RESEARCH ARTICLE

Antibacterial and Antitumor Potentials of Some Novel Coumarins

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ABSTRACT

The growing rise of resistance developed by highly contagious microorganisms and malignant cells is a frightening threat to mankind. The search for novel bioactive molecules and their biological potentials may replenish our armory with weapons capable of combating this danger. The Bargellini reaction was used to convert hymecromone into a new Bargellini-based molecule, however its applicability to further functionalize hydroxycoumarins has not been reported. The latter was reacted with a variety of halophenols to produce the **N1-N12** congeners. By looking at and studying the spectrum charts of the produced compounds, the molecular structures were clearly recognized. The antitumor and antibacterial potentials of the produced compounds were evaluated. The first potential was tested using an MTT-based experiment versus 6 malignant tumor cells, namely MCF-7, SK-OV-3, HeLa, KYSE-30, AMN3, and SKG. A broth-dilution test was used to assess the antibacterial potential versus 6 gram-negative bacterial pathogens, including including *Pseudomonas aeruginosa*, *Salmonella typhi*, *Haemophilus influenza*, *Shigella dysenteriae*, *Escherichia coli,* and *Klebsiella pneumonia*. The findings of evaluating these potentials indicated that the fluorinated-congeners (**N1, N5, N9**) seemed to have a robust and wider antitumor impact against the cancerous-cell-lines under investigation. In the meanwhile, the chlorinated-congeners (**N2, N6, N10**) demonstrated good antibacterial activity against the pathogens studied. The fluorinated-congeners were shown to be promising scaffolds for the development of chemotherapeutic drugs that are more effective in cancer therapy. Furthermore, the chlorinated-congeners might be used as antibacterial agents against gram-negative bacteria.

Keywords: Antibacterial, Antitumor Coumarins, Broth-dilution method, MTT-based assay.

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INTRODUCTION

Cancer and pathogenic bacterial infections are two of the critical human life-menacing potentials. These health issues have been negatively affected by the resistance that mountingly advanced versus the drugs currently in utilization.[1,2] In an attempt to modulate this situation, many investigators focused their efforts to isolate novel natural-based products and explore their bio-potentials.[3-5] Other strains have been aimed to molecularly modify the synthetically bioactive compounds' chemical backbones to improve their potentials.[6,7] This aim attracted a considerable wealth of research because of its role in providing the detailed structural insights of the specific pharmacophore.[8,9]

In 1820, a principal hetero-aromatic phytochemical named coumarin has been extracted from the seeds of a flowering plant known as tonka beans. This natural product and most of its synthetic derivatives have revealed many characteristic medicinal properties.[10] Some of these documented bioactivities include the antithrombotic,[11] hypoglycemic,[12] antispasmodic,[13] hypolipidemic,[14] antiviral,[15] antimicrobial,[16] cytotoxic,[17] and free-radical scavengenic actions.[18]

The compound was chemically identified from various synthetic coumarins as a 7-hydroxy-4-methyl-2*H*-chromen-2-one (imecromone, cantabiline, hymecromone, 4-methylumbelliferon) and herein symbolized as HY has magnetized much interest from medicinal chemists.[19,20] This interest is based on HY's facile preparation and derivatization and the various biological potentials of its congeners.[21] Among many derivatization reactions involving the phenolic hydroxyl moiety of HY, Bargellini reaction has been avoided by the researchers.[22] This avoidance is because one of the Bargellini reagents, sodium hydroxide, can destroy the coumarin backbone of HY by hydrolyzing its lactone.[23] To make a difference, the work-team was initiated this report to perform Bargellini reaction by applying its classical method but in a different manner.

This work aims to synthesize several 7-functionalized coumarins with improved antitumor and antibacterial

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potentials. This aim was realized by synthesizing **HY** via the Pechmann reaction and its Bargellini-based compound (**N0**). The latter was used as a starting point for coupling with different halophenols resulting in the synthesis of 12 congeners. The preliminary cytotoxic potential of the synthesized compounds was assessed via an MTT-based assay versus six tumorous-cell lines. Besides, the antibacterial potential of these compounds was tested basing on a well-identified broth-dilution method versus six gram-negative pathogenic-standard bacteria.

EXPERIMENTAL SECTION

Chemicals and Instruments

The chemicals expended for the synthesis of **HY** and its congeners were assumed from documented international resources and utilized without additional purification. The synthesized congeners' melting points (Mp) were detected based on the USP-dependent capillary method via an electro-thermal CIA 9300 instrument. Thin-layer chromatography (TLC) consists of standard silica gel aluminum-based plates and an eluting mixture of $CHCl_3$: propanone (4:1) was used to assure the fulfillment of reactions and the purity of synthesized chemical compounds. Instruments employed to scan the UV, FTIR, ^{1}H-, ^{13}C-NMR spectra of the synthesized congeners were included UV-1600PC UV-Vis, Bruker-α- ATR-FTIR, Bruker Avance DRX-400 MHz spectrophotometers, respectively. The tumorous-cell lines and pathogenic standard bacteria employed in this work were purchased from Sigma- Aldrich and prepared for utilization according to each leaflet's instructions.

Synthetic Protocol

The schematic steps adopted for synthesizing **HY** and its congeners are displayed in Scheme 1.

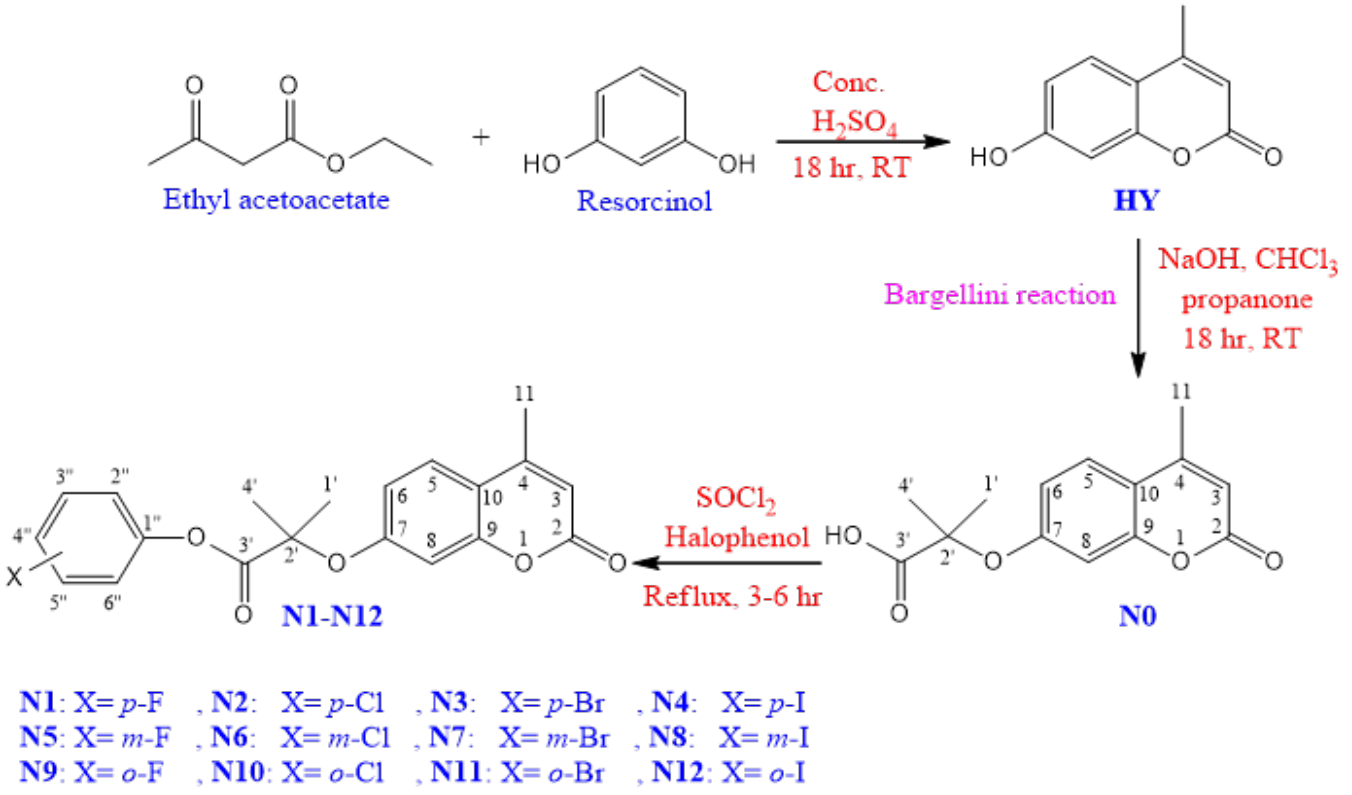
Synthesis of 7-hydroxy-4-methyl-2H-chromen-2-one (HY)

Resorcinol (2.75 g, 25 mmol) was dissolved in ethyl acetoacetate (3.44 mL, 27 mmol) under the influence of heat. The resultant solution was added drop by drop over 30 min to a pre-cold concentrated H_2SO_4 (25 mL) placed in an ice-bath. The reaction temperature was maintained below 10°C by adjusting the addition frequency. The reaction mixture was constantly stirred for 18 hours at room temperature (RT), rained onto a crushed ice-H_2O mixture, stirred vigorously, and filtered. The crude was gathered by filtration, washed several times with cold H_2O, and re-crystalized from 70% EtOH.[24]

HY: Creamy powder; Yield= 88% (3.87 g); Mp= 185-187°C; λ_{max} (EtOH)= 266 nm; R_f= 0.65.

Synthesis of 2'-methyl-2'-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)propanoic acid (N0)

Fine powdered NaOH (90 mmol, 3.45 g) was added to CHCl3 (90 mmol, 7 mL) in small portions with genital stirring. The reaction mixture was placed in a salt-ice bath, and propanone (60 mmol, 4.60 mL) was added drop by drop for 1 hour. Next to 30 minutes from the last addition, a solution of HY (12 mmol, 2.11 g) in 50 mL $CHCl_3$ was added slowly to the reaction mixture. After stirring for 18 hours at RT, H_2O (25 mL) was added to the mixture, and the aqueous layer was acidified with 2N HCl, extracted with diethyl ether (20×3). The collected



Scheme 1: The synthetic steps of HY and its congeners.

organic layer was dried over sodium sulfate, filtered, vaporized, and the crude was re-crystalized from EtOAc.[25]

N0: White powder; Yield= 67% (3.14 g); Mp= 196–198°C; λ_{max} (EtOH)= 268 nm; R_f= 0.48; IR ν_{max} (cm^{-1}): 3057 (m, C-H, alkene), 3002 (br, O-H, carboxylic acid), 2858 (w, C-H, alkane), 1734 (s, C=O, lactone), 1665 (m, C-C, *cis*-alkene), 1595 (m, C-C, aryl), 1263, 1025 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 11.26 (1H, s, O-H), 7.85 (1H, d, H-5), 7.11 (1H, s, H-8), 6.97 (1H, d, H-6), 6.45 (1H, s, H-3), 3.63 (3H, s, H-11), 1.52 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 181.2 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 127.4 (CH, C-5), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 91.6 (C, C-2'), 27.1 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

General Method for the Synthesis of HY Congeners (N1-N12)

In a salt-ice bath, a two-nick round-bottomed flask contained **N0** (5 mmol, 1.31 g) in 25 mL refreshed $SOCl_2$ was immersed. The side-nick was enclosed by a stopper provided with blue litmus-paper, while the central nick was enveloped by a condenser. The mixture was slowly stirred under anhydrous conditions over 30 min, then for the same period at RT, and refluxed for 3 hours. The course of the reaction was detected depending on the color change of litmus-paper, replaced regularly every 30 minutes. As the color of the blue litmus-paper no longer changed, the excess of $SOCl_2$ was distilled-off. The white solid material that remained in the concave of the flask represented the acyl-chloride derivative of **N0**.

To the same flask containing the white residue, a solution of halophenol (4.8 mmol) and pyridine (1 mL) in 50 mL anhydrous diethyl ether was added in one portion at RT and stirred under dehydrated conditions for 30 min. The reaction was refluxed for a period evidenced by changing the color of the litmus-paper as described above. As the reaction was finished, H_2O (50 mL) was added to the mixture, and the organic layer was separated, dehydrated, and vaporized. The **HY** congener was acquired by the recrystallization from a mixture of propanone: CH_2Cl_2 (1:2).[26]

N1: 4''-Fluorophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 62% (1.06 g); Mp= 164-166°C; λ_{max} (EtOH)= 272 nm; R_f= 0.68; IR ν_{max} (cm^{-1}): 3095 (m, C-H, alkene), 2916, 2820 (w, C-H, alkane), 1731 (s, C=O, lactone), 1709 (s, C=O, ester), 1664 (m, C-C, *cis*-alkene), 1599 (m, C-C, aryl), 1076 (s, C-F), 1264, 1025 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.83 (1H, d, H-5), 7.51 (2H, d, H-2'', 6''), 7.31 (2H, d, H-3'', 5''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 160.5 (C, C-4''), 155.9 (C, C-9), 154.7 (C, C-4), 148.9 (C, C-1''), 127.4 (CH, C-5), 125.2 (CH, C-2'', 6''), 117.9 (CH, C-3'', 5''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.4 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N2: 4''-Chlorophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 65% (1.16 g); Mp= 158-160°C; λ_{max} (EtOH)= 271 nm; R_f= 0.69; IR ν_{max} (cm^{-1}): 3093 (m, C-H, alkene), 2914, 2818 (w, C-H, alkane), 1729 (s, C=O, lactone), 1708 (s, C=O, ester), 1664 (m, C-C, *cis*-alkene), 1596 (m, C-C, aryl), 1066 (s, C-Cl), 1263, 1028 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.83 (1H, d, H-5), 7.64 (2H, d, H-2'', 6''), 7.54 (2H, d, H-3'', 5''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 151.4 (C, C-1''), 133.1 (C, C-4''), 132.3 (CH, C-3'', 5''),127.4 (CH, C-5), 125.0 (CH, C-2'', 6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.4 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N3: 4''-Bromophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 66% (1.32 g); Mp= 147-149°C; λ_{max} (EtOH)= 272 nm; R_f= 0.70; IR ν_{max} (cm^{-1}): 3093 (m, C-H, alkene), 2915, 2821 (w, C-H, alkane), 1733 (s, C=O, lactone), 1709 (s, C=O, ester), 1663 (m, C-C, *cis*-alkene), 1595 (m, C-C, aryl), 1000 (s, C-Br), 1263, 1025 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.83 (1H, d, H-5), 7.65 (2H, d, H-3'', 5''), 7.42 (2H, d, H-2'', 6''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 152.3 (C, C-1''), 134.0 (CH, C-3'', 5''), 127.4 (CH, C-5), 125.8 (CH, C-2'', 6''), 121.9 (C, C-4''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.4 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N4: 4''-Iodophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 59% (1.31 g); Mp= 144-146°C; λ_{max} (EtOH)= 272 nm; R_f= 0.71; IR ν_{max} (cm^{-1}): 3093 (m, C-H, alkene), 2914, 2824 (w, C-H, alkane), 1734 (s, C=O, lactone), 1710 (s, C=O, ester), 1662 (m, C-C, *cis*-alkene), 1596 (m, C-C, aryl), 920 (s, C-I), 1265, 1028 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.92 (2H, d, H-3'', 5''), 7.83 (1H, d, H-5), 7.30 (2H, d, H-2'', 6''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 152.2 (C, C-1''), 140.0 (CH, C-3'', 5''), 127.4 (CH, C-5), 125.2 (CH, C-2'', 6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 92.2 (C, C-4''), 90.2 (C, C-2'), 27.4 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N5: 3''-Fluorophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 69% (1.18 g); Mp= 142-144°C; λ_{max} (EtOH)= 272 nm; R_f= 0.68; IR ν_{max} (cm^{-1}): 3096 (m, C-H, alkene), 2917, 2820 (w, C-H, alkane), 1731 (s, C=O, lactone), 1710 (s, C=O, ester), 1665 (m, C-C, *cis*-alkene), 1596 (m, C-C, aryl), 1076 (s, C-F), 1265, 1027 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 8.09 (1H, d, H-4''), 7.83 (1H, d, H-5), 7.69 (1H, s, H-2''), 7.67 (1H, t, H-5''), 7.30 (1H, d, H-6''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9),

155.3 (C, C-3''), 154.7 (C, C-4), 151.7 (C, C-1''), 131.4 (CH, C-5''), 127.4 (CH, C-5), 119.2 (CH, C-6''), 116.3 (CH, C-4''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 110.1 (CH, C-2''), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N6: 3''-Chlorophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 71% (1.27 g); Mp= 136-138°C; λ_{max} (EtOH)= 268 nm; R_f = 0.70; IR ν_{max} (cm^{-1}): 3091 (m, C-H, alkene), 2913, 2818 (w, C-H, alkane), 1732 (s, C=O, lactone), 1710 (s, C=O, ester), 1664 (m, C-C, *cis*-alkene), 1593 (m, C-C, aryl), 1065 (s, C-Cl), 1263, 1028 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.83 (1H, d, H-5), 7.72 (1H, s, H-2''), 7.60 (1H, t, H-5''), 7.49 (1H, d, H-4''), 7.41 (1H, d, H-6''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 153.2 (C, C-1''), 137.9 (C, C-3''), 132.5 (CH, C-5''), 127.4 (CH, C-5), 126.9 (CH, C-4''), 124.4 (CH, C-2''), 121.7 (CH, C-6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N7: 3''-Bromophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 70% (1.40 g); Mp= 143-145°C; λ_{max} (EtOH)= 269 nm; R_f = 0.68; IR ν_{max} (cm^{-1}): 3090 (m, C-H, alkene), 2912, 2826 (w, C-H, alkane), 1733 (s, C=O, lactone), 1711 (s, C=O, ester), 1664 (m, C-C, *cis*-alkene), 1593 (m, C-C, aryl), 999 (s, C-Br), 1260, 1024 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.83 (1H, d, H-5), 7.77 (1H, s, H-2''), 7.59 (1H, t, H-5''), 7.49 (1H, d, H-4''), 7.24 (1H, d, H-6''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 155.5 (C, C-1''), 154.7 (C, C-4), 132.3 (CH, C-5''), 130.4 (CH, C-4''), 127.4 (CH, C-5), 127.0 (CH, C-2''), 124.3 (C, C-3''), 122.6 (CH, C-6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N8: 3''-Iodoophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 64% (1.42 g); Mp= 149-151°C; λ_{max} (EtOH)= 270 nm; R_f = 0.73; IR ν_{max} (cm^{-1}): 3091 (m, C-H, alkene), 2912, 2823 (w, C-H, alkane), 1732 (s, C=O, lactone), 1710 (s, C=O, ester), 1662 (m, C-C, *cis*-alkene), 1595 (m, C-C, aryl), 917 (s, C-I), 1264, 1026 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.97 (1H, s, H-2''), 7.93 (1H, d, H-5), 7.79 (1H, d, H-4''), 7.52 (1H, d, H-6''),7.42 (1H, t, H-5''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 154.2 (C, C-1''), 136.4 (CH, C-4''), 133.2 (CH, C-5''), 127.4 (CH, C-5), 123.3 (CH, C-2''), 122.7 (CH, C-6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 96.1 (C, C-3''), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N9: 2''-Fluorophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 40% (0.68 g); Mp= 166-168°C; λ_{max} (EtOH)= 271 nm; R_f = 0.68; IR ν_{max} (cm^{-1}): 3095 (m, C-H, alkene), 2915, 2820 (w, C-H, alkane), 1731 (s, C=O, lactone), 1710 (s, C=O, ester), 1665 (m, C-C, *cis*-alkene), 1598 (m, C-C, aryl), 1076 (s, C-F), 1266, 1028 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.83 (1H, d, H-5), 7.25-7.29 (4H, m, H-3'', 4'', 5'', 6''), 7.12 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 161.4 (C, C-2''), 155.9 (C, C-9), 154.7 (C, C-4), 139.2 (C, C-1''), 129.1 (CH, C-4''), 127.4 (CH, C-5), 126.7 (CH, C-5''), 126.0 (CH, C-6''), 118.1 (CH, C-3''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N10: 2''-Chlorophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 41% (0.73 g); Mp= 172-174°C; λ_{max} (EtOH)= 274 nm; R_f = 0.73; IR ν_{max} (cm^{-1}): 3089 (m, C-H, alkene), 2914, 2817 (w, C-H, alkane), 1735 (s, C=O, lactone), 1712 (s, C=O, ester), 1665 (m, C-C, *cis*-alkene), 1593 (m, C-C, aryl), 1063 (s, C-Cl), 1266, 1028 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.84 (1H, d, H-5), 7.28-7.32 (4H, m, H-3'', 4'', 5'', 6''), 7.14 (1H, s, H-8), 6.99 (1H, d, H-6), 6.43 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 149.6 (C, C-1''), 133.6 (CH, C-3''), 130.2 (C, C-2''), 129.3 (CH, C-5''), 128.5 (CH, C-4''), 127.4 (CH, C-5), 126.0 (CH, C-6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N11: 2''-Bromophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 40% (0.80 g); Mp= 157-159°C; λ_{max} (EtOH)= 273 nm; R_f = 0.70; IR ν_{max} (cm^{-1}): 3090 (m, C-H, alkene), 2912, 2819 (w, C-H, alkane), 1733 (s, C=O, lactone), 1709 (s, C=O, ester), 1663 (m, C-C, *cis*-alkene), 1595 (m, C-C, aryl), 1001 (s, C-Br), 1260, 1023 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm): δ= 7.84 (1H, d, H-5), 7.28-7.32 (4H, m, H-3'', 4'', 5'', 6''), 7.14 (1H, s, H-8), 6.99 (1H, d, H-6), 6.44 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 150.8 (C, C-1''), 135.6 (CH, C-3''), 129.9 (CH, C-4''), 129.6 (CH, C-5''), 127.4 (CH, C-5), 125.5 (CH, C-6''), 118.4 (C, C-2''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

N12: 2''-Iodophenyl-2'-methyl-2'-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)propanoate. White powder; Yield= 35% (0.78 g); Mp= 180-182°C; λ_{max} (EtOH)= 271 nm; R_f = 0.72; IR ν_{max} (cm^{-1}): 3093 (m, C-H, alkene), 2910, 2822 (w, C-H, alkane), 1733 (s, C=O, lactone), 1711 (s, C=O, ester), 1663 (m, C-C, *cis*-alkene), 1598 (m, C-C, aryl), 917 (s, C-I), 1266, 1023 (s, C-O-C, aryl-alkyl ether); ^{1}H-NMR (DMSO-d_6, 400 MHz,

ppm): δ= 7.84 (1H, d, H-5), 7.36-7.40 (4H, m, H-3'', 4'', 5'', 6''), 7.14 (1H, s, H-8), 7.01 (1H, d, H-6), 6.44 (1H, s, H-3), 3.62 (3H, s, H-11), 1.54 (6H, s, C-1', 4'); ^{13}C-NMR (DMSO-d_6, 100 MHz, ppm): δ= 173.5 (C, C-3'), 162.8 (C, C-2), 162.0 (C, C-7), 155.9 (C, C-9), 154.7 (C, C-4), 154.0 (C, C-1''), 142.0 (CH, C-3''), 130.6 (CH, C-5''), 129.1 (CH, C-4''), 127.4 (CH, C-5), 125.8 (CH, C-6''), 114.5 (CH, C-3), 113.2 (C, C-10), 111.0 (CH, C-6), 106.1 (CH, C-8), 92.9 (C, C-2''), 90.2 (C, C-2'), 27.5 (CH_3, C-1', 4'), 21.4 (CH_3, C-11).

In-vitro Biological Potentials

Assessment of the Preliminary Cytotoxic Potential

Cancerous-line accounting 10^4 cells were planted for 24 hours over a growth-assisted medium positioned in each pit of 96-well tray. Each pit was treated individually with one of the duplicate-reduced concentrations of the tested compounds. The concentrations of each compound were prepared from a stock solution using DMSO as a diluent and ranged between 200–6.25 μM. Later 72 hours of treatment, a preliminary cytotoxic assay was performed by applying MTT (28 μL, 3.27×10^3 μM) as a colored indicator for the cell-viability as the growth medium was removed. Behind the incubation at 37°C for 90 min, a microplate reader adapted at 492 nm was employed to detect the absorbance of each pit. The cytotoxic potential of each tested compound was expressed as a growth-suppression % equal to (**Au** − **At**)/**Au** ×100. The symbols **Au** and **At** were reflected the absorbances of the untreated- and treated-pit, respectively.[18]

Assessment of the Antibacterial Potential

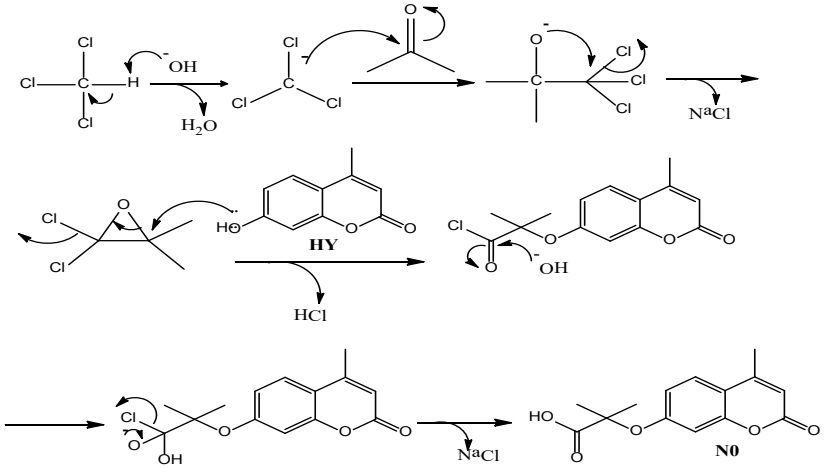
From an ancestry solution prepared by mixing 1 mg of the tested compound with 1 ml DMSO, duplicate-reduced concentrations were organized using an aqueous diluent and ranked between 512–0.25 μg/mL. To a marked test tube, inoculum of the turbidity adapted at 0.5 basing on the standard McFarland scale (0.2 mL), nutrient milieu represented by Mueller-Hinton-broth (3 mL), and labeled concentration of the tested compound were sequentially added. Next to an incubational period at 37°C for 24 hours, the cultured-tubes were scanned virtually to observe the resultant bacterial expansion. These cultural steps were repeated as the concentration exhibiting no bacterial expansion has identified. From this concentration, a second series of duplicated-reduced concentrations was prepared basing on the value of 4, 1, 0.5, or 0.05 to identify the least-restrained concentration (LRC). The least-bactericidal concentration (LBC) was identified by breeding 0.5 ml of the second-prepared concentrations separately in an expansion milieu (3 mL) at 37°C for 24 hours to expand this assessment. Eventually, another measure named bactericidal index (BI) was also calculated by dividing the acquired value of LRC over that of LBC.[27]

RESULTS AND DISCUSSION

Strategy of the Schematic Chemical Synthesis

As illustrated in Scheme 1, **HY** was synthesized by condensing ethyl acetoacetate with resorcinol under the classical conditions of the Pechmann reaction. Concerning the synthesis of the compound symbolized as **N0**, there are two typical methods for generating Bargellini-based products. The first one involves mixing $CHCl_3$, propanone, NaOH, and phenolic derivative concurrently in the reaction mixture.[28,29] The second method involves the portion-wise addition of $CHCl_3$ to the stirred mixture of the other components.[30-32] In both, several drawbacks have been found and primarily resulted from the basic/nucleophilic character of NaOH. This reagent can deprotonate the $CHCl_3$ and the α-carbons of the propanone, and also assault the carbonyl carbon of the latter. These events may result in the generation of many side products that are difficult to eliminate and scarcity in the yield percentage. In the case of employing **HY** in a Bargellini reaction, these drawbacks are aggravated by the nucleophilic assaulting of NaOH on the lactone ring of the coumarin backbone resulting in its hydrolysis.[22]

In this report, the aforementioned drawbacks were excluded by ordering the addition steps according to the classical mechanism of the NaOH-facilitated Bargellini reaction (Scheme 2)[25] and carrying them out at a freezing temperature. In this reaction-phenotype, NaOH deprotonates the acidic proton of the $CHCl_3$ generating a carbanion ion



Scheme 2: Mechanistic steps for the synthesis of N0 compound under the conditions of NaOH-facilitated Bargellini reaction.

Table 1: The data acquired from MTT-based assay evaluated the preliminary antitumor activity of the synthesized compounds.

Derivative symbol	*IC_{50} (μM) ± SD (n=3)*					
	MCF-7	*HeLa*	*SKG*	*AMN3*	*SK-OV-3*	*KYSE-30*
FU	12.46 ± 1.10	13.11 ± 0.81	22.19 ± 1.04	24.67 ± 1.01	22.16 ± 1.02	30.19 ± 0.98
N1	45.85 ± 1.08	50.45 ± 1.01	23.25 ± 1.01	47.81 ± 1.05	13.12 ± 0.96	53.92 ± 1.02
N2	48.02 ± 1.21	98.11 ± 0.97	99.12 ± 1.12	52.67 ± 0.92	23.40 ± 1.01	103.66 ± 0.96
N3	50.75 ± 0.96	198.22 ± 1.05	102.87 ± 0.96	101.18 ± 1.10	48.71 ± 0.94	125.94 ± 1.12
N4	54.91 ± 1.04	103.02 ± 1.16	194.23 ± 1.09	50.46 ± 0.90	24.38 ± 1.08	115.34 ± 1.02
N5	11.16 ± 0.98	12.03 ± 1.01	25.88 ± 1.12	46.12 ± 1.00	13.33 ± 0.96	53.98 ± 1.04
N6	47.15 ± 1.01	51.29 ± 1.19	52.23 ± 0.96	94.88 ± 0.97	24.41 ± 1.06	110.62 ± 1.04
N7	47.87 ± 1.09	100.54 ± 0.95	96.90 ± 1.06	104.98 ± 1.04	48.05 ± 0.89	120.34 ± 0.96
N8	49.71 ± 1.00	96.88 ± 1.06	100.74 ± 0.95	49.23 ± 0.88	25.25 ± 1.00	112.88 ± 0.92
N9	12.01 ± 0.99	12.71 ± 1.16	24.32 ± 1.02	13.57 ± 1.02	11.39 ± 0.98	51.68 ± 0.95
N10	50.74 ± 1.02	103.07 ± 0.95	102.61 ± 1.11	54.76 ± 0.94	23.87 ± 0.94	105.76 ± 1.00
N11	53.93 ± 1.12	198.43 ± 1.08	109.95 ± 0.96	103.27 ± 1.00	47.86 ± 1.02	117.78 ± 0.96
N12	56.42 ± 1.10	198.72 ± 1.14	104.79 ± 1.02	26.36 ± 0.91	24.76 ± 0.98	120.95 ± 1.05
N0	107.21 ± 1.07	199.02 ± 0.91	137.52 ± 086	120.64 ± 1.04	52.06 ± 1.00	129.37 ± 0.97
HY	95.35 ± 1.00	194.35 ± 0.98	101.92 ± 0.92	97.28 ± 1.08	47.10 ± 0.97	115.16 ± 1.02

Table 2: Order of decreasing antitumor activity of the tested compounds in consideration of each cancerous-cell line.

Abbreviations of the cancerous-cell lines					
MCF-7	*HeLa*	*SKG*	*AMN3*	*SK-OV-3*	*KYSE-30*
N5	N5	FU	N9	N9	FU
N9	N9	N1	FU	N1	N9
FU	FU	N9	N12	N5	N1
N1	N1	N5	N5	FU	N5
N6	N6	N6	N1	N2	N2
N7	N8	N7	N8	N10	N10
N2	N2	N2	N4	N4	N6
N8	N7	N8	N2	N6	N8
N10	N4	HY	N10	N12	HY
N3	N10	N10	N6	N8	N4
N11	HY	N3	HY	HY	N11
N4	N3	N12	N3	N11	N7
N12	N11	N11	N4	N7	N12
HY	N12	N0	N7	N3	N3
N0	N0	N4	N0	N0	N0

(intermediate I), which is partially stabilized by the inductive effect exerted by three chloride atoms. This ion nucleophilically assaults propanone's electrophilic carbonyl carbon, affording an epoxide product (intermediate II). The latter is highly susceptible to nucleophilic assault by the phenolic hydroxyl group of **HY** forming the **N0** compound. The low percentage of yield may be the reduced nucleophilicity of the phenolic hydroxyl group because it participates in a high-conjugated system of **HY**.[33]

The final step in the synthetic project involved transforming the carboxylic acid moiety of **N0** under the influence of $SOCl_2$ into an acid chloride-derived product. The latter was reacted with different halophenols to afford the final congeners. Congeners **N1**-**N4** had halogens substituted at para-position to the phenoxy moiety; those had meta-halo substitution termed congeners **N5**-**N8**, while the congeners **N9**-**N12** possessed ortho-halo group.

Valuation of the Preliminary Cytotoxic Potential

The evaluation of **HY**, **N0**, and the synthesized congeners (**N1**-**N12**) as antitumor candidates was performed using an MTT-facilitated cell viability assay. In this method, 5-fluorouracil (**FU**) was employed as a principal cytotoxic relative, DMSO as a negative criterion, and six cancerous-cell lines. The last attribute included MCF-7 (86012803, Caucasian breast adenocarcinoma), HeLa (93021013, epithelioid cervix carcinoma), SKG (C27676, human papillomavirus-related cervical squamous cell carcinoma), AMN3(CVCL-M395, murine mammary adenocarcinoma), SK-OV-3 (91091004, Caucasian ovary adenocarcinoma), and KYSE-30 (94072011, human Asian esophageal squamous cell carcinoma). The data gathered from this assay were listed in Tables 1. Besides, Table 2 highlighted the order of decreasing antitumor activity of the tested compounds concerning each cancerous-cell line.

The outcomes recorded in Table 2 revealed four inferences; first, the fluorinated-congeners (**N1**, **N5**, **N9**) have a more potent and broader antitumor activity than **HY**, **N0**, and other congeners. Secondly, the congener **N5** was better influenced than **FU** against two cancerous-cell lines named MCF-7 and HeLa. Thirdly, the congener **N9** exhibited the best potential compared to **FU** against two cancerous-cell lines, AMN3 and SK-OV-3. In the literature, many reports highlighted the positive impact of an aromatic ring substituted with fluoride on the antitumor activity of the synthesized analogues.[34-37] Finally, the Bargellini-based compound (**N0**) showed the least antitumor effect against the investigated cancerous-cell lines. The inefficiency of this compound may refer to its carboxylic acid entity that enhances the aqueous-solubility

and consequently drops the permeation into cancerous-cells.[38-41]

Valuation of the Antibacterial Potential

The consideration of **HY**, **N0**, and the synthesized congeners as elected antibacterials was assessed by applying a well-identified broth-dilution method. In which, ciprofloxacin (**CP**) was applied as a definite antibacterial drug, DMSO as a negative criterion, and six Gram-negative pathogenic-standard bacteria. These infectious agents were *Pseudomonas aeruginosa* (***GN-Pa***, ATCC 27853), *Shigella dysenteriae* (***GN-Sd***, ATCC 13313), *Haemophilus influenza* (***GN-Hi***, ATCC 49247), *Klebsiella pneumonia* (***GN-Kp***, ATCC 700603), *Escherichia coli* (***GN-Ec***, ATCC 25922), and *Salmonella typhi* (***GN-St***, ATCC 6539). This assay was conducted to calculate three antibacterial-measures including LRC, LBC, and BI. The results acquired from investigating the first measure are listed in Table 3. Those gathered from evaluating the second measure were recorded in Table 4. Besides, the data obtained from calculating the third measure were reported in Table 5. The order of dropping the antibacterial potential of the synthesized compounds corresponding to each investigated infectious agent was displayed in Table 6.

The scores entered in Table 6 showed four reasoning yields; first, the synthesized compounds exhibited less

Table 3: The LRC data of the synthesized compounds measured against the investigated infectious agents.

Compound symbol	*Standard pathogenic bacterial strains*					
	GN-Pa	*GN-Sd*	*GN-Hi*	*GN-Kp*	*GN-Ec*	*GN-St*
CF	0.80	0.95	0.70	0.60	0.80	0.55
N1	5.00	5.00	5.50	4.50	5.50	4.50
N2	2.00	2.50	2.00	1.50	2.00	1.00
N3	13.00	14.00	12.00	11.00	13.00	11.00
N4	10.00	10.00	11.00	9.00	9.00	8.00
N5	6.50	7.50	7.50	5.50	6.50	5.50
N6	4.00	4.00	3.50	3.00	4.00	2.50
N7	15.00	14.00	15.00	13.00	15.00	14.00
N8	12.00	11.00	12.00	10.00	12.00	10.00
N9	6.50	6.00	6.00	5.00	7.00	5.00
N10	3.50	3.50	3.00	2.50	3.50	2.50
N11	14.00	13.00	14.00	13.00	14.00	13.00
N12	10.00	10.00	12.00	9.00	13.00	9.00
N0	20.00	22.00	26.00	18.00	22.00	18.00
HY	15.00	14.00	15.00	14.00	15.00	14.00

Table 4: The LBC data of the synthesized compounds measured against the investigated infectious agents.

Compound symbol	*Standard pathogenic bacterial strains*					
	GN-Pa	*GN-Sd*	*GN-Hi*	*GN-Kp*	*GN-Ec*	*GN-St*
CF	0.95	1.00	0.95	0.85	0.85	0.60
N1	6.50	6.00	7.50	5.00	7.00	5.00
N2	3.00	3.00	3.50	1.50	3.00	2.00
N3	15.00	14.00	14.00	12.00	15.00	13.00
N4	12.00	11.00	11.00	10.00	11.00	8.00
N5	7.50	7.50	7.50	5.50	7.00	5.50
N6	4.00	4.00	3.50	3.50	4.00	2.50
N7	15.00	15.00	16.00	13.00	16.00	14.00
N8	14.00	13.00	14.00	11.00	13.00	11.00
N9	7.50	7.50	6.50	5.50	7.50	5.00
N10	3.50	3.50	3.00	2.50	4.00	3.00
N11	15.00	13.00	15.00	13.00	15.00	13.00
N12	12.00	14.00	13.00	9.00	13.00	10.00
N0	28.00	30.00	28.00	24.00	26.00	20.00
HY	15.00	16.00	15.00	14.00	15.00	15.00

Table 5: The BI values of the synthesized compounds measured against the investigated infectious agents.

Compound symbol	*Standard pathogenic bacterial strains*					
	GN-Pa	*GN-Sd*	*GN-Hi*	*GN-Kp*	*GN-Ec*	*GN-St*
CF	1.19	1.05	1.36	1.42	1.06	1.09
N1	1.30	1.20	1.36	1.11	1.27	1.11
N2	1.50	1.20	1.75	1.00	1.50	2.00
N3	1.15	1.00	1.17	1.09	1.15	1.18
N4	1.20	1.10	1.00	1.11	1.22	1.00
N5	1.15	1.00	1.00	1.00	1.08	1.00
N6	1.00	1.00	1.00	1.17	1.00	1.00
N7	1.00	1.07	1.07	1.00	1.07	1.00
N8	1.17	1.18	1.17	1.10	1.08	1.10
N9	1.15	1.25	1.08	1.10	1.07	1.00
N10	1.00	1.00	1.00	1.00	1.14	1.20
N11	1.07	1.00	1.07	1.00	1.07	1.00
N12	1.20	1.40	1.08	1.00	1.00	1.11
N0	1.40	1.36	1.08	1.33	1.18	1.11
HY	1.00	1.14	1.00	1.00	1.00	1.07

Table 6: The order of declining the antibacterial effect of the synthesized compounds corresponding to each investigated bacterium.

Abbreviations of the standard pathogenic bacterial strains					
GN-Pa	*GN-Sd*	*GN-Hi*	*GN-Kp*	*GN-Ec*	*GN-St*
CF	CF	CF	CF	CF	CF
N2	N2	N2	N2	N2	N2
N10	N10	N10	N10	N10	N6
N6	N6	N6	N6	N6	N10
N1	N1	N1	N1	N1	N1
N5	N9	N9	N9	N5	N9
N9	N5	N5	N5	N9	N5
N4	N4	N4	N4	N4	N4
N12	N12	N3	N12	N8	N12
N8	N8	N8	N8	N3	N8
N3	N11	N12	N3	N12	N3
N11	N3	N11	N7	N11	N11
N7	N7	N7	N11	N7	N7
HY	HY	HY	HY	HY	HY
N0	N0	N0	N0	N0	N0

antibacterial effect than **CF** against the investigated pathogens. Secondly, the synthesized compounds showed a similar fashion in their antibacterial potential concerning the tested microorganisms. Thirdly, the chlorinated-congeners (**N2**, **N6**, **N10**) have a powerful antibacterial activity than **HY**, **N0**, and other congeners; among these chlorinated derivatives, congener **N2** was the best.[42-44] Finally, compounds **N0** and **HY** have the least effect as antibacterial candidates than the synthesized congeners. The authors proposed that the presence of hydrophilic functional groups involving the hydroxyl and carboxyl moieties in compounds **N0** and **HY**, respectively, may be the reason for this finding.[27,45,46]

CONCLUSION

Despite its limitations concerning hydroxycoumarins, this work reported the success in transforming **HY** into Bargellini-based compound, **N0**. The latter was esterified with various halophenols affording 12 congeners. The outcomes acquired from exploring the antitumor and antibacterial potentials of the synthesized compounds showed that the fluorinated-congeners could serve as potent antitumor agents with a broad-spectrum of activity. Also, the chlorinated-congeners exhibited a promising effect as antibacterial agents against Gram-negative bacteria. Finally, these two congener-phenotypes can be considered privileged platforms and biomedical pre-validated scaffolds in exploring new biologically active compounds.

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