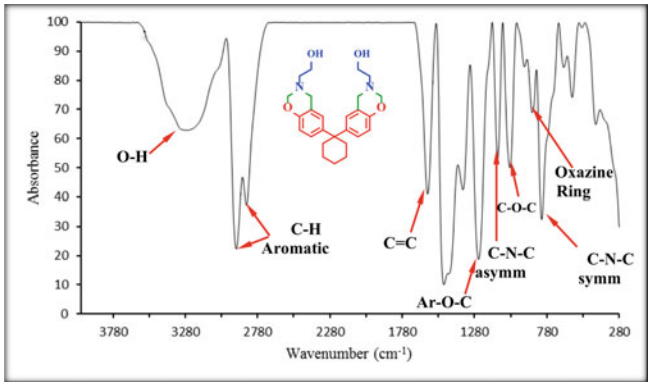

Figure 16. Spectrum of FTIR of 6,6'-(cyclohexane-1,1-diyl) bis (3-(ethanol-2-yl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2d

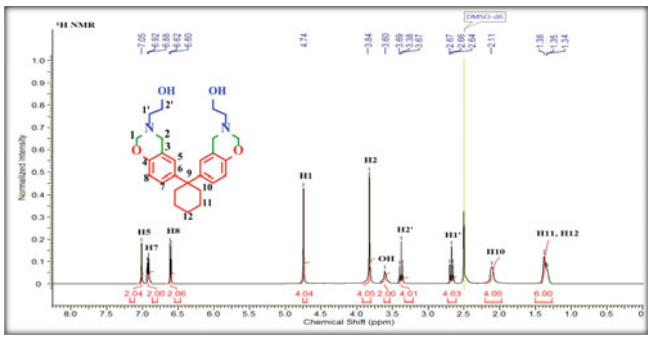

Figure 17. Spectrum of ^{1}H NMR of 6,6'-(cyclohexane-1,1-diyl) bis (3-(ethanol-2-yl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2d

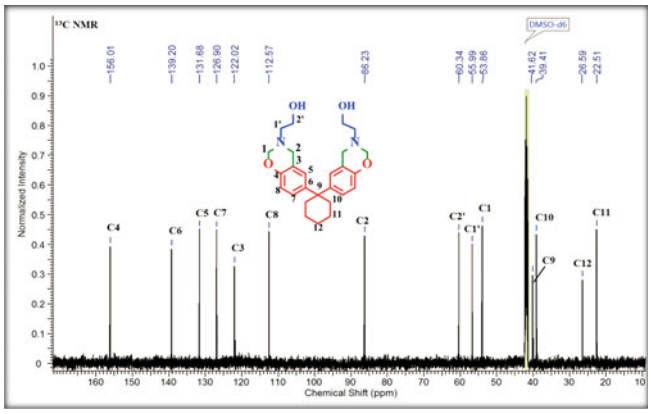

Figure 18. Spectrum of ^{13}C NMR of 6,6'-(cyclohexane-1,1-diyl) bis (3-(ethanol-2-yl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2d

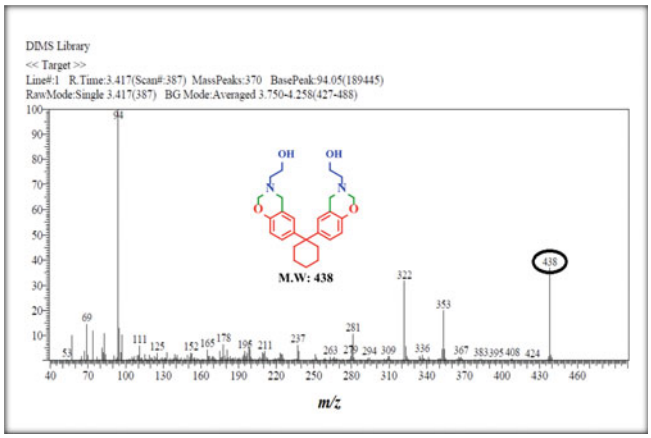

Figure 19. Spectrum of GCMS of 6,6'-(cyclohexane-1,1-diyl) bis (3-(ethanol-2-yl)-3,4-dihydro-2*H*-1,3-benzoxazine) 2d

(C-N-C) unsymmetric and symmetric and 934 cm^{-1} (C-H) benzene stretch attached to ring of oxazine. Examinations can be noted for ^{1}H NMR, ^{13}C NMR, GCMS (Figs. 16, 17, 18, 19), Anal. Calcd. of $C_{26}H_{34}N_2O_2$: H (7.81%) and C (71.21%) and N (6.39%), Found H (7.83%) and C (71.17%) and N (6.36%).

Antimicrobial analyzes

The antibacterial and anti-fungal activity test aims to determine how well the provided sample and other medications treat infections caused by bacteria and fungi. This test aims to determine the relative success of these antibiotics in inhibiting the growth or killing of the targeted microorganisms. Microbes exhibit varying responses, either sensitivity or resistance, towards antimicrobial compounds. Sensitivity refers to the susceptibility

of the microbes to the action of the antibiotic, resulting in growth inhibition or death. In contrast, how well did the microorganisms resist the tested substances or avoid antimicrobial effects? Testing follows established procedures to ensure that the results are accurate and reliable. Traditionally, a culture medium supporting the growth of microorganisms is used to ingest various concentrations of the sample antibiotics. A culture medium containing antibiotics is used to purify clinical isolates represented by known bacterial or fungal species. Methods for these tests can range from automated systems to cycle diffusion testing and broth dilution methods. The methodology is governed by considerations including the type of microorganism studied, the intended purpose of the study, and available resources.

Standard methods for assessing antibiotic activity include measuring the buffer zone according to the disc size of the antibiotic or calculating the minimum concentration (MIC) required for inhibition of microbial growth. A low minimum inhibitory concentration (MIC) or large inhibitory zone (IZ) indicates a strong antibiotic resistance to tested bacteria. The results of this research assist in enhancing comprehension of antibiotic efficacy and prospective applications in treating bacterial and fungal infections. Researchers and clinicians can choose antibiotics for the most appropriate infections, thereby providing treatment options.

Microorganisms

During this investigation, a total of four bacterial hosts were used. Staphylococcus aureus S276 and Bacillus subtilis B29 were Gram-positive strains, whereas Escherichia coli E266 and Pseudomonas aeruginosa ATCC 15442 were Gram-negative. Additionally, Aspergillus was the sole fungus that was included in this group. This is ATCC 16404. All of the formulations were examined to determine their level of action. To cultivate and sustain bacterial cultures, nutrient agar (NA) media[41–43] was utilized. In contrast, the fungus was cultured on potato dextrose agar (PDA) medium[44, 45]. Bacteria were cultured on agar slants and incubated at 37 °C, while fungi were grown at 28 °C. These temperature settings were chosen to provide optimal growth for each microorganism.

The selection of specific bacterial and fungal species in this study is important because they represent relevant human pathogens commonly associated with disease. Species of fungi and bacteria (Gram-positive and Gram-negative) are consumed in combination use of; the study aimed to provide a comprehensive analysis of the antimicrobial activity of compounds synthesized -The selection of agar and potato dextrose agar as culture medium was based on the ability to support the growth and multiplication of bacteria and fungi; respectively Nutrient agar provides the necessary nutrients for bacterial growth, while potato dextrose agar provides the right conditions for fungal growth. Temperatures of bacteria 37 °C and fungi 28 °C correspond to optimal growth conditions for the selected microorganisms. These temperatures promote colony growth and ensure microbial presence in the antimicrobial activity analysis's most active surrogate state. By carefully selecting microorganisms and growth conditions using these standardized protocols, the study aims to obtain reliable and comparable results on the efficacy of formulations against bacterial and fungal pathogens.

Determination of Antimicrobial Activity

The cycle diffusion method was utilized in this investigation to assess the efficiency of the antibacterial and antifungal properties[46, 47]. For the experiments, paper disks of 6 mm diameter were placed on the agar plates where the microorganisms were growing. The microbial cultures stood standardized to 0.5 McFarland standard before testing, corresponding to 10^8 cells/mL. For comparison, commercially available antibiotics, such as streptomycin for bacteria and nystatin for fungi, remained used as reference standards. These antibiotics were dissolved in dimethyl sulfoxide (DMSO) in 1990 and developed into high-quality antibiotics. DMSO was used as a solvent to dissolve the formulations and included as a negative control. Plates of agar that contained bacterial and fungal germs were turned over and incubated at temperatures ranging from 30–37 °C for 18–24 hr for bacteria and 6–7 days for fungus. The incubation period was continued until it was seen that the microorganisms had grown significantly. After plating, each plate was carefully inspected, and the total diameter of the inhibition zones was measured. Areas were examined with the unaided eye and defined as areas where no visible improvement was observed with the paper material.

Considerations include the paper size of the disc itself. Field diameters were recorded to the nearest whole millimeter using a sliding caliper or ruler. These measuring instruments were placed on inverted petri plates for accurate readings. By employing the cycle diffusion method and carefully measuring field diameters, the study proposes to quantitatively assess the efficacy of the formulations in inhibiting the growth of the tested microorganisms; the results have been compared and validated using positive and negative controls with reference antibiotics.

The final step was to analyze the antibacterial and antifungal activity using the disc diffusion method. This procedure involved placing paper discs containing antibiotics on agar plates that contained fixed microbiological cultures. Components of commercial antibiotics were utilized as reference standards. After incubation, the areas of absolute inhibition were visually inspected and evaluated with appropriate equipment to obtain accurate results.

RESULT AND DISCUSSION

The synthetic process generated sequences of unique symmetrical 1,3-benzoxazine derivatives (2a–d) starting with bisphenol-C synthesis. Conventional protocols were followed to use a variety of aromatic and aliphatic primary amines in conjunction with formaldehyde using the Mannich condensation process[48, 49]. For the synthesis of all the compounds, a solvent-assisted method employing dioxane was employed, which proved to be suitable. The reactions exhibited short reaction times and were conducted at low temperatures, yielding high product yields, as detailed in Table 1. Based on an extensive literature search, the synthesized 1,3-benzoxazine derivatives (2a–d) presented herein are novel compounds with no

Table 1. Tocopherol concentration (mg/kg oil) and total carotenoids (mg/kg oil) in AVO submitted at 120 °C and 130 °C for 30 minutes

Compound	MF.	MW.	M. P. °C	Yield(%)	Time	Elemental Analysis		
						%C	%H	%N
2a	$C_{36}H_{38}N_2O_2$	530	153–155	83%	6 hr	(81.43) (81.47)	(7.20) (7.22)	(5.25) (5.28)
2b	$C_{36}H_{38}N_2O_2$	530	130–132	81%	6 hr	81.44 (81.47)	(7.22) (7.22)	(5.29) (5.28)
2c	$C_{32}H_{46}N_2O_2$	490	191–193	80%	10 hr	(77.84) (77.88)	(9.12) (9.15)	(6.00) (6.05)
2d	$C_{26}H_{34}N_2O_2$	438	122–123	78%	10 hr	(71.17) (71.21)	(7.83) (7.81)	(6.36) (6.39)

previously published reports on their synthesis. Following purification, the chemical structures of all newly obtained products were characterized and confirmed through various analytical techniques, including TLC, melting point determination, elemental analysis, ^{1}H NMR, ^{13}C NMR, FTIR, and GCMS analysis. Synthesis of new symmetrical 1,3-benzoxazine derivatives proceeded through a two-step procedure. In the initial step, the reaction temperature was maintained below –10 °C for approximately 30 minutes when utilizing aromatic amines, whereas for aliphatic amines, the reaction was completed within (1 h). Subsequently, the temperature was raised to 40 °C in the final step, forming 1,3-benzoxazine derivatives after 6–10 h for compounds containing aromatic or aliphatic R groups, respectively.

In this condensation reaction, several substituted amines were used, including p-toluidine, m-toluidine, tert-amylamine, and ethanolamine. The experimental outcomes revealed a strong dependence of 1,3-benzoxazine ring formation on the structural characteristics of the starting amines. Specifically, 1,3-benzoxazine could not be formed from amines with electron-withdrawing groups or extensive substitutions in the same orientation as the amine[50, 51]. Such compounds as methyl-2-aminobenzoate, 3,4-dihydro-2H-1,3-benzoxazines have not remained successfully produce utilizing 2-chloroaniline, 2-amino-4-chlorophenol, ethyl-2-aminobenzoate, 2-methoxyaniline and 4-amino-5-thioxodihydrothiophen--3(2H)-one. Even after changing the methods to increase the temperature up to reflux and taking advantage of various solvents, such as methanol, ethanol, and tetrahydrofuran (THF), there was still no production of 1,3-benzoxazines.

ASSESSMENT OF ANTIMICROBIAL ACTIVITY

The newly synthesized symmetrical 1,3-benzoxazines outscored the original parent chemical, which exhibited substantial activity, in a study examining the activity of antibacterial of two gram-negative and two gram--positive bacteria. Four bacteria were tested. There were three compounds that demonstrated comparable effectiveness against Bacillus subtilis B29. These compounds were 2a (R=4- CH3C6H4), 2c (R=C5H11) and 2b (R=3- CH3C6H4). Nevertheless, compound 2b (R=3- CH3C6H4) outperformed all other compounds in terms of effectiveness against the studied bacteria. Compound 2b showed marginally more significant activity than 2a against all examined bacteria, indicating that the methyl group was positioned meta > para. However, all symmetrical 1,3-benzoxazine compounds, beginning with bisphenol-C, were verified for their anti--fungal activity against Aspergillus brasilliensis ATCC 16404 in a laboratory setting. It was disappointing that the examined microbe showed no activity in the results.

According to the findings summarised in Table 2, all newly synthesized compounds showed antibacterial activity over the validated microorganisms. The activity was higher than that of the initial parent drug. These compounds inhibited gram-negative and gram-positive bacteria, but gram-positive bacteria were particularly effective. This difference in activity may be attributed to the higher impermeability of the cell walls of gram-bacteria that are gram-negative as opposed to gram-positive species. Newly produced symmetrical 1,3-benzoxazine compounds exhibited significantly better antibacterial activity levels against gram-negative and gram-positive bacteria when compared to the parent molecule that was initially manufactured. On the other hand, *Aspergillus brasiliensis* ATCC 16404 did not show any anti-fungal

Table 2. Composition (%) of fatty acids analyzed by gas chromatography of AVO submitted at 120 °C and 130 °C for 30 minutes

Samples (100 µg/ml)	Target Bacteria				
	Millimeters (±1 mm) that constitute the inhibition zone's diameter				
	The Antibacterial				The Anti-fungal
	B. subtilis B29 (+)	S. aureus S276 (+)	P. aeruginosa ATCC 15442 (–)	E. coli E266 (–)	Aspergillus brasilliensis ATCC 16404
BC	10	–	7	–	–
2a	21	16	14	11	–
2b	24	17	16	17	–
2c	22	15	10	10	–
2d	19	13	11	15	–
Streptomycin	31	31	33	28	–
Nystatin	–	–	–	–	25
DMSO	–	–	–	–	25

Indicators of (–) gram-negative bacteria and the (+) gram-positive.
Standard bacteria, including streptomycin, nystatin, andstandard fungi, are included in this category. (BC) stands for bisphenol-C. The positive control, which consisted of streptomycin and nystatin, was compared to the negative control, dimethyl sulfoxide (DMSO).

activity. These results warrant additional research and optimization of these compounds for possible use in antimicrobial treatments since they show promise as effective antibacterial agents, especially against gram--positive bacteria.

CONCLUSIONS

Utilizing a simple method that made use of inexpensive and easily accessible raw ingredients, we were able to synthesize and analyze 1,1'-bis(4-hydroxyphenyl) cyclohexane. The synthesis was carried out utilizing the Friedel-Craft process. In the presence of formaldehyde, the 1,1'-bis (4-hydroxyphenyl)cyclohexane that had been produced was subsequently put through a reaction of the Mannich type with several different amines, those of both the aliphatic and aromatic varieties. A high yield of novel symmetrical 3-substituted-3,4-dihydro-2H-1,3-benzoxazine derivatives be situated produced as a result of this process. The structures of the newly generated compounds were validated by utilizing TLC and spectroscopic techniques, including GCMS, FTIR, CHNS, ^{1}H NMR, and ^{13}C NMR study. To evaluate the potential antibacterial activity of the newly synthesized 1,3-benzoxazine compounds (2a–d) and the intermediate bisphenol-C, they were tested in vitro against four strains of human pathogenic bacteria, including two gram-negative bacteria and (2) gram-positive bacteria, and one fungus. The consequences of the antimicrobial screening showed that the formulations were less effective against the variety of microorganisms examined. This is compared to streptomycin, which is considered the standard. All the compounds in the study showed antimicrobial action against the bacteria; several were even more effective than the original drug. This suggests that these compounds have considerable promise as antimicrobials. These results demonstrate that new symmetrical 1,3-benzoxazine derivatives with verified chemical structures were synthesized successfully. These chemicals have been shown to encourage antibacterial efficacy against harmful microorganisms in antimicrobial screenings. If these compounds are further studied and optimized, they may be effective antibacterial agents in various therapeutic settings.

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