Applied Chemical Engineering (2024) Volume 7 Issue 3
doi: 10.59429/ace.v7i3.5527

ORIGINAL RESEARCH ARTICLE

Coumarins from toxic phenol: An algorithm of their synthesis and assessment as biosafe, wide-spectrum, potent antimicrobial prospects

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ABSTRACT

The existential war between pathogens and humans has heavily intensified during the last few decades. The former war side has been strengthened by developing various mechanisms of resistance to the currently-in-use antimicrobial drugs. To overcome the consequences of this development, it becomes an urgent global request to explore new potent, wider-ranging, and biosafe prospects as antimicrobial medications. In response to this request, this work was designed to include three parts. In the first one, coumarin-based compounds were created using a toxic material named 2-methyl-3,5-dinitrophenol as a starting block. The Pechmann condensation reaction was conducted to convert this building block to the precursor, **P-MDNP**, which was esterified with various phenols to create **MDNPU1–MDNPU10**. The antimicrobial function was evaluated in the second study part using a broth microdilution approach and three standards, including ciprofloxacin, metronidazole, and nystatin. The studied pathogens were four-infectious bacterial aerobes, four-infectious bacterial anaerobes, and two-infectious fungi. Given the third study part, the biosafety of the synthesized compounds was quantified on the three healthy cellular species, two non-infectious aerobic bacteriomers, and human blood processed in the lab. The synthesized compounds showed strong, wide-ranging, and biosafe antimicrobial properties versus the pathogens examined, according to the outcomes. Moreover, the study showed that some of these compounds demonstrated anti-anaerobic bacterial activity that is superior to metronidazole. Furthermore, the study found a connection between the number and distribution of chlorides in the off-side aromatic rings, antimicrobial activity, and biosafety. Finally, it is determined that the health-damaging effects of the toxicant under study can be mitigated by grafting it into coumarin frameworks. These are potent, ascribed to **MDNPU9**, and have great levels of biosafety and wider-ranging antimicrobial efficacy. Furthermore, this approach offered the chance to turn the health-detrimental effects of the nitrophenols into potential benefits. Coumarin-4-acetic acid and **MDNPU9** can be employed as a synthetic fragment and a bioactive scaffold, respectively, to accomplish this.

Keywords: Antimicrobial; Biosafety; Coumarin; Molecular hybridization; Toxic dinitrophenol

ARTICLE INFO

Received: 16 August 2024
Accepted: 4 September 2024
Available online: 13 September 2024

1. Introduction

2-Methyl-3,5-dinitrophenol (MDNP, 3,5-dinitro-2-hydroxytoluene) is a synthetic dinitrophenol that is extremely toxic to mammals since it hinders the capacity of cells to create adenosine triphosphate, which serves as a cellular fuel. MDNP, one of the earliest pesticides ever developed, has been utilized as an insecticide and as an herbicide, but the USA has forbidden its utilization since 1991 because of increased poisoning cases[1]. Medical characteristics of MDNP acute poisoning include confusion, weariness, difficulty of breath, and sweating, which might come from intake or other contact pathways[2].

The structural transformation of forbidden-utilized materials enables them to be reused into valuable items for human applications.

The changed molecules may have had the same or different biological functions under this technique, but the adverse effects will always be mitigated or minimized[3]. This technique can be implemented by changing existing substituents, adding functionality, or enclosing the entire material under study in a separate structure[4]. The most notable characteristics are the wide range of biological properties and a long tradition of biosafety [5–7]. Both of those characteristics are typically found in natural-generated platforms, such as sugars, flavonoids, and coumarins[8–10]. The researcher and his fellow investigators have been exploring the biological profiling of coumarin-based products for more than two decades; hence, the last category, coumarins, has been chosen for the purpose of this study[11–13].

Since the initial identification of coumarin over 140 years ago, molecules based on coumarin cores have been extensively investigated. Natural coumarins have been found in a variety of phytoorganisms, microorganisms, and mammals[14]. Traditional and modern catalysts have been employed in a number of procedures to create man-made coumarins[15–18]. Multiple studies have proven the diverse biological functions of both natural-found and lab-synthesized coumarins. Coumarins in both forms have a diverse functional group inventory. Anti-oxidative stress[19], antibacterial[20], antifungal[21], anti-dementia[22], anti-Alzheimer's[23], anti-inflammatory[24], anticholinergic[25], anticancer[25], and analgesic attributes[26] were among the identified biomedical traits.

The struggle of humans against infectious agents has begun but is still ongoing, with the tendency fluctuating between the two sides[27]. The powerful fighting capacity of these microbes was enhanced thanks to the benefits of incorrect antibiotic use[28], the development of resistance to currently-in-clinical use drugs[29], acute and chronic off-target effects[30], and many immunity-dropping internal/ external variables[31]. So, this struggle creates a worldwide health instance, demanding the manufacture of antimicrobial medicines with two crucial features: extensive spectrum actions and biosafe profiles[32]. To the greatest extent of my comprehension, no natural or manmade substance has biosafe antimicrobial properties against infection-induced aerobic and anaerobic bacteria and fungi.

Given the knowledge provided above, the purpose of this project is to convert the hazardous dinitrophenol MDNP into antimicrobial agents with three key assets: synthesis, wide-ranging action, and biosafety. To do this, MDNP and 2-acetyl-2-chloromalonic acid were combined via the Pechmann process, yielding the precursor known as **P-MDNPU**. $SOCl_2$-facilitated esterification yielded ten **P-MDNPU** aromatic esterified counterparts, named **MDNPU1**-**MDNPU10**. The aromatic equivalents used were various mono-, di-, and trisubstituted phenol-containing compounds.

The **P-MDNPU** and its aromatic esters were subjected to both microbiology and biosafety tests. Using a developing medium-diluted technique, the created coumarins were evaluated in the previous assessment for their potential as wide-ranging antimicrobials. The gold standards for four-infectious bacterial aerobes, four-infectious bacterial anaerobes, and two-infectious fungi were ciprofloxacin (Cipro), metronidazole (Met), and nystatin (Nys), respectively. *Escherichia coli* (A-Ec), *Shigella dysenteriae* (A-Sd), *Salmonella typhi* (A-St), and *Klebsiella pneumoniae* (A-Kp) are the names and identification codes for the infectious bacterial aerobes. *Fusobacterium necrophorum* (N-Fn), *Clostridium perfringens* (N-Cp), *Prevotella melaninogenica* (N-Pm), and *Bacteroides fragilis* (N-Bf) were the infectious bacterial anaerobes employed. As infectious fungi, *Aspergillus niger* (F-An) and *Candida albicans* (F-Ca) were employed in the study.

Determining if the synthesized coumarins were chemicals that were compatible with the biological environment was the aim of the biosafety assessment. This goal was achieved by observing the effects of these coumarins on three healthy cellular species, two non-infectious aerobic bacteriomers, and human blood processed in the lab. The former species were recognized and given identification codes: HEK-293 (H-1, human embryonic renal-derived cells-293), RWPE-1 (H-2, human prostate-derived epithelial cells), and MCF-

A10 (H-3, human mammary gland-derived cells). On the other hand, the identified and certified bacteriomers in question were normal flora-derived *E. coli* MG1655 (NF-1) and BAA-1427 (NF-2).

2. MATERIALS AND METHODS

P-MDNPU and its corresponding aromatic esterified counterparts were created using ingredients from numerous international-recognized resources, and the ability of the compounds under study to provide broad-spectrum, biosafe antimicrobial qualities was evaluated. BT-LAB, Bioworld, BioVision, Haihang, Sigma-Aldrich, Scharlau, Chem-Lab, and Labcorp are a few of these vendors. The melting points (mp) of **P-MDNPU** and its aromatic esters were determined using a single tip vessel approach with CIA 9300 research equipment that was computer-operated. The researcher tracked the changes in the molecular chemistry and verified that all impurities had been eliminated from the counterfeits using thin-layer chromatography (TLC). This technique employed silicon dioxide on a Millipore SigmaTM Chromatogram as the solid phase and benzene-to-MeOH (3:1) as the fluid in movement. Using the spectrum-detecting devices Bruker ATR, Avance III HD (Bruker, DMSO-d_6, 100 MHz for ^{13}C-NMR and 400 MHz for ^{1}H-NMR), Shimadzu Single Quadrupole-2020 LC-MS (taken in plus-ve settings while employing CH_4 as an ESI gas), and UV-1600PC UV-Vis, the IR, NMR, mass, and UV/Vis spectra of **P-MDNPU** and its aromatic esters were determined.

2.1. Synthetic pathway

2.1.1. Synthesis of P-MDNPU

A 20-ml-concentrated H_2SO_4 was used to dissolve 2-acetyl-2-chloromalonic acid (1.08 g, 6 mmol) and **MDNP** (0.99 mg, 5 mmol). The thermostatically-controlled sonicator (410-Power Sonic, Korea) was adjusted at 45 °C and used to irradiate the working solution for 40 minutes. The sonicated solution was one-portion-poured into a blend of ice and H_2O; the formed solid was filtered, washed multiple times with cold H_2O, and left to water off in a lab setting[33].

P-MDNPU: Yellow powder, %yield = 56%, R_f = 0.10, λ_{max} (MeOH) = 502 nm, and mp = 159-161 °C. IR (cm^{-1}): Broad band centered at 3002 carboxylic acid OH, 3065 alkene CH, 2912 as well as 2856 alkane CH, 1730 lactone ester C=O, 1690 carboxylic acid dimer C=O, 1585 alkene C=C, 1562 aromatic C=C, 1510 nitro group, and 888 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-d_6): 11.08 (1H, s, H-12), 9.12 (1H, s, H-6), 3.06 (2H, s, H-11), and 2.53 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-d_6): 170.4 C-12, 161.6 C-2, 157.5 C-4, 154.3 C-9, 152.7 C-7, 145.6 C-5, 133.8 C-8, 130.6 C-10, 118.1 C-6, 114.6 C-3, 36.8 C-11, and 18.9 8-CH_3. LC-MS (ESI, m/z): 365 $[M+Na]^+$, 357 $[M+CH_3]^+$, 343 $[M+H]^+$, and 342 $[M]^+$.

2.1.2. General method for synthesizing MDNPU1–MDNPU10

A 15-ml-redistilled $SOCl_2$ was used in excess to dissolve **P-MDNPU** (0.68 g, 2 mmol), and the resulted solution was stirred under dry conditions in an ice-water bath for 30 minutes. For the same time frame, the working solution was stirred at 25 °C and then refluxed for 3 hours. The excess of $SOCl_2$ was subjected to reduced pressure evaporation, and the solid was treated with a 15-ml-dried diethyl ether solution of 2 mmol of a particular phenol. The blend was refluxed for 3 hours, poured into an ice-H_2O mixture, and the organic phase was separated, H_2O-dried, and vaporized. A MeOH-toluene crystallization was used to purify the final compound[34].

MDNPU1: Yellowish powder, %yield = 76%, R_f = 0.22, λ_{max} (MeOH) = 519 nm, and mp = 122-124 °C. IR (cm^{-1}): 3067 alkene CH, 2907 as well as 2862 alkane CH, 1732 lactone ester C=O, 1711 alkyl-arene ester C=O, 1590 alkene C=C, 1556 aromatic C=C, 1511 nitro group, 886 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-d_6): 9.11 (1H, s, H-6), 7.30 (2H, d, J = 8 Hz, H-16 and H-18), 7.18 (2H, d, J = 8 Hz, H-15 and H-19), 3.05 (2H, s, H-11), 2.51 (3H, s, 8-CH_3), and 2.46 (3H, s, 17-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-d_6): 169.3 C-12, 161.7 C-2, 157.4 C-4, 154.4 C-9, 152.7 C-7, 146.2 C-14, 145.6 C-5, 137.2 C-17, 133.3 C-8, 131.9

C-16 as well as C-18, 130.6 C-10, 122.8 C-15 as well as C-19, 118.0 C-6, 114.6 C-3, 36.7 C-11, 23.6 17-CH_3, and 18.9 8-CH_3. LC-MS (ESI, m/z): 455 $[M+Na]^+$, 447 $[M+CH_3]^+$, 433 $[M+H]^+$, and 432 $[M]^+$.

MDNPU2: Yellowish powder, %yield = 80%, R_f = 0.26, λ_{max} (MeOH) = 526 nm, and mp = 138-140 °C. IR (cm^{-1}): 3065 alkene CH, 2906 as well as 2862 alkane CH, 1734 lactone ester C=O, 1711 alkyl-arene ester C=O, 1588 alkene C=C, 1555 aromatic C=C, 1511 nitro group, 1235 as well as 1066 alkyl-arene ether C-O-C, and 889 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-*d*$_6$): 9.10 (1H, s, H-6), 7.12 (2H, d, *J* = 8 Hz, H-15 and H-19), 7.03 (2H, d, *J* = 8 Hz, H-16 and H-18), 3.96 (3H, s, 17-OCH_3), 3.05 (2H, s, H-11), and 2.51 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-*d*$_6$): 169.2 C-12, 161.6 C-2, 158.2 C-17, 157.4 C-4, 154.3 C-9, 152.7 C-7, 145.9 C-14, 145.6 C-5, 133.3 C-8, 130.6 C-10, 123.8 C-15 as well as C-19, 118.0 C-6, 116.1 C-16 as well as C-18, 114.6 C-3, 56.4 17-OCH_3, 36.7 C-11, and 18.8 8-CH_3. LC-MS (ESI, m/z): 471 $[M+Na]^+$, 463 $[M+CH_3]^+$, 449 $[M+H]^+$, and 448 $[M]^+$.

MDNPU3: Yellowish powder, %yield = 54% (0.45 g), R_f = 0.15, λ_{max} (MeOH) = 518 nm, and mp = 146-148 °C. IR (cm^{-1}): 3063 alkene CH, 2905 as well as 2860 alkane CH, 1734 lactone ester C=O, 1710 alkyl-arene ester C=O, 1589 alkene C=C, 1558 aromatic C=C, 1513 nitro group, 1103 arene fluoride C-F, 885 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-*d*$_6$): 9.11 (1H, s, H-6), 7.37 (2H, d, *J* = 8 Hz, H-15 and H-19), 7.26 (2H, d, *J* = 8 Hz, H-16 and H-18), 3.05 (2H, s, H-11), and 2.52 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-*d*$_6$): 169.1 C-12, 161.5 C-2, 160.3 C-17, 157.4 C-4, 154.3 C-9, 152.8 C-7, 149.2 C-14, 145.6 C-5, 133.4 C-8, 130.6 C-10, 125.3 C-15 as well as C-19, 118.0 C-6, 117.1 C-16 as well as C-18, 114.6 C-3, 36.7 C-11, and 18.7 8-CH_3. LC-MS (ESI, m/z): 459 $[M+Na]^+$, 451 $[M+CH_3]^+$, 437 $[M+H]^+$, and 436 $[M]^+$.

MDNPU4: Yellowish powder, %yield = 57%, R_f = 0.13, λ_{max} (MeOH) = 516 nm, and mp = 140-142 °C. IR (cm^{-1}): 3066 alkene CH, 2902 as well as 2863 alkane CH, 1734 lactone ester C=O, 1708 alkyl-arene ester C=O, 1590 alkene C=C, 1556 aromatic C=C, 1514 nitro group, 993 arene chloride C-Cl, and 885 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-*d*$_6$): 9.12 (1H, s, H-6), 7.56 (2H, d, *J* = 8 Hz, H-16 and H-18), 7.49 (2H, d, *J* = 8 Hz, H-15 and H-19), 3.05 (2H, s, H-11), and 2.51 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-*d*$_6$): 169.2 C-12, 161.4 C-2, 157.4 C-4, 154.3 C-9, 152.8 C-7, 151.5 C-14, 145.7 C-5, 140.1 C-16 as well as C-18, 139.2 C-17, 133.4 C-8, 130.6 C-10, 124.6 C-15 as well as C-19, 118.0 C-6, 114.5 C-3, 36.7 C-11, and 18.7 8-CH_3. LC-MS (ESI, m/z): 475 $[M+Na]^+$, 467 $[M+CH_3]^+$, 453 $[M+H]^+$, and 452 $[M]^+$.

MDNPU5: Yellowish powder, %yield = 48%, R_f = 0.17, λ_{max} (MeOH) = 511 nm, and mp = 127-129 °C. IR (cm^{-1}): 3065 alkene CH, 2900 as well as 2861 alkane CH, 1735 lactone ester C=O, 1711 alkyl-arene ester C=O, 1587 alkene C=C, 1555 aromatic C=C, 1515 nitro group, 889 alkene C-Cl, and 823 arene bromide C-Br. ^{1}H-NMR (400 MHz, ppm, DMSO-*d*$_6$): 9.11 (1H, s, H-6), 7.67 (2H, d, *J* = 8 Hz, H-16 and H-18), 7.28 (2H, d, *J* = 8 Hz, H-15 and H-19), 3.05 (2H, s, H-11), and 2.51 (3H, s, 8-CH_3). ^{13C}NMR (100 MHz, ppm, DMSO-*d*$_6$): 169.1 C-12, 161.4 C-2, 157.3 C-4, 154.3 C-9, 152.8 C-7, 151.9 C-14, 145.7 C-5, 134.7 C-16 as well as C-18, 133.4 C-8, 130.6 C-10, 125.0 C-15 as well as C-19, 121.3 C-17, 118.1 C-6, 114.5 C-3, 36.8 C-11, and 18.9 8-CH_3. LC-MS (ESI, m/z): 519 $[M+Na]^+$, 511 $[M+CH_3]^+$, 497 $[M+H]^+$, and 496 $[M]^+$.

MDNPU6: Yellowish powder, %yield = 45%, R_f = 0.19, λ_{max} (MeOH) = 509 nm, and mp = 121-123 °C. IR (cm^{-1}): 3065 alkene CH, 2901 as well as 2864 alkane CH, 1734 lactone ester C=O, 1710 alkyl-arene ester C=O, 1588 alkene C=C, 1556 aromatic C=C, 1511 nitro group, 892 alkene C-Cl, and 804 arene iodide C-I. ^{1}H-NMR (400 MHz, ppm, DMSO-*d*$_6$): 9.10 (1H, s, H-6), 7.85 (2H, d, *J* = 8 Hz, H-16 and H-18), 7.12 (2H, d, *J* = 8 Hz, H-15 and H-19), 3.04 (2H, s, H-11), and 2.52 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-*d*$_6$): 169.1 C-12, 161.4 C-2, 157.3 C-4, 154.3 C-9, 153.8 C-14, 152.6 C-7, 145.7 C-5, 140.9 C-16 as well as C-18, 133.4 C-8, 130.6 C-10, 124.4 C-15 as well as C-19, 118.2 C-6, 114.5 C-3, 89.8 C-17, 36.7 C-11, and 18.6 8-CH_3. LC-MS (ESI, m/z): 567 $[M+Na]^+$, 559 $[M+CH_3]^+$, 545 $[M+H]^+$, and 544 $[M]^+$.

MDNPU7: Yellowish powder, %yield = 73%, R_f = 0.23, λ_{max} (MeOH) = 523 nm, and mp = 150-152 °C. IR (cm^{-1}): 3066 alkene CH, 2905 as well as 2860 alkane CH, 1734 lactone ester C=O, 1710 alkyl-arene ester

C=O, 1587 alkene C=C, 1556 aromatic C=C, 1510 nitro group, 1235 as well as 1066 alkyl-arene ether C-O-C, 1002 arene-chloride C-Cl, and 887 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-d_6): 9.10 (1H, s, H-6), 7.33 (2H, s, H-15 and H-19), 3.94 (3H, s, 17-OCH_3), 3.06 (2H, s, H-11), and 2.52 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-d_6): 169.2 C-12, 161.6 C-2, 157.4 C-4, 154.3 C-9, 153.8 C-17, 152.7 C-7, 146.7 C-14, 145.6 C-5, 133.3 C-8, 130.6 C-10, 125.7 C-16 as well as C-18, 123.6 C-15 as well as C-19, 118.0 C-6, 114.6 C-3, 63.4 17-OCH_3, 36.7 C-11, and 18.9 8-CH_3. LC-MS (ESI, m/z): 539 $[M+Na]^+$, 531 $[M+CH_3]^+$, 517 $[M+H]^+$, and 516 $[M]^+$.

MDNPU8: Dark-yellow powder, %yield = 82%, R_f = 0.30, λ_{max} (MeOH) = 550 nm, and mp = 143-145 °C. IR (cm^{-1}): 3065 alkene CH, 2904 as well as 2860 alkane CH, 1734 lactone ester C=O, 1709 alkyl-arene ester C=O, 1587 alkene C=C, 1556 aromatic C=C, 1510 nitro group, 1238 as well as 1070 alkyl-arene ether C-O-C, 998 arene-chloride C-Cl, and 885 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-d_6): 9.09 (1H, s, H-6), 7.30 (2H, s, H-15 and H-19), 3.91 (6H, s, 16-OCH_3 and 18-OCH_3), 3.06 (2H, s, H-11), and 2.51 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-d_6): 169.2 C-12, 161.7 C-2, 159.1 C-16 as well as C-18, 157.4 C-4, 154.3 C-9, 152.3 C-14, 152.7 C-7, 145.6 C-5, 133.3 C-8, 130.6 C-10, 118.0 C-6, 114.6 C-3, 107.8 C-17, 103.5 C-15 as well as C-19, 61.2 16-OCH_3 as well as 18-OCH_3, 36.7 C-11, and 18.9 8-CH_3. LC-MS (ESI, m/z): 535 $[M+Na]^+$, 527 $[M+CH_3]^+$, 513 $[M+H]^+$, and 512 $[M]^+$.

MDNPU9: Yellowish powder, %yield = 36%, R_f = 0.20, λ_{max} (MeOH) = 505 nm, and mp = 165-167 °C. IR (cm^{-1}): 3065 alkene CH, 2904 as well as 2860 alkane CH, 1734 lactone ester C=O, 1710 alkyl-arene ester C=O, 1587 alkene C=C, 1555 aromatic C=C, 1512 nitro group, 1235 as well as 1064 alkyl-arene ether C-O-C, 1010 arene-chloride C-Cl, 884 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-d_6): 9.12 (1H, s, H-6), 7.60 (2H, s, H-15 and H-19), 3.05 (2H, s, H-11), and 2.54 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-d_6): 169.3 C-12, 161.6 C-2, 157.4 C-4, 156.1 C-14, 154.3 C-9, 152.7 C-7, 145.6 C-5, 137.6 C-16 as well as C-18, 133.3 C-8, 132.0 C-17, 130.6 C-10, 123.4 C-15 as well as C-19, 118.0 C-6, 114.5 C-3, 36.7 C-11, and 18.9 8-CH_3. LC-MS (ESI, m/z): 543 $[M+Na]^+$, 535 $[M+CH_3]^+$, 521 $[M+H]^+$, and 520 $[M]^+$.

MDNPU10: Dark-yellow powder, %yield = 86%, R_f = 0.35, λ_{max} (MeOH) = 560 nm, and mp = 173-175 °C. IR (cm^{-1}): 3066 alkene CH, 2902 as well as 2864 alkane CH, 1733 lactone ester C=O, 1711 alkyl-arene ester C=O, 1585 alkene C=C, 1558 aromatic C=C, 1515 nitro group, 1239 as well as 1073 alkyl-arene ether C-O-C, and 889 alkene C-Cl. ^{1}H-NMR (400 MHz, ppm, DMSO-d_6): 9.12 (1H, s, H-6), 6.35 (2H, s, H-15 and H-19), 3.93 (9H, s, 16-OCH_3, 17-CH_3, and 18-OCH_3), 3.06 (2H, s, H-11), and 2.51 (3H, s, 8-CH_3). ^{13}C-NMR (100 MHz, ppm, DMSO-d_6): 169.1 C-12, 161.7 C-2, 157.4 C-4, 155.2 C-16 as well as C-18, 154.3 C-9, 152.7 C-7, 148.4 C-14, 145.5 C-5, 138.4 C-17, 133.3 C-8, 130.5 C-10, 118.0 C-6, 114.6 C-3, 99.1 C-15 as well as C-19, 65.2 17-OCH_3, 61.3 16-OCH_3 as well as 18-OCH_3, 36.6 C-11, and 18.4 8-CH_3. LC-MS (ESI, m/z): 531 $[M+Na]^+$, 523 $[M+CH_3]^+$, 509 $[M+H]^+$, and 508 $[M]^+$.

2.2. Antimicrobial activity assessments

2.2.1. Methodology for anti-infectious aerobic bacteriomers

To put it briefly, 7.5 mg of a research compound was dissolved in 5 ml of DMSO to create a starting solution. From then on, thirteen diluted concentrations ranging in quantity from 1024 to 0.25 µg/ml were obtained using a H_2O-doubling continual approach. The following were added to a designated research container: a 1 ml-designated concentration, 2 ml of a 0.5 McFarland-calibrated bacterial inoculant, and a 3 ml-broth of Mueller-Hinton (bacterial growth medium). Following a 24-hour lab incubation period at 37 °C, the turbidity resulting from bacterial growth was monitored by eye. The Minimum Inhibitory Concentration (MIC) value represented the concentration of the eye-detected clear research container. By increasing the MIC concentration in an ascending order at 4, 1, 0.5, or 0.05 frequencies, the Minimum Bactericidal Concentration (MBC) value was found. With the second round of dilutions, the previously specified organized steps were

carried out once more. Finally, by dividing the value of MBC over that of the MIC, the potency detector (PD) statistic for each research compound was determined[35].

2.2.2. Methodology for anti-infectious anaerobic bacteriomers

Upon considering some noteworthy distinctions, a methodology analogous to that employed in assessing the role of anti-infectious aerobes was picked. The environment used to lab-incubate anaerobic microorganisms for 48 hours at 37 °C has some maintenance issues. These comprise an anaerobe probe, an oxygen-free atmosphere (10% H_2 gas, 10% CO_2 gas, and 80% N_2 gas), Brucella blood agar (5% sheep blood) as a growth medium, and palladium metal as an inducer[36].

2.2.3. Methodology for anti-infectious fungiomers

This assessment involved two changes to the abovementioned anti-infectious aerobes functioning approach. These included the employment of Sabouraud-dextrose broth and 48-hour lab incubation at 30 °C as parts of the handling conditions[37].

2.3. Biosafety inspection

2.3.1. Detection of cytocompatibility

For every item under research, a DMSO (1 mg/ml) solution was made and used to prepare seven H_2O-diluted concentrations, ranging from 400 to 6.25 µg/ml. Subsequently, 10,000 designated healthy cells were plated on a 96-well plate, capped with a growth medium, and allowed to lab-develop for 24 hours. Subsequently, an already assembled dilute concentration was introduced to each well individually. Following the growing medium's discharge, the MTT reagent (28.0 µl, 3.27 mM) was incorporated to assess the vitality of the cells after a 72-hour contact. After that, the wells in question were kept at 37 °C for an extra 1.5 hours and investigated spectrophotometrically at 492 nm. The absorbance readings for the treated and control wells were used to compute the inhibition percentages by applying the following math equation: absorbance of the control well minus that of the treated one, over the control absorbance, multiplied by 100. Graphing these percentages versus their corresponding logarithmic concentrations, the IC_{50} score for the research compound was calculated using GraphPad Prism software. To validate the results and the applied methodology, the abovementioned workflows were repeated independently three times to specify the scores of the standard deviation (SD)[38].

2.3.2. Detection the compatibility with microbiota

With one exception—the kind of bacterial strains studied—the methodology used to evaluate the anti-infectious aerobes activity of the research compounds was applied here[39].

2.3.3. Biocompatibility with human blood erythrocytes

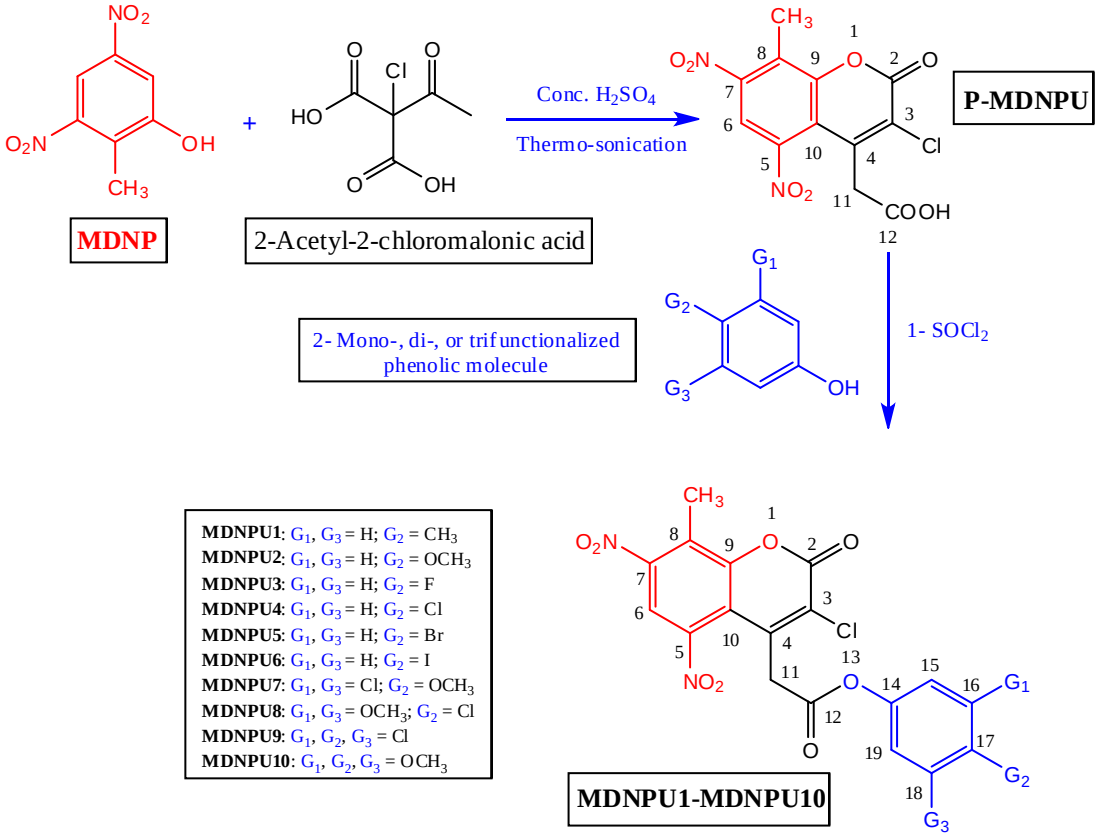
Healthy colleagues aged between 24-28 years were provided the blood samples with the permission (No. DPC-2024-55170E) of the scientific committee of the Pharmaceutical Chemistry Department, College of Pharmacy, University of Mosul. Briefly, a 1 ml blood specimen was quickly added to an EDTA-lab vessel. By spinning the unclotted blood at 14,000 rpm for 5 minutes, the erythrocyte dispersion was obtained and subsequently diluted by a phosphate buffer solution (pH = 7.2) up to 0.2 ml. This volume was mixed equivocally with a research compound (50, 100, 200, or 400 µg/ml), and the resultant mixture was lab-incubated for 1 hour at 37 °C and spun at 10,000 rpm for 10 minutes. When these workflows were applied to the compounds under research, the collected specimens were added to a 96-well array. The absorbance values of these species were detected at 540 nm via a microarray detector (BioTek-405, USA) and used to quantify the erythrocyte hemolysis percentage (EH%)[40]. This was computed by applying the following formula: (sample absorbance minus negative control absorbance) / (positive control absorbance minus negative control

absorbance). Triton X-100 and DMSO represent the positive and negative controls, respectively. For three independent trials, the SD values were calculated to validate the outcomes of this experiment[41].

3. RESULTS AND DISCUSSION

3.1. Synthetic workflow

Scheme 1 illustrates the easy-to-perform processes employed to create the **P-MDNPU** and its aromatic esters (**MDNPU1**–**MDNPU10**). Given a synthesized compound, its physical and chemical properties, together with the interpretive data derived from the analyzed spectra, are recorded next to the synthetic methodology.



Scheme 1. The synthetic plan utilized to create **P-MDNPU** and its aromatic esters (**MDNPU1**–**MDNPU10**). The in-side and off-side rings are drawn in red and blue, respectively.

3.2. Findings of antimicrobial activity evaluation

Given the antimicrobial qualities of **P-MDNPU** and its aromatic esters (**MDNPU1**–**MDNPU10**), three investigations were initiated. These include two-infectious fungiomers, four-infectious aerobic bacteriomers, and four-infectious anaerobic bacteriomers. In addition, three established standards (Cipro, Met, and Nys), thirteen aqueous-diluted concentrations with determinations ranging from 1024 to 0.25 g/ml, and broth microdilution procedures were included in this sort of study.

The antimicrobial qualities of the research compounds are shown in **Table 1**, with the findings indicating both broad and particular features. Given the broad ones, the compounds under research have demonstrated satisfactory effectiveness against the examined microbes as compared to the standards. Furthermore, compared to the **MDNPU7**–**MDNPU10** (trisubstituted off-side aromatics), the effectiveness of the **P-MDNPU** and the **MDNPU1**–**MDNPU6** (monosubstituted off-side aromatics) is reduced. Moreover, **MDNPU9**, which includes three chlorides, is the outstanding compound for the former category, whereas the range of activity fluctuates slightly for the latter category. Furthermore, the compounds containing chloride functionalities on the off-side

aromatics had the strongest antimicrobial qualities. These are increased when the number of these functionalities rises[42–45], and as a result, **MDNPU9**, **MDNPU7**, **MDNPU8**, and **MDNPU4** are the activity configurations within chloride-containing compounds. Lastly, taking into account predicted levels of PD with values below 4, the research compounds' effect against the studied microbes may be designated as microbiocidal in contrast to microbiostatic[46–48].

The researcher made a number of remarks about the particular features. According to their MIC values, **MDNPU9** and **MDNPU7** are assigned preferred characteristics, and the research compounds perform stronger against N-Bf than Met. Additionally, of the eleven produced products, five work better against N-Fn than Met, with the same two compounds responsible for the best achievements. Furthermore, of all the research compounds, **MDNPU9** is the only one that demonstrates a more robust function against N-Pm than the reference. However, compared to Nys, all of the compounds under research performed better against infectious fungiomers, with **MDNPU9** and **P-MDNPU** demonstrating the most promising outcomes.

It is possible to draw two conclusions from the general and specific features stated above. The first is the strong and wide-ranging antimicrobial capacity of the research compounds against the pathogens being studied. The second is that against three prevalent contagious anaerobic bacteria, this is the first study describing coumarin-derived compounds that perform better than Met[49]. It is possible to draw two conclusions from the general and specific features stated above. The first is the strong and wide-ranging antimicrobial capacity of the research compounds against the pathogens being studied. The second is that, against three prevalent contagious anaerobic bacteria, this is the first study describing coumarin-derived compounds that perform better than Met. In reference to the latter matter, it is suggested that two structural traits confer this preferred role on the research compound, **MDNPU9**, in comparison to those with comparable frameworks[36,38,50]. These are the two nitrofunctionalities on the in-side aromatic ring and the three chlorides on the off-side aromatic ring. The latter characteristic might be in charge of the activity's strength, while the former might define the kind of activity[51–53]. This is because the principal amine congeners that these nitro groups produce inside anaerobic bacteriomers appear to be fatal to these organisms through a reduction reaction[54]. **Figure 1** displays the computed MIC values regarding the research compounds and their corresponding references against the pathogens studied.

Table 1. Numbering the antimicrobial qualities of the research compounds and their standards.

Code	Identification codes of the assessed infectious aerobes and their computed microbiological factors											
	A-St			A-Ec			A-Sd			A-Kp		
	MBC	MIC	PD	MBC	MIC	PD	MBC	MIC	PD	MBC	MIC	PD
Cipro	1.25	1.05	1.18	1.30	1.05	1.24	0.65	0.55	1.18	0.70	0.55	1.25
P-MDNPU	4.35	4.00	1.08	4.10	3.85	1.06	4.25	3.90	1.09	4.05	3.55	1.13
MDNPU1	3.70	3.45	1.06	3.45	3.20	1.08	3.50	3.25	1.08	3.40	3.15	1.06
MDNPU2	3.35	3.10	1.07	3.20	3.05	1.05	3.35	3.10	1.08	3.20	3.05	1.07
MDNPU3	3.95	3.60	1.09	3.61	3.40	1.06	3.55	3.45	1.03	3.60	3.30	1.08
MDNPU4	3.00	2.80	1.06	2.85	2.65	1.08	2.95	2.65	1.11	3.00	2.70	1.10
MDNPU5	4.10	3.80	1.09	3.90	3.55	1.10	3.85	3.60	1.07	3.80	3.45	1.12
MDNPU6	4.00	3.65	1.11	3.70	3.45	1.07	3.85	3.50	1.10	3.70	3.45	1.04
MDNPU7	3.10	2.85	1.08	2.90	2.70	1.07	2.85	2.60	1.10	2.70	2.55	1.02
MDNPU8	3.10	2.90	1.05	2.90	2.75	1.05	2.70	2.55	1.06	2.85	2.60	1.11
MDNPU9	2.45	2.15	1.13	2.20	2.10	1.05	2.40	2.25	1.07	2.30	2.20	1.06
MDNPU10	3.70	3.30	1.11	3.30	3.15	1.05	3.40	3.20	1.06	3.20	3.10	1.05

Code	Identification codes of the assessed infectious anaerobes and their computed microbiological factors											
	N-Pm			N-Fn			N-Cp			N-Bf		
	MBC	MIC	PD	MBC	MIC	PD	MBC	MIC	PD	MBC	MIC	PD
Met	1.00	0.85	1.17	2.05	1.90	1.07	0.95	0.75	1.26	3.55	3.05	1.17
P-MDNPU	3.95	3.80	1.03	3.20	3.05	1.04	2.35	2.20	1.05	2.70	2.30	1.15
MDNPU1	2.65	2.45	1.07	2.95	2.70	1.08	2.05	1.35	1.50	1.55	1.35	1.14
MDNPU2	1.50	1.25	1.13	0.95	0.80	1.14	1.45	1.20	1.20	1.60	1.40	1.17
MDNPU3	2.30	2.10	1.12	2.75	2.55	1.05	1.85	1.70	1.08	2.15	1.65	1.22
MDNPU4	1.55	1.30	1.17	0.90	0.65	1.37	1.95	1.70	1.16	1.45	1.30	1.11
MDNPU5	2.50	2.25	1.12	3.05	2.80	1.08	2.10	1.25	1.61	2.40	2.10	1.16
MDNPU6	2.45	2.15	1.11	2.95	2.70	1.04	1.40	1.20	1.13	2.30	2.15	1.09
MDNPU7	1.30	1.20	1.04	0.85	0.55	1.53	1.70	1.60	1.02	1.25	1.20	1.05
MDNPU8	1.45	1.10	1.33	1.00	0.80	1.22	1.50	1.35	1.10	1.50	1.25	1.22
MDNPU9	0.35	0.30	1.15	0.60	0.45	1.30	1.85	1.60	1.12	1.30	1.15	1.15
MDNPU10	2.15	1.90	1.12	2.45	2.20	1.17	1.60	1.35	1.13	1.75	1.50	1.19

Code	Identification codes of the assessed infectious fungi and their computed microbiological factors					
	F-An			F-Ca		
	MFC	MIC	PD	MFC	MIC	PD
Nys	12.05	8.05	1.48	6.05	4.05	1.47
P-MDNPU	4.70	4.25	1.14	2.10	1.90	1.13
MDNPU1	5.25	5.00	1.03	2.55	2.30	1.12
MDNPU2	5.20	4.90	1.02	2.15	2.05	1.06
MDNPU3	5.60	5.20	1.07	2.95	2.70	1.08
MDNPU4	4.75	4.50	1.04	2.50	2.25	1.10
MDNPU5	6.10	5.85	1.02	3.10	2.80	1.12
MDNPU6	6.15	5.80	1.05	3.20	2.80	1.16
MDNPU7	4.45	4.30	1.06	2.60	2.15	1.22
MDNPU8	5.25	5.15	1.07	2.45	2.20	1.15
MDNPU9	4.95	3.80	1.27	2.00	1.75	1.12
MDNPU10	4.30	4.15	1.03	2.50	2.35	1.10

The unit of µg/ml was used to expressed the microbiological factors MBC, MIC, and MFC (Minimum Fungicidal Concentration).

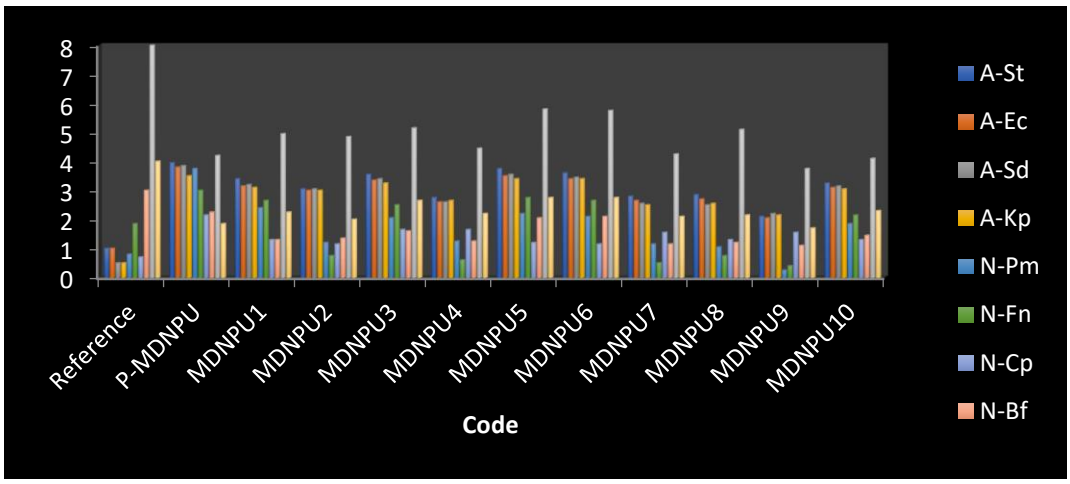


Figure 1. Graphical representation of the MIC values of the research compounds against the pathogens studied.

3.3. Findings of the biosafety evaluation

To evaluate the biosafety of **P-MDNPU** and its aromatic esters, **MDNPU1**–**MDNPU10**, three investigations were carried out. The findings of these investigations are displayed visually in **Figures 2-4** and in **Table 2**. The first investigation studied how the research compounds affected the regular multiplication of three healthy cellular species. MTT was used in this work as a visual analytical tool, while 5-fluorouracil (5-FU) served as the standard. In the second investigation, the impact of the compounds under research on the typical bacterial growth of two non-infectious populations was assessed. It used Cipro as a point of reference and broth-diluting as a working protocol. In the third and last investigation, actual human blood was used to examine the impact of the research compounds on the integrity of erythrocytes in lab conditions.

In light of the first investigation, the Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA) concluded in 2018 (https://www.atsdr.cdc.gov/ToxProfiles/tp63.pdf) that MDNP is not carcinogenic. Nevertheless, a number of investigations on both human and animal models have established this dinitrophenol's toxicity, which depends on the quantity, types of organisms, and duration of exposur[55–58]. There are three principal conclusions that were derived from the investigative ratings that are displayed in **Table 2**. To begin with, the research compounds showed more biocompatibility than conventional 5-FU. Additionally, the biosafety patterns displayed by these compounds were nearly identical to those of the three examined healthy cellular populations. Ultimately, **Figure 2** illustrates the superior results of the compounds with chloride-substituted off-side aromatics. These results improve as the number of chlorofunctionalities increases[59–61]. These three main conclusions are also included in the second investigation, as displayed in **Figure 3**, which looks at how the research compounds affect the regular development of two common flora species. Additionally, it was shown that these compounds produced a bactericidal effect rather than a bacteriostatic one on the microbes under study, according to the PD readings[62].

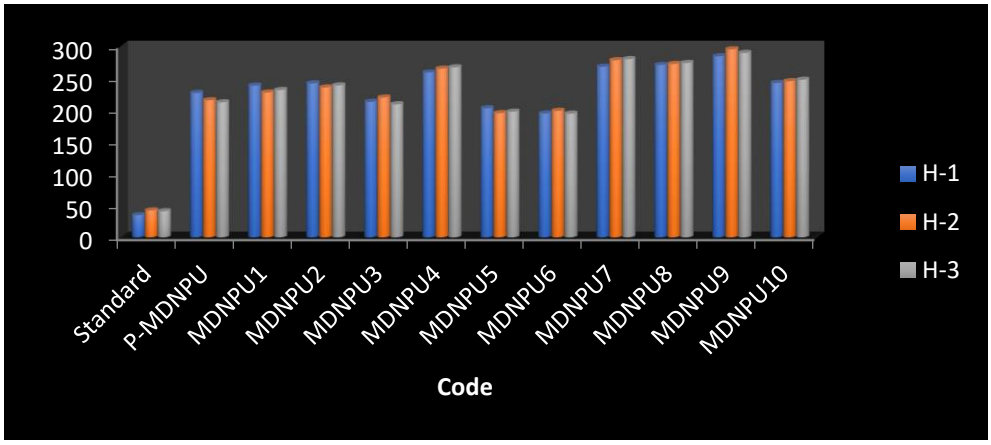


Figure 2. Graphical representation of the IC_{50} values of the research compounds toward the healthy cellular populations studied.

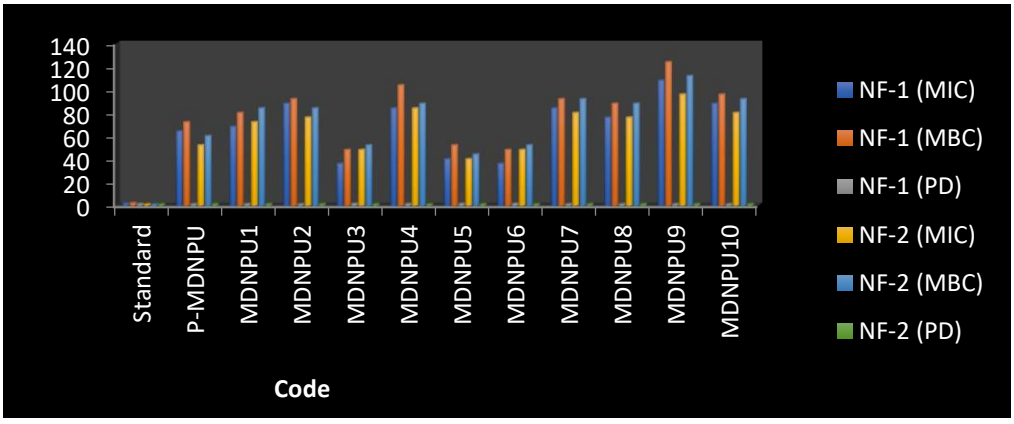


Figure 3. Graphical representation of the microbiological factors regarding the research compounds toward non-infectious aerobes studied.

The final investigation examined the potential of the research compounds to degrade or preserve red blood cells in a laboratory setting, as illustrated in **Figure 4**. This work used a 540 nm microarray detection device, four twofold-diluted dosages of the compounds under investigation, and normal human erythrocytes[63]. Additionally, three ASTM International (http://www.astm.org/) guidelines were used to specify the erythrocyte-hydrolyzing settings. The research compound falls into one of three categories: as a fully erythrocyte hemolyzer when the EH% number is greater than 5, as a moderately hemolytic reagent when the number rates between 2 and 5, and as an entirely erythrocyte maintainer when the value is less than 2[64]. Three conclusions are drawn from these guidelines, and an analysis of the percentage statistics associated with this investigation is shown in **Table 2** and **Figure 3**. First off, in comparison to **P-MDNPU**, the other research compounds showed perfect erythrocyte-maintaining capability even at the highest dose applied. The top-performing compounds are listed in descending order: **MDNPU9**, **MDNPU8**, **MDNPU2**, and **MDNPU7**. The second realization is that, at the highest dosage used, **P-MDNPU**, **MDNPU5**, and **MDNPU6** had a significant potential to damage erythrocytes. Lastly, keeping in mind the preceding statement, the EH% readings of the research compounds vary from one another somewhat rarely.

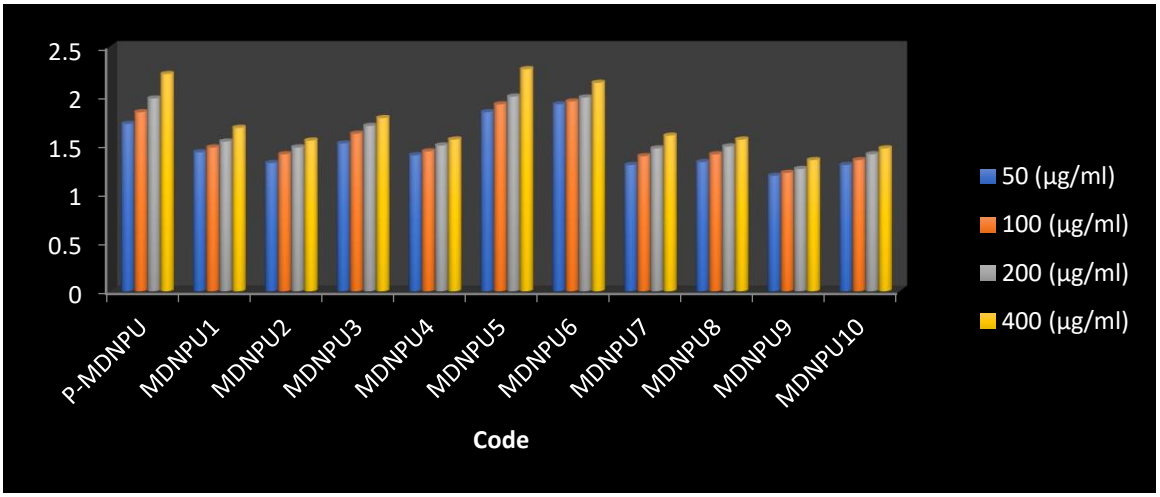


Figure 4. Graphical representation of the EH% values of the research compounds using normal human blood.

Table 2. Numbering the biosafety qualities of the research compounds and their standards.

Code	Identification codes of the assessed healthy cellular populations and their obtained IC_{50} (µg/ml) ± SD (n = 3)			Identification codes of the assessed non-aerobes and their obtained microbiological factors					
				NF-1			NF-2		
	H-1	H-2	H-3	MBC	MIC	PD	MBC	MIC	PD
Standard	35.78 ± 1.05	43.85 ± 1.04	41.47 ± 0.98	2.10	1.70	1.24	1.00	0.95	1.05
P-MDNPU	225.33 ± 0.88	213.54 ± 1.06	211.32 ± 1.04	72.05	64.05	1.12	60.05	52.05	1.15
MDNPU1	238.69 ± 1.03	227.34 ± 0.86	231.29 ± 1.10	80.05	68.05	1.18	84.05	72.05	1.17
MDNPU2	241.44 ± 1.01	235.23 ± 1.10	238.08 ± 1.07	92.05	88.05	1.05	84.05	76.05	1.11
MDNPU3	212.57 ± 0.89	219.79 ± 0.91	209.78 ± 1.08	48.05	36.05	1.33	52.05	48.05	1.08
MDNPU4	258.22 ± 1.02	265.54 ± 1.02	267.29 ± 1.01	104.05	84.05	1.24	88.05	84.05	1.05
MDNPU5	202.79 ± 1.04	195.22 ± 0.91	197.97 ± 0.85	52.05	40.05	1.30	44.05	40.05	1.10
MDNPU6	194.12 ± 1.08	198.60 ± 0.90	194.51 ± 1.07	48.05	36.05	1.33	52.05	48.05	1.08
MDNPU7	268.45 ± 1.08	278.94 ± 0.89	280.79 ± 0.85	92.05	84.05	1.10	92.05	80.05	1.15
MDNPU8	270.10 ± 0.95	272.54 ± 0.92	273.32 ± 0.93	88.05	76.05	1.16	88.05	76.05	1.16
MDNPU9	284.25 ± 0.96	295.98 ± 1.03	289.25 ± 1.01	124.05	108.05	1.15	112.05	96.05	1.17
MDNPU10	242.89 ± 1.07	245.23 ± 1.06	247.71 ± 1.09	96.05	88.05	1.09	92.05	80.05	1.15

Code	EH% ± SD (n = 3)			
	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml
P-MDNPU	1.54 ± 0.97	1.83 ± 0.88	1.99 ± 0.89	2.26 ± 0.94
MDNPU1	1.12 ± 0.79	1.47 ± 1.05	1.54 ± 0.91	1.69 ± 0.97
MDNPU2	1.58 ± 0.92	1.40 ± 0.97	1.50 ± 0.94	1.55 ± 0.89
MDNPU3	1.13 ± 0.95	1.58 ± 1.09	1.70 ± 0.92	1.78 ± 0.91
MDNPU4	1.22 ± 0.88	1.44 ± 1.09	1.51 ± 0.97	1.59 ± 0.89
MDNPU5	1.90 ± 0.91	1.95 ± 0.92	1.99± 0.829	2.29 ± 1.01
MDNPU6	1.67 ± 0.89	1.96 ± 0.94	1.97 ± 0.89	2.16 ± 0.96
MDNPU8	1.45 ± 0.85	1.39 ± 1.01	1.49 ± 1.08	1.61 ± 1.05
MDNPU7	1.56 ± 0.91	1.43 ± 0.99	1.49 ± 0.98	1.56 ± 0.89
MDNPU9	1.22 ± 1.08	1.24 ± 1.05	1.28 ± 0.89	1.36 ± 0.85
MDNPU10	1.32 ± 1.09	1.38 ± 0.89	1.43 ± 0.95	1.47 ± 0.91

The research compounds, in particular **MDNPU7**, have exceptional biocompatibility properties toward healthy cellular species, non-infectious aerobes, and human erythrocyte viability under examination settings, according to the findings of the biosafety investigations.

4. Conclusion

The results of this investigation have led to the conclusion that MDNP's harmful health effects can be lessened by turning it into coumarins. Additionally, the creation of efficient, wider-ranging, and biosafe antimicrobial alternatives was a consequence of this change in structure. Additionally, the number of chlorofunctionalities on the off-side aromatics of the research compounds is closely correlated with both biocompatibility and action against the studied microbes. Consequently, the structural modifications that have been made can create chances for the conversion of toxic phenolic compounds into biosafe and medicine-effective drug candidates.

5. Declarations

Availability of data The data are available from the author upon request.

Ethics approval Not applicable.

Consent to participate The corresponding author is the sole one in this work.

Consent for publication The author gives consent to the publication of the study.

Funding The article has not received any funds.

Conflict of interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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