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New Zwitterionic Imidazolones with Enhanced Water Solubility and Bioavailability: Synthesis, Anticancer Activity, and Molecular Docking

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Abstract: Finding an effective anticancer drug to combat cancer cell resistance remains a challenge. Herein, we synthesized a new series of imidazolone derivatives **4a–4i** and assessed their anticancer activities against liver cancer cells (Hep3B), Hela cells, and normal LX2 cells. The imidazolne derivatives were synthesized by the condensation cyclization reaction using the natural product vanillin as a starting material. Among the synthesized imidazolones are those with an alkyl sulfate moiety that are water-soluble and showed enhanced anticancer activity against the tested cancer cells. The anticancer testing results showed that compound **4d** with the NO_2 group at position 4 of the benzene ring was superior to the other compounds; it showed an IC_{50} value of 134.2 ± 4.4 μM against Hep3B cells, while compound **4h** with the pyridyl moiety showed the highest cytotoxicity against Hela cells with an IC_{50} of 85.1 ± 2.1 μM. The anticancer activity of some imidazolones was greatly enhanced by adding to them the zwitterionic properties that made them more polar and water-soluble. DNA binding studies with compounds **4a**$_1$, **4d**, and **4g** indicated a docking score ranging from approximately −6.8 to −8.7 kcal/mol. This could be attributed to the outstanding interaction between the molecule and the DNA binding sites, which primarily relies on its inherent capability to establish hydrogen bonds, facilitated by the electron pair present at the oxygen atoms and the drug's amino group. In conclusion, water-soluble imidazolone with zwitterionic functionality could be a promising tool for the development of anticancer medication. To outline the general idea and the relationships for the effect of the developed compounds under study, as well as their mechanism of action, further extensive research is also necessary.

Keywords: hippuric acid; oxazolone; imidazolones; anticancer; molecular docking

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1. Introduction

Nitrogen-containing heterocyclic compounds have been desirable targets for synthetic organic chemists because of their diverse biological activities [1–5]. Among them were imidazoles, and imidazolones garnered the most interest since they are recognized to offer a wide range of pharmacological and biological potentials [6–8], including anti-convulsant activity [9,10], monoamine oxidase (MAO) inhibitory effect [11], anti-bacterial, anti-tubercular [12], anti-asthmatic, and cardiovascular activities [13,14]. Moreover, several

other imidazole and imidazolone derivatives showed other various bioactivities such as anti-muscarinic, antimicrobial [15], anti-histamine, anti-inflammatory, and anticancer [10]. For the most recent review on the synthesis and biological activities of imidazole and imidazoline, one can refer to a review by Tolomeu et al. [16]. Imidazole is also known as the basic core of natural products like purine, histamine, histidine, and DNA structures [17]. Many of the chemotherapeutic drugs known for their potent actions, such as Methotrexate, Etoposide, and Paclitaxel, exhibit several drawbacks, including drug resistance, toxicity, and severe side effects. Some published work showed that imidazole compounds could have the potential to overcome the mentioned obstacles and could hold promise as anticancer drugs with multiple potential mechanisms of action [18,19]. A summary of methods used to synthesize imidazole can be found in several published review articles [20–26]. However, many reported methods still have some disadvantages, like using severe reaction conditions, long reaction periods, and low yields [9]. In addition, some imidazole and imidazole derivatives suffer from limited bioactivities due to insolubility and low bioavailability. The synthesis of a new bioactive pharmaceutical ingredient with multiple components could be an acceptable approach to solving these issues. Adding a highly polar substituent like one with salt functionality has been considered a reliable method to improve solubility and bioavailability [27].

To identify new anticancer candidates that may be potent, less toxic, water-soluble, and more selective, we report in the current work the synthesis of vanillin-based and zwitterionic vanillin-based imidazolone derivatives with an alkyl sulfonate moiety starting from the natural product vanillin. The anticancer activity and cytotoxicity of the prepared imidazolone derivatives were investigated against the human cancer liver cells Hep3B, Hela cells, and the normal liver cell LX2. The obtained results suggest that further research on this type of heterocycle would be beneficial to finding highly efficient anticancer drugs, as some of the imidazolone derivatives reported in this work show strong anticancer activity.

2. Materials Methods

2.1. Reagents and Instruments

All materials used in this research were of analytical grade and purchased from Sigma-Aldrich (Milwaukee, WI, USA). The materials included glycine, 4-chloroaniline, 2-aminopyridine, 4-bromoaniline, 5-fluorouracil (5-FU), p-phenylenediamine, 4-aminophenol, 4-nitroaniline, benzoyl chloride, 4-amino benzoic acid, and vanillin. A Stuart Scientific Melting Point SMP3 apparatus was used for the melting point determinations with degrees, and all melting points are reported in Celsius (°C). (FT-IR) was performed on the Nicolet iS5 by Thermo-Fisher Scientific Company (Waltham, MA, USA). Analysis by Nuclear Magnetic Resonance (NMR) was carried out using Bruker Avance 500 spectrometers (Fällanden, Switzerland) at the University of Jordan (Amman, Jordan). The MS/MS analysis was performed using a Thermo-Fisher Scientific LCQ Fleet ion trap mass spectrometer (Waltham, MA, USA) that was run in a positive electrospray mode with a voltage of 5.0 kV, a capillary temperature of 295.0 °C, and a gas flow of 30.0 units. An isolation width of 2.0 Da with a 20.0 msec activation time was used for the M.S. method. All scans were acquired with an ionization time of 250.0 ms. The purification of the prepared curcumin derivatives was performed by recrystallization.

Hepatocellular carcinoma cell line (Hep3B) cells (HB-8064) and cervical adenocarcinoma (Hela) cells (CCL-2) were purchased from ATCC (Manassas, VA, USA), whereas normal liver cells (LX2) cells (SCC064) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Dulbecco's free Ca^{+2} phosphate-saline buffer (REF # 02-023-1A), Pen-Strep Solution (catalogue #030311B), and L-glutamine solution (REF # 03-020-1B) were obtained from the Biological Industries (Milwaukee, WI, USA). Sigma Life Science (Milwaukee, WI, USA) provided us with a Trizma base (Lot SLBF2864V). The viability of cells was determined by the Cell Tilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay (Promega Corporation, Madison, WI, USA).

An Esco CO_2 cell culture incubator was used to incubate the cell line. Moreover, an Accumax Variable micropipette with normal and long, narrow gel loading tips was used. Eppendorf Thermo mixer (Eppendorf, Hamburg, Germany), PH/ORP meter (Hanna Instruments, Woonsocket, RI, USA), XB-30 Flak ice maker (MRC laboratory instruments, Harlow, UK).

2.2. Synthesis and Characterization

2.2.1. Synthesis of Benzoyl Glycine (**1**)

A mixture of glycine (0.5 g, 6.6 mmol) and benzoyl chloride (1.0 g, 7.11 mmol) was dissolved in a solution of $NaHCO_3$ (10 mL, 10%). The solution was mixed vigorously until the smell of the benzoyl chloride disappeared completely. The $NaHCO_3$ was neutralized by the addition of concentrated HCl dropwise. The resulting solid was collected through vacuum filtration, rinsed with cold water, and subjected to recrystallization in approximately 20.0 mL of boiling water. The produced benzoylglycine was collected by vacuum filtration and dried in an oven at 60 °C under a vacuum. The mass of the product was 0.85 g (yield 71%), m.p 188–190 °C (Lit = 190 °C). IR (ν cm^{-1}): 3339 (N-H stretching), 3071 (OH stretch of carboxylic acid), 2930–2670 (CH_2 group), 1743 (C=O), 1600 (aromatic ring), 600–580 (C-C=O).

2.2.2. Synthesis of Oxazolone (Z)-4-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyloxazol-5-(4H)-One (**3**)

Benzoyl glycine **1** (0.4 g, 2.23 mmol) and vanillin **2** (0.34 g, 2.23 mmol) were placed in a flask (50 mL), followed by the addition of acetic anhydride (3.0 mL, 6.69 mmol) and sodium acetate (0.18 g, 2.23 mmol). The mixture was stirred for 2 h at 110 °C, cooled to room temperature, and diluted with 10 mL of ethanol. The reaction mixture was cooled in a refrigerator overnight, and the formed yellow solid was collected by vacuum filtration and dried in an oven at 60 °C. The product mass was 0.35 g (yield 54%), m.p 189–190 °C (Lit m.p. 192–193 °C). IR (ν cm^{-1}): 3630 (O-H), 1790–1750 (C=O, lactone), 1650 (C=N), 1606–1553 (C=C), 1326–1286 (C-N), 1271 (C-O, OCH_3), 1119 (C-O-C).

^{1}H NMR (500 MHz, DMSO) δ in ppm: 9.81 (s, 1H, OH), 8.09 (s, 1H, CH=C), 7.62, 7.51 (m, 5H, Ar), 7.26–6.96 (m, 3H, Ar), 3.91 (s, 3H, OCH_3). C-13 NMR (500 MHz, DMSO) δ in ppm: 167.3 (O-C=O), 163.6 (O-C=N), 151.4 (Ar-O), 142.0 (Ar-OH), (132.7–133.4) (=C-N), (126.0–130.5) (C_6H_5-), 56.3 (CH_3). m/z: (M+) for $C_{17}H_{13}NO_4$ calcd.: 295.08, found: 295.16.

2.2.3. General Procedure: Synthesis of Imidazolones (**4a**–**i**)

A solution of amine (5 mmol), ethanol (20 mL), and acetic acid (3 drops) was prepared in a round-bottomed flask (50 mL). A sample of oxazolone **3** (5 mmol) was dissolved in the solution. The reaction components were refluxed for 2 h and cooled in a refrigerator for 2 h. The produced crystals were collected by vacuum filtration, dried, and recrystallized in methanol.

(Z)-3-(4-Bromophenyl)-5-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyl-3,5-Dihydro-4H-Imidazol-4-One (**4a**)

Following the general procedure for the synthesis of imidazolone**4**, product **4a** was obtained in a yield of 68%, m.p 238–240 °C. IR (ν cm^{-1}): 3400–3260 (O-H), 2929 (C-H, OCH_3), 1758 (C=O, lactam), 1640 (C=N), 1617–1510 (C=C), 1315 (C-N), 1280–1160 (CO,Ar-O-CH_3), 1200 (C-O,OH), 800–600 (C-Br). ^{1}H NMR (500 MHz, DMSO) δ (ppm):10.19 (s, 1H, OH), 8.06 (d, 2H, *J* = 7.6 Hz, Ar-Br), 7.73 (d, 2H, *J* = 8.8 Hz, Ar-Br), 7.61–7.51 (m, 5H, Ar), 7.42 (s, 1H, CH=C), 7.25 (d, 1H, *J* = 8.4 Hz, Ar-H-C-OH), 7.19 (s, 1H, Ar-H-C-OCH_3), 7.12 (d, 1H, *J* = 8.0 Hz, Ar-H-C-C=C), 3.62 (s, 3H, OCH_3). ^{13}CNMR (500 MHz, DMSO) δ (ppm):166.5 (N-C=O), 164 (N-C=N), 139.99 (Ar-OH), 151.06 (Ar-O), 132.39–131.83 (=C-N), 131.28–128.37 (C_6H_5-),122.54 (Ar-Br), 55.96 (CH_3). m/z: (M+) for $C_{23}H_{17}BrN_2O_3$ calcd.: 448.04, found: 448.12.

(Z)-3-(4-Chlorophenyl)-5-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyl-3,5-Dihydro-4H-Imidazol-4-One (**4b**)

Product **4b** was obtained in a yield of 74%, m.p =248–250 °C. R_f0.41 (hexane/ethyl acetate: 4:2) IR (ν cm^{-1}): 3400–3220 (O-H), 2968 (=C-H), 2880 (C-H,OCH_3), 1759 (C=O, lactam), 1639 (C=N), 1617–1510 (C=C), 1315 (C-N), 1280–1160 (CO, Ar-O-CH_3), 1215 (C-O,OH), 800–600 (C-Cl). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.20 (s, 1H, OH), 8.06 (d, 2H, *J* = 7.6 Hz, Ar-Cl), 7.78 (d, 2H, *J* = 8.4 Hz, Ar-Cl), 7.61–7.37 (m, 5H, Ar), 7.29 (s, 1H, CH=C), 7.25 (d, 1H, *J* = 8.4 Hz, Ar-H-C-OH), 7.20 (s, 1H, Ar-H-C-OCH_3), 7.12 (d, 1H, *J* = 8.3 Hz, Ar-H-C=C), 3.62 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 166.54 (N-C=O), 164.86 (N-C=N), 139.99 (Ar-OH), 151.07 (Ar-O), 133.43 (Ar-Cl), 133.43–132.40 (=C-N), 131.27–128.37 (C_6H_5-), 55.95 (CH_3). *m*/*z*: (M+) for $C_{23}H_{17}ClN_2O_3$ calcd.: 404.09, found: 448.15.

(Z)-3-(4-Aminophenyl)-5-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyl-3,5-Dihydro-4H-Imidazol-4-One (**4c**)

Product **4c** was obtained with a yield of 52%, m.p 208–212 °C. IR (ν cm^{-1}): 3620 (OH), 3367–3261 (primary amine), 3060–3020 (=C-H), 2881–2837 (C-H, OCH_3), 1764 (C=O, lactam), 1640 (C=N), 1614–1511 (C=C), 1322 (C-N), 1276–1162 (C-O, Ar-O-CH_3), 1204 (C-O,OH). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 9.73 (s, 1H, OH), 7.60–7.50 (m, 5H, Ar), 7.39 (s, 1H, CH=C) 7.33 (d, 1H, *J* = 8.2 Hz, Ar-H-C=C), 7.25 (s, 1H, Ar-H-C-OCH_3) 7.20 (d, 1H, *J* = 10.1 Hz, Ar-H-C-OH), 7.09 (d, 2H, *J* = 8.3 Hz, Ar-NH_2), 6.53 (d, 2H, *J* = 8.2 Hz, Ar-NH_2), 4.89 (s, 2H, NH_2), 3.58 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 168.92 (N-C=O), 163.76 (N-C=N), 145.51 (Ar-OH), 151.00 (Ar-O), 140 (C_6H_4-NH_2), 133.70–132.26 (=C-N), 131.54–128.27 (C_6H_5-), 123.36–122.52 (OH-C6H4-), 55.89 (CH_3). *m*/*z*: (M+) for $C_{23}H_{19}N_3O_3$ calcd.: 385.14, found: 385.21.

(Z)-5-(4-Hydroxy-3-Methoxybenzylidene)-3-(4-Nitrophenyl)-2-Phenyl-3,5-Dihydro-4H-Imidazol-4-One (**4d**)

Product **4d** was obtained with a yield of 72%, m.p 105–109 °C. IR (ν cm^{-1}): 3420 (OH), 3350 (tertiary amine), 3064–3014 (=C-H), 2881–2837 (C-H, OCH_3), 1766–1713 (C=O, lactam), 1665 (C=N), 1598–1508 (C=C), 1550–1350 (NO_2), 1375 (C-N), 1271–1151 (C-O, Ar-O-CH_3), 1221 (C-O, OH). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.16 (s, 1H, OH), 9.96 (s, 1H, CH=C), 7.99 (d, 2H, *J* = 7.7 Hz, Ar-NO_2), 7.94 (d, 2H, *J* = 8.8 Hz, Ar-NO_2), 7.60–7.29 (m, 5H, Ar), 7.10 (d, 1H, *J* = 8.3 Hz, Ar-H-C-OH), 6.73 (s, 1H, Ar-H-C-OCH_3), 6.60 (d, 1H, *J* = 8.8 Hz, Ar-H-C=C), 3.62 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 168.86 (N-C=O), 156.2 (N-C=N), 151.1 (Ar-O), 140.53 (Ar-NO_2), 132.41 (=C-N), 136.09–128.08 (C_6H_5-), 56.02 (CH_3). *m*/*z*: (M+) for $C_{23}H_{17}N_3O_5$ calcd.: 415.12, found: 415.18.

(Z)-4-(4-(4-Hydroxy-3-Methoxybenzylidene)-5-Oxo-2-Phenyl-4,5-Dihydro-1H-Imidazol-1-yl)benzoicacid (**4e**)

Compound **4e** was obtained in a yield of 53%, m.p 220–225 °C. IR (ν cm^{-1}): 3600 (OH), 3300 (O-H carboxylic acid), 3050 (=C-H), 1731 (C=O, lactam), 1671 (C=N), 1600–1519 (C=C), 1320 (C-N), 1287–1157 (C-O,Ar-O-CH_3), 1264 (C-O,OH). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.31 (s, 1H, COOH), 9.98 (s, 1H, OH), 8.20 (s, 1H, CH=C), 8.01–7.85 (m, 4H, Ar-COOH), 7.66–7.39 (m, 5H, Ar), 7.25–6.85 (m, 3H, Ar-OCH_3), 3.85 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 169.91 (N-C=O), 169.36 (COOH),185.67 (N-C=N), 147.86 (Ar-OH), 149.29 (Ar-O), 131.62–130.5 (=C-N), 135.94–130.67 (C_6H_5-), 130–123.85 (-C_6H_4-COOH), 55.69 (CH_3). *m*/*z*: (M+) for $C_{24}H_{18}N_2O_5$ calcd.: 414.42, found: 414.51.

(Z)-5-(4-Hydroxy-3-Methoxybenzylidene)-3-(4-Hydroxyphenyl)-2-Phenyl-3,5-Dihydro-4H-Imidazol-4-One (**4f**)

Imidazolone **4f** was obtained in a yield of 67%, m.p 208–212 °C. R_f 0.82 (ethyl acetate/methanol 9.5:0.5) IR (ν cm^{-1}): 3430 (O-H), 3270 (tertiary amine), 3100–3020 (=C-H), 2842 (C-H,OCH_3), 1770–1735 (C=O, lactam), 1638 (C=N), 1612–1509 (C=C), 1320 (C-N), 1273–1161 (C-O,Ar-O-CH_3), 1211 (C-O,OH). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.09

(s, 1H, OH), 9.89 (s, 1H, N-Ar-OH), 9.23 (s, 1H, CH=C), 8.05–7.57 (m, 4H, Ar-OH), 7.54–7.40 (m, 5H, Ar), 7.20 (s, 1H, Ar-H-C-OCH_3), 7.10 (d, 1H, *J* = 8.2 Hz, Ar-H-C=C), 6.73 (d, 1H, *J* = 8.3 Hz, Ar-H-C-OH), 3.59 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 168.9 (N-C=O), 164.08 (N-C=N), 154.04 (-C_6H_5-OH), 151.01 (Ar-O), 132.29–131.23 (=C-N), 133.63–128.31 (C_6H_5-), 56.52 (CH_3). *m*/*z*: (M+) for $C_{23}H_{18}N_2O_4$ calcd.: 386.13, found: 386.19.

(Z)-5-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyl-3-(2-(Pyridin-2-yl)ethyl)-3,5-Dihydro-4H-Imidazol-4-One (**4g**)

Imidazolone **4g** was obtained with a yield of 50%, m.p 147–151 °C. R_f 0.33 (ethyl acetate/methanol 9.5:0.5) IR (ν cm^{-1}): 3700 (OH), 3210 (tertiary amine of pyridine), 2900 (=C-H), 2800 (C-H,OCH3), 1762 (C=O, lactam), 1641 (C=N), 1619–1513 (C=C), 1311 (C-N), 1209 (C-O,OH), 1200–1163 (C-O,Ar-O-CH_3). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 9.98 (s, 1H, OH), 8.04 (d, 1H, *J* = 8.0 Hz, Pyridine-H), 7.67–7.54 (m, 5H, Ar), 7.52 (t, 1H, *J* = 7.5 Hz, Pyridine-H), 7.34 (s, 1H, CH=C), 7.28–719 (m, 2H, Pyridine-H), 7.14–7.06 (m, 3H, Ar-OCH_3), 3.53 (m, 2H, Pyridine-CH_2), 3.36 (s, 3H, OCH_3), 2.93 (q, 2H, Pyridine-CH_2). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 166.36 (N-C=O), 159.78 (=C-N in pyridine), (149.39 (N-C=N), 150.99 (Ar-OH), 131.33–130.98 (=C-N), 133.96–128.3 (C_6H_5-), 55.85 (CH_3). *m*/*z*: (M+) for $C_{24}H_{24}N_3O_3$ calcd.: 399.16, found: 399.21.

(Z)-5-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyl-3-(Pyridin-2-Ylmethyl)-3,5-Dihydro-4H-Imidazol-4-One (**4h**)

Imidazolone **4h** was obtained with a yield of 62.5%. R_f 0.37 (ethyl acetate/methanol 9.5:0.5) IR (ν cm^{-1}): 3430 (OH), 3288 (tertiary amine), 3064 (=C-H), 2972 (C-H,OCH_3), 1760 (C=O, lactam), 1648 (C=N), 1598–1512 (C=C), 1371 (C-N), 1201 (C-O,OH). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 9.98 (s, 1H, OH), 8.83–8.09 (m, 4H, Pyridine), 7.76–7.39 (m, 5H, Ar), 8.23 (s, 1H, CH=C), 7.24–7.07 (m, 3H, Ar-OCH_3), 6.77 (d, 2H, Pyridine-CH_2), 3.56 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 168.91 (N-C=O), 159.16 (N-C=N), 151.04 (Ar-O), 132.30 (=C-N), 137.06–128.06 (C_6H_5-), 56.55 (CH_3). *m*/*z*: (M+) for $C_{23}H_{19}N_3O_3$ calcd.: 385.14, found: 385.17.

(Z)-5-(4-Hydroxy-3-Methoxybenzylidene)-2-Phenyl-3-(Pyridin-2-yl)-3,5-Dihydro-4H-Imidazol-4-One (**4i**)

Imidazolone **4i** was obtained with a yield of 55%. IR (ν cm^{-1}): 3370 (O-H), 3167 (tertiary amine of pyridine), 2980 (=C-H), 2880 (C-H,OCH_3), 1700 (C=O, lactam), 1644 (C=N), 1567–1500 (C=C), 1410 (C-N), 1286–1165 (C-O,Ar-O-CH_3), 1244 (C-O,OH). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.00 (s, 1H, OH), 8.04–7.87 (m, 4H, Pyridine), 7.58–7.47 (m, 5H, Ar), 7.45 (s, 1H, CH=C), 7.15–6.48 (m, 3H, Ar-OCH_3), 3.58 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO) δ in ppm: 166.80 (N-C=O), 160.01 (N-C=N), 149.72 (Ar-O), 132.34 (=C-N), 137.74–128.03 (C_6H_5-), 55.64 (CH_3). *m*/*z*: (M+) for $C_{22}H_{17}N_3O_4$ calcd.: 371.13, found: 371.17.

2.2.4. General Procedure for Synthesis of Imidazolone Alkyl Sulfonate

A sample of imidazolone (0.245 mmol) was dissolved in THF (15 mL). 1,3-propane sultone (0.245 mmol) was added to the solution in the flask. The reaction mixture was refluxed until the starting material completely disappeared (1 h). The reaction was monitored by TLC. The solvent was then removed under vacuum using a rotary evaporator. The collected solid was rinsed with ethanol to remove any residual starting materials.

(Z)-3-(1-(4-Bromophenyl)-4-(4-Hydroxy-3-Methoxybenzylidene)-5-Oxo-2-Phenyl-4,5-Dihydro-1H-Imidazol-3-Ium-3-yl)propane-1-Sulfonate(**4a**$_1$)

Product **4a$_1$** was obtained with a yield of 71.5%. IR (ν cm^{-1}): 3500–3230 (O-H), 2930 (=C-H), 1758 (C=O, lactam), 1639 (C=N), 1618–1531 (C=C), 1315 (C-N), 1248 (C-O, OH), 1278–1160 (CO,Ar-O-CH_3), 1194 (SO_3^- group), 800–600 (C-Br). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.28 (s, 1H, OH), 8.04 (d, 2H, *J* = 7.7 Hz, Ar-Br), 7.71 (d, 2H, *J* = 8.6 Hz, Ar-Br),

7.60–7.40 (m, 5H, Ar), 7.35 (s, 1H, CH=C), 7.27–7.21 (m, 1H, Ar-H-C-OH), 7.17 (s, 1H, Ar-H-C-OCH_3), 7.11 (d, 1H, *J* = 8.2 Hz, Ar-H-C-C=C), 4.45 (t, 2H, *J* = 6.8 Hz, propyl group C**H$_2$**CH_2CH_2), 3.61 (s, 3H, OCH_3), 3.51–3.40 (m, 2H, propyl group CH_2CH_2C**H$_2$**), 2.00–1.67 (m, 2H, propyl group CH_2C**H$_2$**CH_2). *m*/*z*: (M+) for $C_{26}H_{23}BrN_2O_6S$ calcd.: 570.03, found: 570.11.

(Z)-3-(1-(4-Chlorophenyl)-4-(4-Hydroxy-3-Methoxybenzylidene)-5-Oxo-2-Phenyl-4,5-Dihydro-1H-Imidazol-3-Ium-3-yl)propane-1-Sulfonate (**4b$_1$**)

Product **4b$_1$** was obtained with a yield of 77%. IR (ν cm^{-1}): 3350–3220 (O-H), 2900 (=C-H), 1760 (C=O, lactam), 1645 (C=N), 1625–1510 (C=C), 1300 (C-N), 1235 (C-O,OH), 1270–1110 (CO,Ar-O-CH3), 1190 (SO_3^- group), 800–600 (C-Cl) ^{1}H NMR (500 MHz, DMSO) δ in ppm: 10.18 (s, 1H, OH), 8.04 (d, 2H, *J* = 7.7 Hz, Ar-Cl), 7.75 (d, 2H, *J* = 8.4 Hz, Ar-Cl), 7.59–7.35 (m, 5H, Ar), 7.00 (s, 1H, CH=C), 7.23 (d, 1H, *J* = 8.2 Hz, Ar-H-C-OH), 7.15 (s, 1H, Ar-H-C-OCH_3), 7.11 (d, 1H, *J* = 8.2 Hz, Ar-H-C=C), 4.45 (t, 2H, *J* = 6.7 Hz, propyl group C**H$_2$**CH_2CH_2), 3.69 (s, 3H, OCH_3), 3.39 (t, 2H, *J* = 7.7 Hz, propyl group CH_2CH_2C**H$_2$**), 2.23–1.96 (m, 2H, propyl group CH_2C**H$_2$**CH_2). *m*/*z*: (M+) for $C_{26}H_{23}ClN_2O_6S$ calcd.: 526.10, found: 526.1.

(Z)-3-(4-(4-Hydroxy-3-Methoxybenzylidene)-1-(4-Hydroxyphenyl)-5-Oxo-2-Phenyl-4,5-Dihydro-1H-Imidazol-3-Ium-3-yl)propane-1-Sulfonate (**4f$_1$**)

Following the general procedure for the synthesis of imidazolones **4f** with alkyl sulfonate, pure product **4f$_1$** was obtained with a yield of 65%. IR (ν cm^{-1}): 3660 (OH), 2971 (=C-H), 1712 (C=O, lactam), 1641 (C=N), 1600–1515 (C=C), 1381 (C-N), 1210 (C-O, OH), 1286–1164 (C-O, Ar-O-CH_3), 1164 (SO_3^- group). ^{1}H NMR (500 MHz, DMSO) δ in ppm: 9.92 (s, 1H, OH), 9.81 (s, 1H, N-Ar-OH), 8.66 (s, 1H, CH=C), 8.06–7.98 (m, 4H, Ar-OH), 7.60–7.49 (m, 5H, Ar), 7.32 (s, 1H, Ar-H-C-OCH_3), 7.17 (d, 1H, *J* = 8.3 Hz, Ar-H-C=C), 7.11 (d, 1H, *J* = 8.3 Hz, Ar-H-C-OH), 4.44 (t, 2H, *J* = 6.6 Hz, propyl group C**H$_2$**CH_2CH_2) 3.72 (s, 3H, OCH_3), 3.3 (t, 2H, *J* = 7.8 Hz, propyl group CH_2CH_2C**H$_2$**), 2.03–1.92 (m, 2H, propyl CH_2C**H$_2$**CH_2). *m*/*z*: (M+) for $C_{26}H_{24}N_2O_7S$ calcd.: 508.13, found: 508.21.

2.3. Anticancer Activity

2.3.1. Cell Line

The prepared compounds were evaluated against human cancer cell lines: liver cancer cells (Hep3B), cervical adenocarcinoma cells (HeLa), and normal liver cells (LX2).

2.3.2. Cell Culture and Cytotoxicity Test

Cell lines were cultured in a T-175 cell culture flask containing RPMI basal medium supplemented with 1% L-glutamine, 10% FBS, and 1% penicillin/streptomycin. The flask was incubated at 37 °C with 99% humidity. The cells were maintained at 5% CO_2 in a cell culture incubator. The prepared medium was rinsed with Ca(II)-free PBS prior to subculture. The cells were incubated with trypsin (0.025%) for 5 min until enough cells were detached. The added trypsin was deactivated using culture growth medium (CGM). The cell suspension was collected, and the viable cell count was determined using trypan blue stain before adjusting the cell concentration to 50.000 cell mL^{-1}. Then the cells were seeded in a 96-well plate with 5000 cells/well. The cells were then permitted to adhere and adapt overnight prior to testing. Various concentrations of the tested compounds were then prepared at 500, 250.0, 125.0, 62.5, and 31.25 ug/mL concentrations at a pH value of 7.4. In addition, 500 ug/mL of 5-FU was used as a positive control. After that, 100.0 µL of each concentration was added to each well. The wells were left under these conditions for 48 h. At the end of this period, 20 µL of MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) solution was added to each well and incubated for 2.0 h. The absorbance (490 nm) was then read using a multi-well plate reader.

2.3.3. Statistical Analysis

The cytotoxicity data was conducted in triplicate and presented as the means ± standard deviation. All data analysis, including the IC50 calculation, was prepared by GraphPad Prism Software 9 (GraphPad Software, La Jolla, CA, USA).

3. Results and Discussion

The imidazolone derivatives presented in this work were synthesized according to the strategy summarized in Figure 1. The multistep process involved three steps, with a total yield ranging from 50 to 70%. The first step involved a direct condensation reaction between glycine and benzoyl chloride in hippuric acid **1**. The second step involved preparing 5-oxazolone 3 by reacting hippuric acid **1** with vanillin in the presence of sodium acetate and acetic anhydride as a dehydrating agent [28]. This step involved a condensation-cyclization reaction with a loss of water molecules to form 5-oxazolone **3**. Compound **3** was then converted to the final product imidazolone (**4a–i**) by reacting it with various primary amines, which made a nucleophilic addition at the carbonyl group in compound **3**, causing it to undergo ring opening, followed by condensation cyclization that results in the loss of water molecules and the formation of the target product. Various spectroscopic methods have identified the structures of all derivatives. Various aromatic primary amines were used to study the effect of various substituents on bioactivity. All prepared compounds were analyzed by ^{1}H and ^{13}C NMR. The obtained NMR raw data for all prepared compounds is available in the Supplementary Materials.

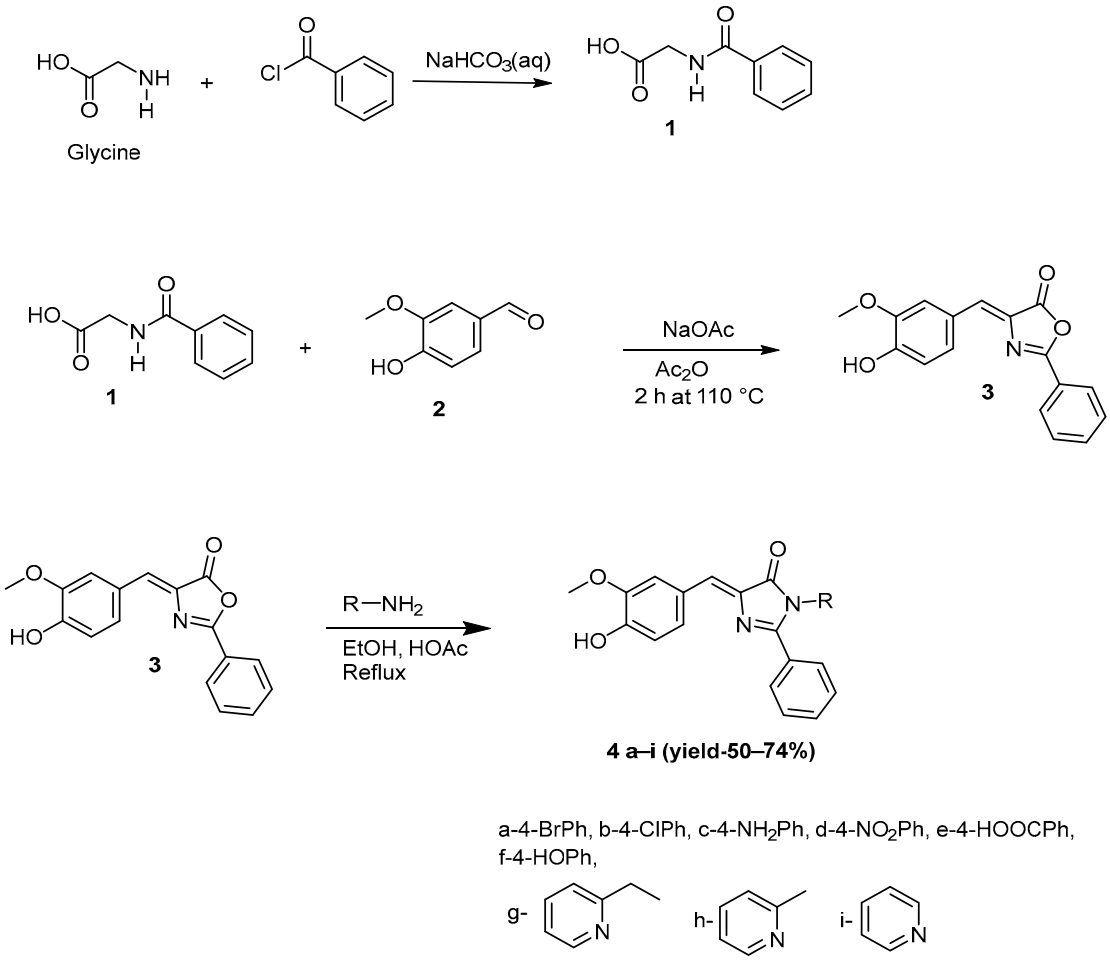


Figure 1. Synthesis of imidazolones **4a–i**.

All prepared compounds were submitted to the pharmaceutical department at An-Najah University (Nablus, Palestine) for anticancer activity against liver cancer (Hep3B) cells, cervical adenocarcinoma (HeLa) cells, and normal liver cells (LX2). The preliminary in vitro anticancer screening results are shown in Table 1. The test was performed using five doses against the three selected cell lines with five series of two-fold dilutions from a stock solution with 500 μg (500.0, 250.0, 125.0, 65.5, and 32.25 μM). A 48 h incubation with the selected cancer cells was conducted. The results were reported for each cell line from triplicate response parameters; the IC_{50} (molar concentration required to kill 50% of cancer cells) was determined.

Table 1. IC_{50} (μM) of imidazolones **4** against the tested cancer cells (Hep3B, Hela, and normal cells).

Imidazolone	IC_{50} μM		
	Hep3B	**Hela**	**LX2**
4a	267.3 ± 2.5	506.5 ± 4.3	264.9 ± 3.3
4b	347.2 ± 3.3	2230 ± 4.2	296.7 ± 3.1
4c	757.6 ± 2.6	760.2 ± 3.2	666.1 ± 2.1
4d	134.2 ± 4.4	236.6 ± 2.6	158.1 ± 3.1
4e	155.5 ± 5.3	200 ± 2.2	240.7 ± 3.7
4f	1273.25 ± 3.4	740.1 ± 4.5	450.5 ± 4.6
4g	255.4 ± 3.6	-	291.8 ± 4.8
4h	340.9 ± 2.9	85.1 ± 2.1	125.9 ± 4.2
4i	1822.8 ± 2.8	1580.2 ± 3.2	853.4 ± 4.4

The majority of the prepared imidazolnes (**4a**, **4b**, **4c**, **4d**, **4e**, **4f**, and **4h**) with the substituents -Br, -Cl, $-NH_2$, $-NO_2$, -COOH, and -OH on position four of the imidazolone ring and the 2-methyl pyridine groups showed good anticancer activities against the tested cancer cells (Hep3B and Hela cells) with low cytotoxicity against the normal cells. Imidazolone **4g** with the 2-ethyl pyridine group showed no activity against Hela cells, and compound **4i** with a pyridine ring showed poor activity against Hep3B and Hela cells. Compound **4h** with a similar structure to **4i** with an extra methylene group showed remarkable activity against Hela cells. It exhibited remarkable growth inhibition with an IC_{50} of 85.1 ± 2.1 μM and cytotoxicity over 95% at 250 μg/mL. These results could be attributed to the steric hindrance that prevented the pyridine from associating with receptors. However, adding methylene groups extended the chain length, reduced the steric hindrance, and enhanced the interaction between the pyridine ring and the target sites. The results also showed that compound **4d** with a NO_2 group in position 4 showed the highest anticancer activity against Hep3B cells with 134.2 ± 4.4 μM. It showed a growth inhibition of over 85% at 250 μg/mL. Same as compound **4h**, compound **4d** showed a 70% cytotoxicity on Hep3b cells at a concentration of 62.5 μg/mL with no cytotoxicity on the normal LX2 cells. Therefore, this compound has the potential to kill liver cancer cells at the mentioned concentration without any cytotoxicity to normal liver cells. The results could be related to the high interaction with the target site of the tested cancer cell. The results also indicated low cytotoxicity of the tested compounds (**4a**, **4b**, **4c**, and **4g**). The cytotoxicity against the tested normal LX2 cells was very low for most compounds, as shown in Figure 2.

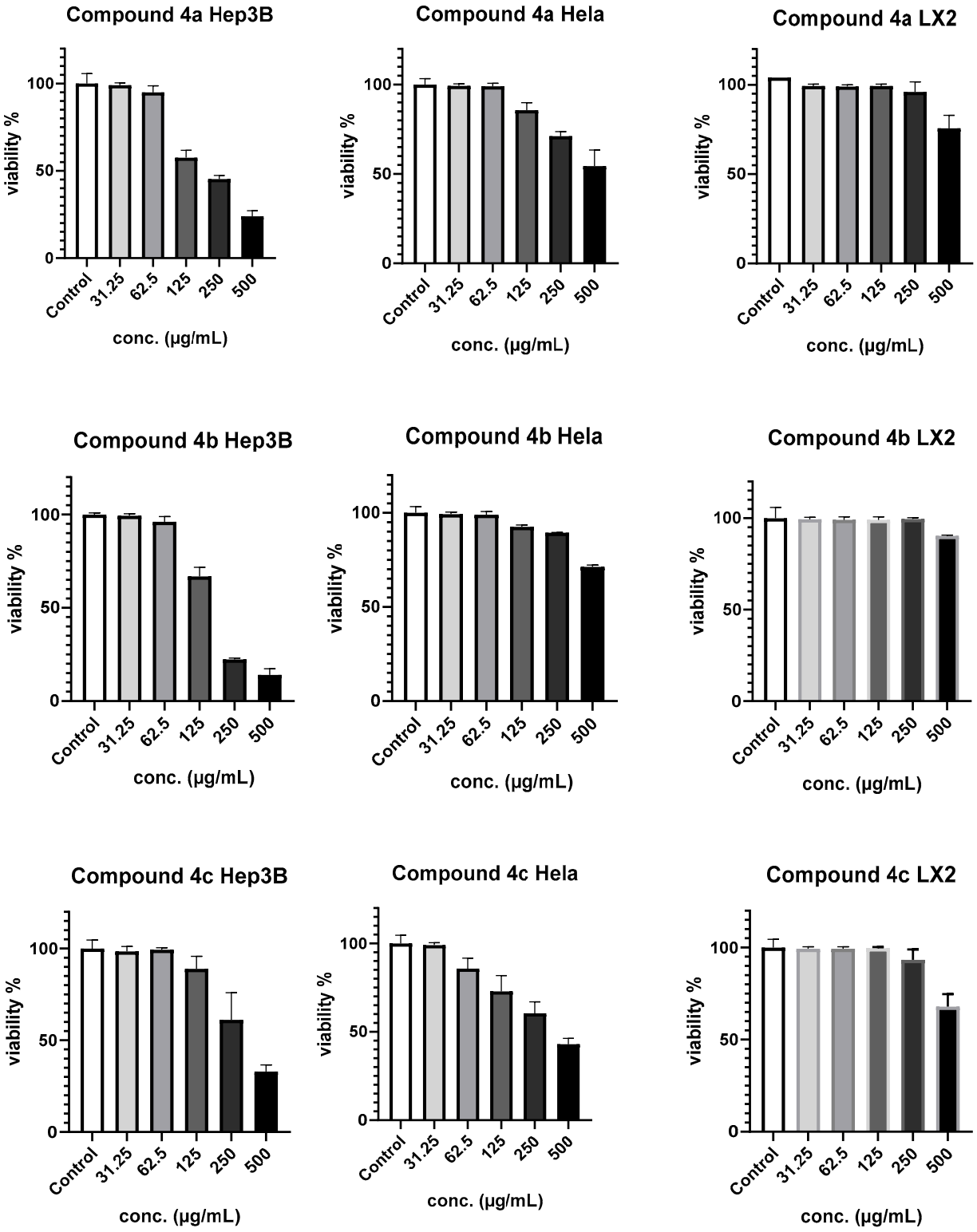
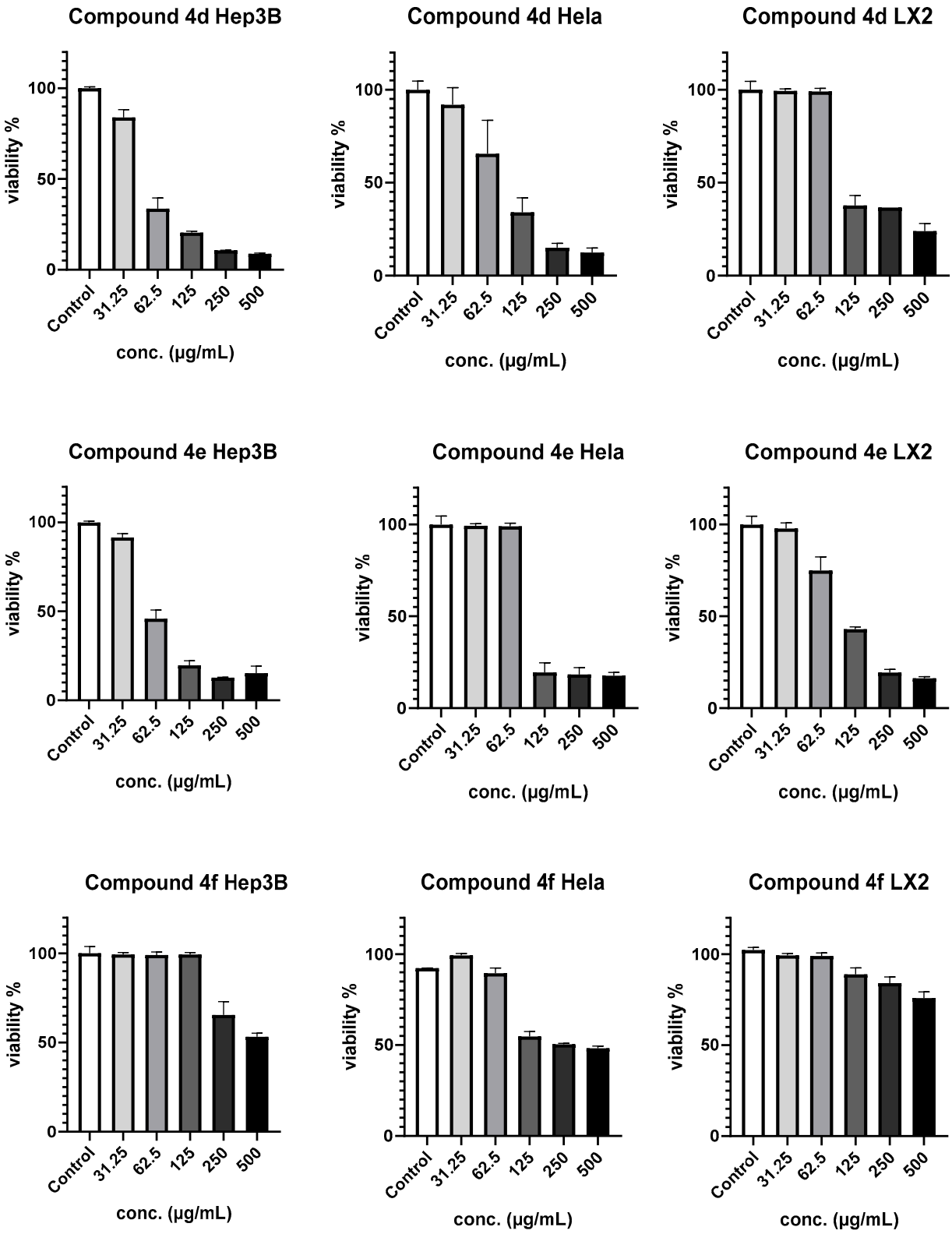


Figure 2. *Cont.*



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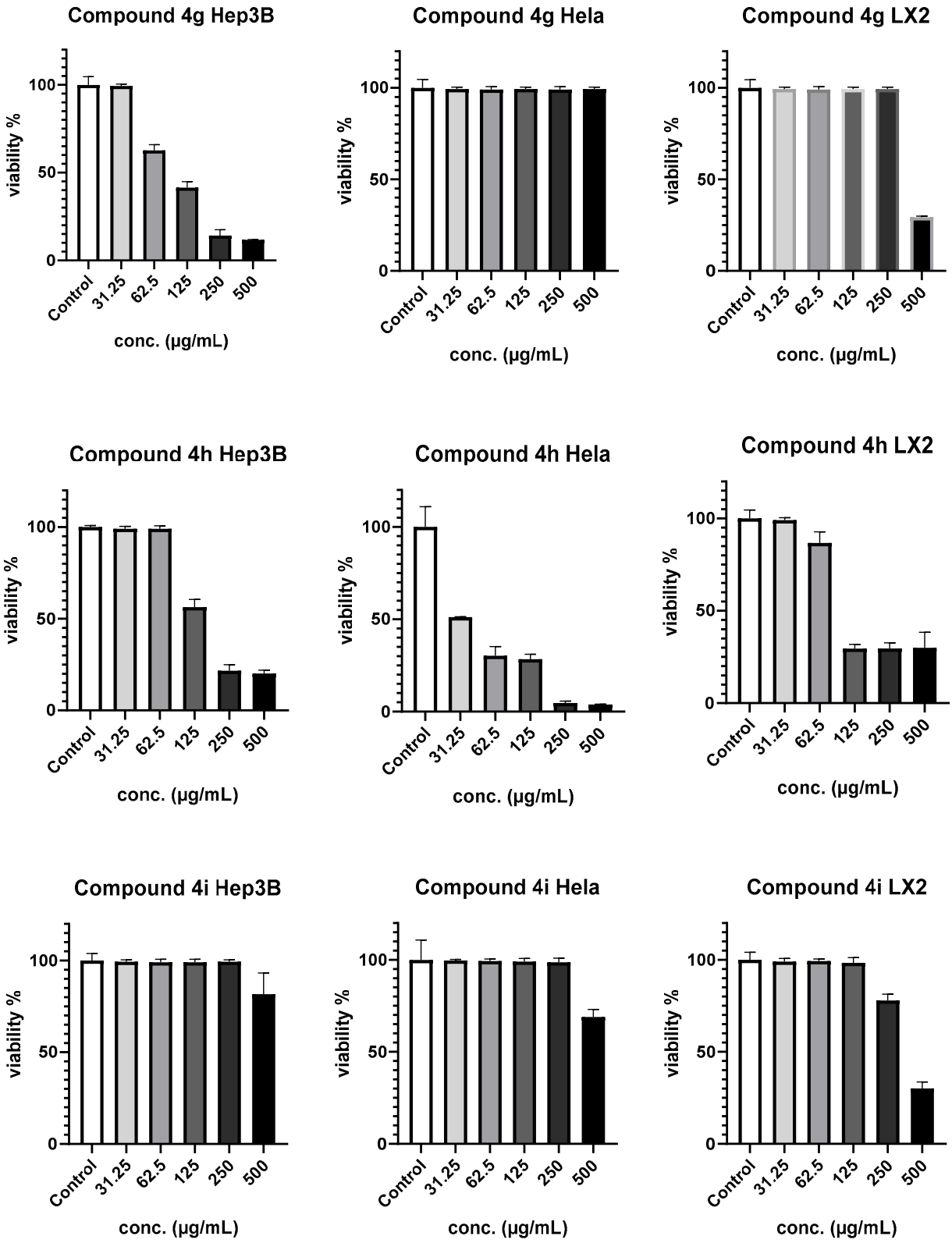

Figure 2. Imidazolone compounds have viability against the tested cells.

After running anticancer activity on all prepared imidzolones, we tested the possibility of enhancing the bioactivity of the imidazolones that showed low anticancer activity by modifying their structure with zwitterionic functionality. This was selected to enhance their water solubility and, hence, their bioavailability. This was carried out by reacting

selected imidazolones with 1,3-propane sultone. The reaction was performed by refluxing a solution of an equimolar amount of imidazolone and sultone in THF for one hour. A summary of the reaction is shown in Figure 3.

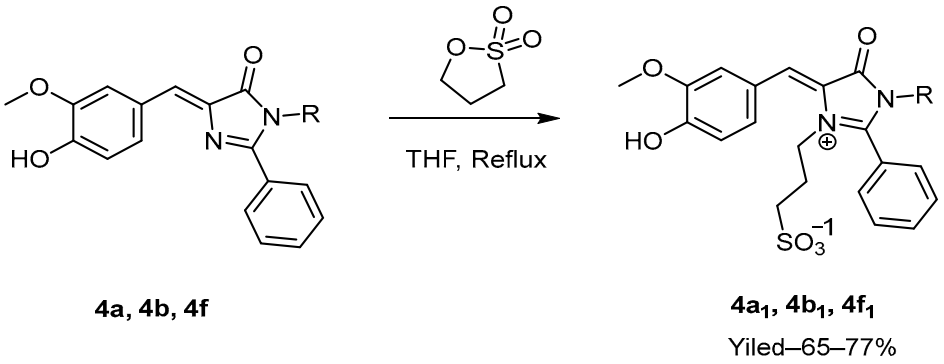

Figure 3. Synthesis of zwitterionic imidazolone with an alkyl sulfonate moiety.

Compounds **4a**$_1$, **4b**$_1$, and **4f**$_1$ were prepared using the zwitterionic approach. The anticancer activities of the zwitterionic imidazolone are shown in Figure 4. All showed improvement in the anticancer activities against the tested cancer cells (Hep3B and Hela cells). Compound **4a**$_1$ showed remarkable activity against Hela cells compared to compound **4a**, and it exhibited remarkable growth inhibition (IC_{50} = 105.4 µg/mL) with a viability of over 70% at a concentration of 250 µg/mL. The results also showed that the compound **4b**$_1$ with the sulfate groups showed the highest anticancer activity against Hep3B cells with an IC_{50} of 134.7 µg/mL; it showed a growth inhibition of tested cancer cells of over 67% at 250 µg/mL. The improvement in activity could be attributed to the enhancement in water solubility. The presence of zwitterionic functionality caused the imidazolones to interact strongly with the cell receptor sites that are available for H-bonding and dipole-dipole interaction.

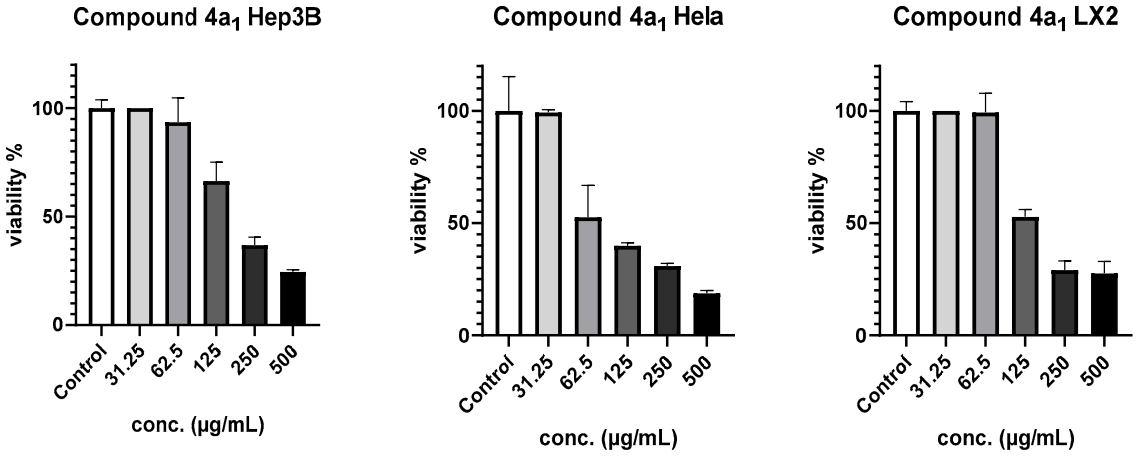

Figure 4. *Cont.*

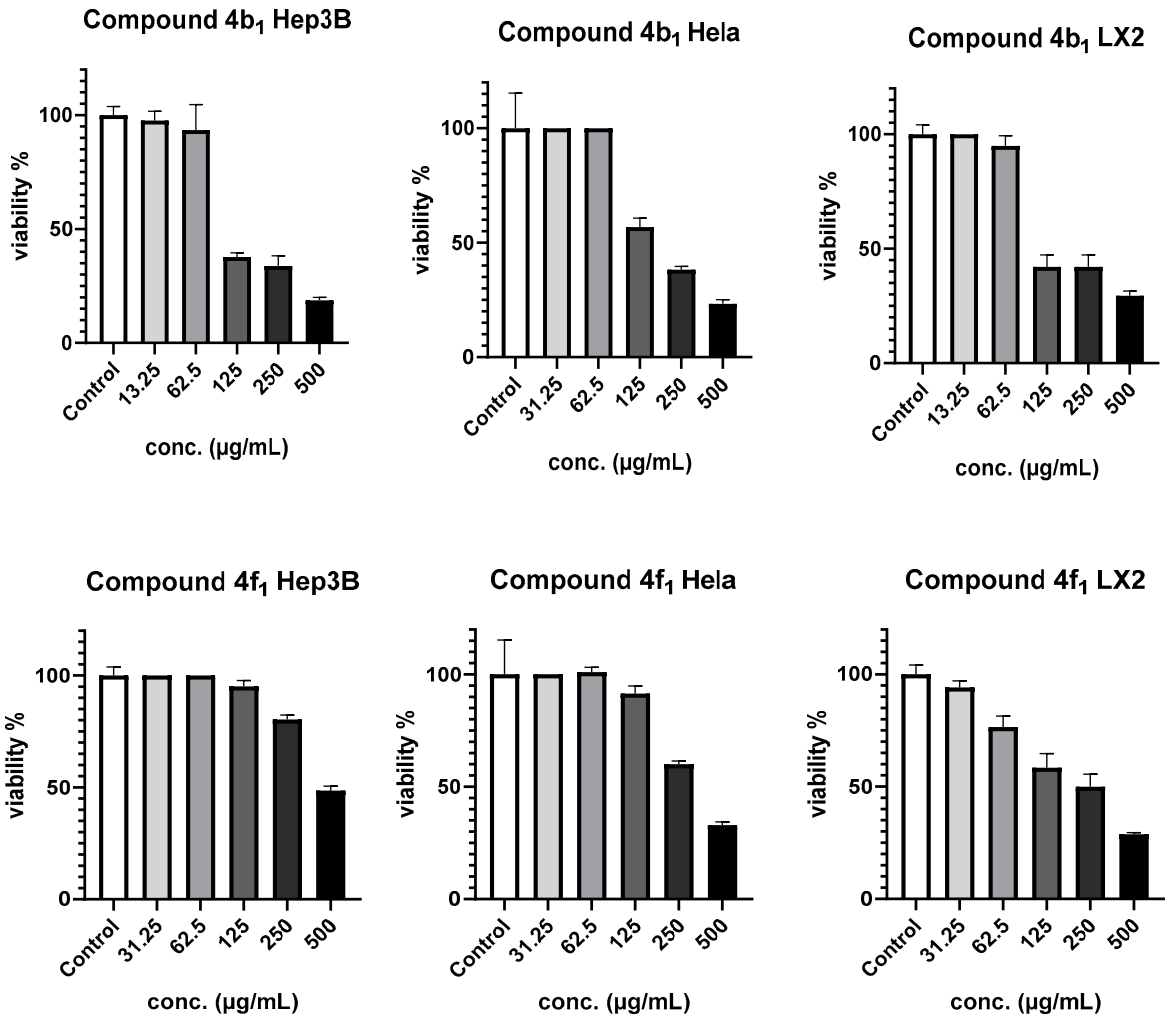

Figure 4. The viability of compounds (**4a**$_1$, **4b**$_1$, and **4f**$_1$) against tested cancer cell lines.

DNA Binding

The aptitude to estimate and comprehend the interactions among proteins and small molecules holds paramount significance within the domain of biology and the development of pharmaceuticals. This prophetic ability empowers scientists to investigate biological processes' complexities, providing a profound understanding that forms the foundation for advancing pharmaceutical research and fostering innovation. By accurately anticipating these interactions, researchers gain access to a wealth of insights within the intricate realm of biology. They can discern the subtle connections between proteins and small molecules, shedding light on the intricate molecular choreography governing various cellular functions. Furthermore, this foresight enables scientists to pinpoint potential targets for therapeutic interventions, ushering in opportunities for creating novel drugs and treatments. The importance of this predictive capability resonates widely throughout the scientific community, serving as a pivotal element in unraveling the multifaceted fabric of cellular mechanisms. Whether it involves elucidating the intricacies of cellular signaling pathways, deciphering the finely tuned mechanisms governing protein regulation, or unraveling the complex pathways underpinning various diseases, predictive modeling of protein-small molecule interactions assumes a central role in advancing our comprehension of these intricate biological processes. Essentially, it acts as a guiding beacon, illuminating the path forward in the pursuit of new therapies and breakthroughs in the field of medicine [29,30]. Additionally, precision in forecasting interactions between proteins and small molecules constitutes an essential requirement in the domain of drug development.

This precision equips researchers with the necessary tools to identify potential drug targets and refine the formulation of innovative medications. Mastery of these interactions empowers scientists to exert control over specific biological pathways, facilitating the creation of highly customized drugs capable of finely tuning protein activities to achieve therapeutic objectives.

The precise three-dimensional DNA coordinates (Pdb id: 1BNA, with a resolution of 1.90 Å) were successfully retrieved from the Protein Data Bank (PDB) database [31]. Subsequently, the three synthesized drug molecules underwent a thorough assessment to determine their potential interactions with specific DNA structures, as elaborated in Figure 5. The resulting docking scores provided insights into their respective affinities for the DNA structure. The ability to predict interactions between proteins or other biological macromolecules (DNA) and molecules holds significant importance in unraveling a broad spectrum of biological processes, comprehending protein functions and DNA interactions, and facilitating drug development. Blind docking of proteins, DNA, and ligands stands as a potent technique for exploring the locations where receptors bind and the orientations of ligand binding [32,33]. This approach has gained widespread acceptance in the fields of pharmacology and biology, and CB-Dock2 was employed for this purpose.

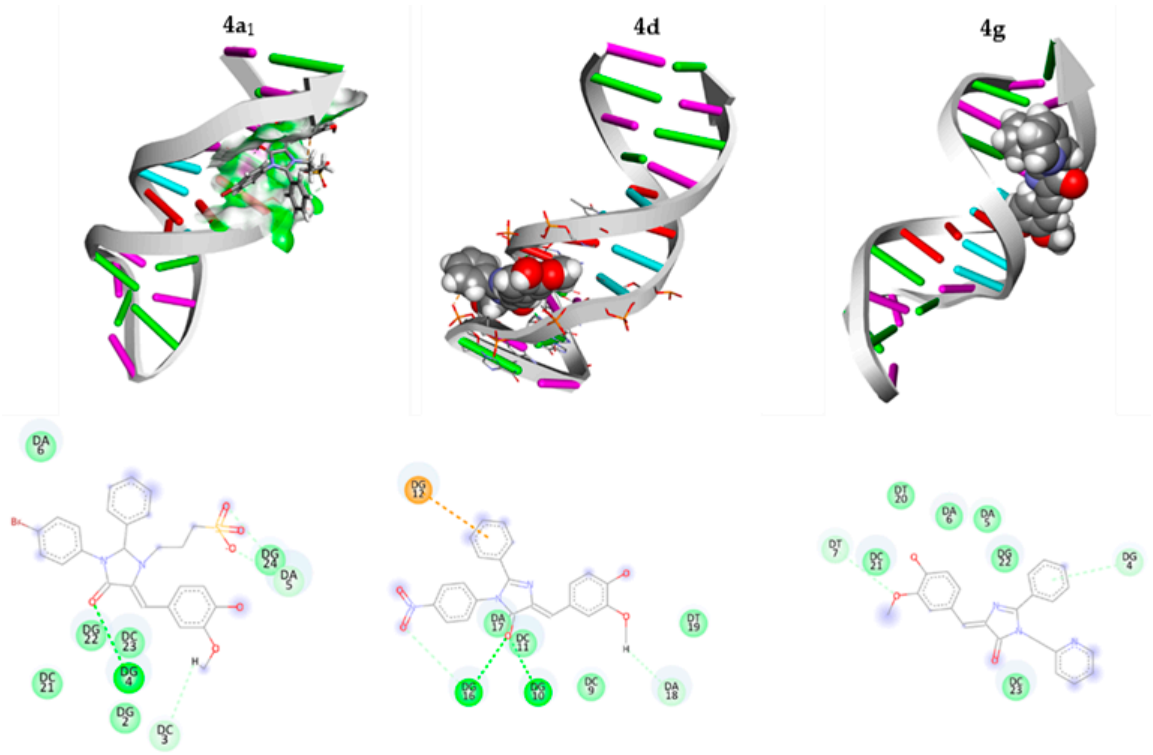

Figure 5. 3D and 2D docking of the three synthesized drugs **4a**$_1$, **4d,** and **4g** with DNA strands from the PDB id 1BNA (definition of atom color: red oxygen, blue nitrogen, gray carbon, white hydrogen).

The findings gain considerable strength from the drug's ability to form hydrogen bonds with DNA chains during the docking process, which aligns perfectly with the exceptionally favorable docking score values observed elsewhere [33,34]. Figure 5 visually illustrates the molecule's robust binding affinity to the DNA loop, emphasizing a remarkable docking score ranging from approximately −6.8 to −8.7 kcal/mol for all three studied molecules and the DNA structure. This outstanding interaction between the molecule and the target DNA strand primarily relies on its inherent capability to establish hydrogen bonds, facilitated by the electron pair at the oxygen atoms and the drug's amino group. Additionally, the molecule actively engages in π-alkyl interactions through its aromatic rings. In summary, these results underscore the drug's potential as a promising candidate for DNA binding and provide insight into the intricate molecular mechanisms behind its strong binding to

specific protein targets. This paves the way for further exploration and development in the field of pharmaceutical research.

4. Conclusions

A new series of imidazolone derivatives was synthesized using vanillin as a starting material in a three-step process. The anticancer activities of the prepared imidazolones against the two cancer cells, Hela cells and human liver cancer cell (Hep3B), in addition to a normal cell LX2, were studied. Most of the prepared compounds showed some activity against the tested cell. Compound **4d** showed exceptional activity against Hep3B cells with an IC_{50} of 134.2 ± 4.4 μM and a cytotoxicity of over 85% against both tested cells with low cytotoxicity. In addition, compound **4h** showed remarkable activity against Hela cells with **an** IC_{50} of 85.1 ± 2.1 μM. Introducing the alkyl sulfonate moiety to the imidazolone enhanced their water solubility and improved their bioactivities against the tested cancer cells. The molecular docking assessment for some of the prepared imidazolne and sulfonate showed a remarkable docking score ranging from approximately −6.8 to −8.7 kcal/mol for protein structures.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry5040169/s1. Figure S1: ^{1}H NMR of (Z)-4-(4-hydroxy-3-methoxybenzylidene)-2-phenyloxazol-5(4H)-one (**3**). Figure S2: ^{13}CNMR of (Z)-4-(4-hydroxy-3-methoxybenzylidene)-2-phenyloxazol-5(4H)-one (**3**). Figure S3: ^{1}H NMR of (Z)-3-(4-bromophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4a**). Figure S4: ^{13}CNMR of (Z)-3-(4-bromophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4a**). Figure S5: ^{1}HNMR of (Z)-3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4b**). Figure S6: ^{13}CNMR of (Z)-3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4b**). Figure S7: ^{1}HNMR of (Z)-3-(4-aminophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4c**). Figure S8: ^{13}CNMR of (Z)-3-(4-aminophenyl)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4c**). Figure S9: ^{1}HNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-nitrophenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4d**). Figure S10: ^{13}CNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-nitrophenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4d**). Figure S11: ^{1}HNMR of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)benzoicacid (**4e**). Figure S12: ^{13}CNMR of (Z)-4-(4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)benzoicacid (**4e**). Figure S13: ^{1}HNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxyphenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4f**). Figure S14: ^{13}CNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxyphenyl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (**4f**). Figure S15: ^{1}HNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(2-(pyridin-2-yl)ethyl)-3,5-dihydro-4H-imidazol-4-one (**4g**). Figure S16: ^{13}CNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(2-(pyridin-2-yl)ethyl)-3,5-dihydro-4H-imidazol-4-one (**4g**). Figure S17: ^{1}HNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-ylmethyl)-3,5-dihydro-4H-imidazol-4-one (**4h**). Figure S18: ^{13}CNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-ylmethyl)-3,5-dihydro-4H-imidazol-4-one (**4h**). Figure S19: ^{1}HNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-yl)-3,5-dihydro-4H-imidazol-4-one (**4i**). Figure S20: ^{13}CNMR of (Z)-5-(4-hydroxy-3-methoxybenzylidene)-2-phenyl-3-(pyridin-2-yl)-3,5-dihydro-4H-imidazol-4-one (**4i**). Figure S21: ^{1}HNMR of (Z)-3-(1-(4-bromophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (**4a$_1$**). Figure S22: ^{1}HNMR of (Z)-3-(1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (**4b$_1$**). Figure S23: ^{1}HNMR of (Z)-3-(4-(4-hydroxy-3-methoxybenzylidene)-1-(4-hydroxyphenyl)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-3-ium-3-yl)propane-1-sulfonate (**4f$_1$**).

Author Contributions: Conceptualization, O.H.; methodology, O.H., M.A. and N.A.; software, D.S.; validation, O.H. and M.A.; formal analysis, D.S., M.A., A.B., W.M., A.J. (Ataa Jaser) and A.O.; investigation, S.A.-J., A.J. (Alaa Janem), N.A., A.G., A.G. and A.O.; data curation, M.A., S.A.-J. and A.B.; writing—original draft preparation, O.H., S.A.-J., M.A. and A.B.; writing—review and editing, O.H., N.A. and M.A.; supervision, O.H., M.A. and N.A.; funding acquisition, N.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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