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Synthesis of disubstituted anisolodipyrone-derived ester compounds: The search for new bioactive candidates

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In this study, ketone di-α-carboxylic acid prepared from the interaction between anhydrous citric acid and strong inorganic acid was included in the Pechmann-related condensation to create a novel disubstituted anisolodipyrone building unit (**MSY1**). This precursor was interacted with different monofunctionalized phenols to afford six disubstituted anisolodipyrone-derived ester compounds, marked here as **MSY2-MSY7**. The molecular chemo-skeletal structures of the precursor and its derived compounds were identified spectrometerically. The potential of these compounds to present bioactive candidates was inspected *in vitro* through evaluation of their capacity to act as antiproliferative, antimicrobial, and glucose-regulating applicants. The authors found that our compounds, notably **MSY4**, have broad potent-to-moderate antiproliferative properties with a tiny harmful impact on normal cells. Also, these compounds showed positive antibacterial properties, particularly **MSY2**, **MSY5,** and **MSY1**, for anaerobic, aerobic gram-negative bacteria, and filamentous fungi, correspondingly. This positivity is endorsed by our chemicals' comparative safety in terms of normal flora bacteria. Moreover, **MSY2** and **MSY3** have the strongest suppression influence on the test glucose-regulating enzymes, with a moderate effect attributed to the other compounds. Based on the findings, the researchers concluded that the synthetic candidates might provide a type of platform, potentially opening up new options for the discovery of novel pharmaceutically active applicants.

KEYWORDS

Reusable catalyst; disubstituted anisolodipyrone-derived ester compounds; antiproliferative; antimicrobial; glucose-regulating capacity.

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Introduction

Carcinoma and microbiological opposition are two most challenging public health problems in the world. As of 1990, their levels have risen remarkably in almost every corner of the world [1]. Regardless of the fact that many investigated alternatives from both natural [2] and synthetic [3] contributions have been created, the efficacious diagnosis and control of several kinds of carcinoma and infection presents a concern. Consequently, an imperative need to generate unique chemical scaffolds is mounting day over night. Likewise, this necessitates investigating their biomedical activities to

identify workable alternatives to these two public health concerns [4].

Coumarin is a term abstracted linguistically from the French word "coumarou," which makes reference to the Tonka plant seeds of *Dipteryx odorata*, which was outlined as one of the initial isolated natural products in 1820. Coumarin has a good smell, which has led to its use in skincare products since 1882 [5,6]. The coumarinic compound phenotype is the lactone moiety fused with a phenyl ring, culminating in a highly conjugated entity with many valence electrons and robust charge-transport attributes [7,8]. Because of its simplification and ability to adapt, the coumarin chemical unit is an enticing launching point for a broad array of medicinal and non-medicinal applications [9,10].

Coumarins have been used in perfumes, personal care products, and as factory ingredients. Many of its equivalents have been used as smell inducers in cigarettes and hard liquor [11,12]. Their greatest vital purpose, nevertheless, is defined by organic chemistry, natural products, and medicinal plant/organic chemistry [13,14].

Moreover, various coumarinic compounds are purposefully being investigated as medical prospects for prescribing medicine development with a considerable therapeutic potential, good biocompatibility and tolerable side effects, fewer drug resistances, rising biodistribution, a broad range of potential health benefits, etc., to consider a spectrum of ailments [15]. Several efforts have been made to develop coumarin-based blood thinners, free-radical scavengers [16], antimicrobials [17], anti-carcinoma [18,19], anti-diabetic, painkiller, antineurodegenerative [20], and anti-inflammatory applicants [21]. Coumarinic compounds are also important in biomedical chemistry due to their unique and flexible oxygen-containing polycyclic aromatic hydrocarbon configuration [22]. Coumarins have been further studied as bioactive candidates [23], biomolecular homeopathic agents, imaging agents, pathophysiologic probes, and biomedical spatters [24].

This study aims to create six disubstituted anisolodipyrone-derived ester compounds, marked here as **MSY2-MSY7**, with wide-ranging antiproliferative, antimicrobial, and glucose-regulating properties. To accomplish this, the Pechmann interaction was used to produce **MSY1**. Then, it was used as a reference point for pairing to various monofunctionalized phenolic moieties, resulting in six esterified analogues recognized here as **MSY2-MSY7**. Our compounds were screened as antiproliferative applicants versus six cancer cell lines, including those of "MCF-7, AMN3, SK-OV-3, HeLa, SKG, and KYSE-30". The antimicrobial properties of these compounds were tested against aerobic bacteria belonging to the gram negative category "*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*, *Haemophilus influenzae, Klebsiella pneumoniae, and Shigella dysenteriae*", anaerobic bacteria "*Clostridium perfringens, Fusobacterium necrophorum, Bacteroides fragilis, and Prevotella melaninogenica*", two filamentous fungi "*Candida albicans and Aspergillus niger*", and a normal flora bacterial strain "*Escherichia coli*, BAA-1427". Moreover, the glucose-regulating impact of the synthetic products was studied *in vitro* by using two glucose-hemostasis involving enzymes known as yeast α-glucosidase (Y-enzyme) and porcine α-amylase (P-enzyme).

Experimental section

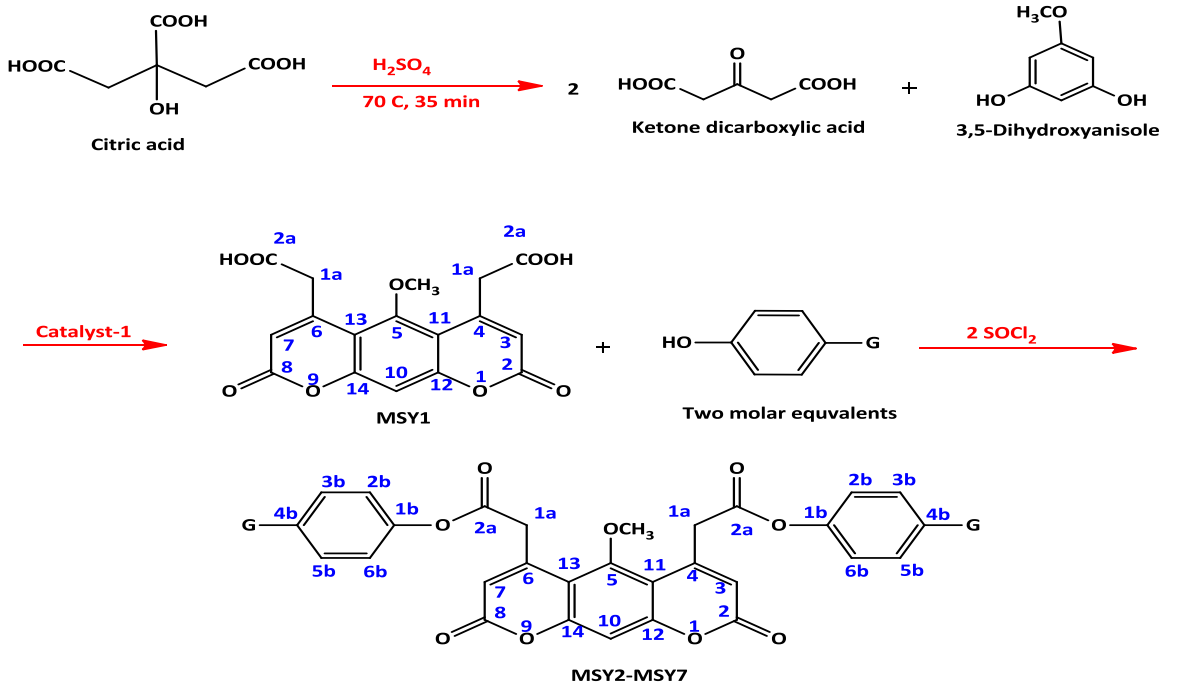
Analytical tools and chemical products

MSY1 and its derivatives **MSY2-MSY7** were synthesized by using chemicals procured from currently accessible worldwide references elsewhere without additional purifying. The melting points (M.P) of the **MSY1** and its derivatives **MSY2-MSY7** were calculated by using a digital CIA-9300

apparatus and the USP-dependent one end glass capillary technique. TLC-based methodology was used to make sure the sanctity of generated intermediate products and derivatives, with pretty standard silica gel coated aluminum trays as a static phase and an elevator combination of consisting of $CHCl_3$ and acetone in the ratio 4:1. The tools employed to analyze and record various UV (Ethanol), FTIR, ^1H-, ^{13}C-NMR spectra of the **MSY1** and its derivatives **MSY2-MSY7** were UV-PC1600 UV-Vis spectroscopy, Bruker-FTIR spectroscopy, Bruker DRX-300 (d_6-DMSO) MHz spectroscopy, correspondingly. The biomaterials and their related reagents used in this study were procured from Sigma Company and were primed and ready for use as per the commands in every handout.

Synthetic catalog

The illustration segments employed for creating **MSY1** and its derivatives **MSY2-MSY7** were displayed in Scheme 1.



MSY1: 2,2'-(5-Methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetic acid
MSY2: G= OCH_3; Bis(4-methoxyphenyl) 2,2'-(5-methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate
MSY3: G= CH_3; Di-*p*-tolyl 2,2'-(5-methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate
MSY4: G=F; Bis(4-fluorophenyl) 2,2'-(5-methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate
MSY5: G= Cl; Bis(4-chlorophenyl) 2,2'-(5-methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate
MSY6: G= Br; Bis(4-bromophenyl) 2,2'-(5-methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate
MSY7: G= I; Bis(4-iodophenyl) 2,2'-(5-methoxy-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate

SCHEME 1 Graphic stages for synthesizing **MSY1** and its based derivatives, **MSY2-MSY7**

Synthesis of ketone di-α-carboxylic acid

A solution of 19.2 g of anhydrous citric acid (0.1 mol) in 30 mL of concentrated H_2SO_4 was vortexed at ambient temperature (At) for 1 hour, and then step by step wormed to 70°C for another 1 hour. The completion of the reaction was indicated by the gas bubble formation stopping and the solution clarity. The solution was mixed with a 250 g of crushed ice/water blend and extracted with ethyl acetate. The lipophilic phase was isolated, dried, vaporized under vacuum, and the acquired light yellow residue represents the titled compound with a yield of 40% [25].

Synthesis of the novel disubstituted anisolodipyrone building unit (MSY1)

The mixture of 0.70 g of 3,5-dihydroxyanisole (5 mmol), 1.46 g of ketone di-α-carboxylic acid (10 mmol), and 25 mg of the initiator

named [Msim]HSO_4 (0.096 mmol) was vortexed for 30 min in a dry environment at 40°C. When TLC documented the reaction finishing, the resulted semi-solid was treated with 10 mL of ethyl acetate, and the initiator can be separated by decanting. The lipophilic mixture was treated with 25 mL of water, and the lipophilic layer was isolated, dehydrated, and vaporized. The ethanol recrystallization of the crude afforded the titled compound as an off-white powder. After dehydrating, the retrieved ionic liquid, the promoter, could be used in the next cycle with no further purification [26].

MSY1: Off-white powder, yield 56%, M.P 212-214°C, λ_{max} 423 nm, R_f value 0.28. IR (cm^{-1}): 3063 (alkene-lactone C-H), 3012 (carboxylic acid O-H), 2893 (aliphatic C-H), 1732 (pyrone C=O), 1694 (cis alkene C=C), and 1588 (aromatic C=C). ^{1}H-NMR (ppm): 11.09 (2H, s, H-2a), 6.70 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.77 (3H, s, 5-OCH_3), and 3.12 (4H, s, H-1a). ^{13}C-NMR (ppm): 173.2 (C-2a), 162.3 (C-2, C-8), 154.3 (C-5), 153.1 (C-4, C-6), 151.8 (C-12, C-14), 115.7 (C-3, C-7), 111.5 (C-11, C-13), 109.3 (C-10), 65.2 (5-OCH_3), and 31.0 (C-1a).

General method for synthesizing disubstituted anisolodipyrone-derived ester compounds (MSY2-MSY7)

A salt-ice bath was used to immerse a two-nick shaped flask enclosing 1.80 g **MSY1** (5 mmol) in 25 mL of restocked $SOCl_2$. The shoulder of the flask was covered with a blocker comprising blue litmus paper, whereas condenser enclosed the middle nick. Under anhydrous circumstances, the combination was lightly vortexed for 30 min, and then at At for the same timeframe before even being refluxed for 3 hours. The color transition of the litmus paper, which was changed each half an hour, was used to track the progress of a project. When the color scheme of the blue litmus paper no longer shifted, the overabundance of $SOCl_2$ was extracted out. The acyl-chloride modified version of **MSY1** emerged as a white solid material that stayed attached to the sides and bottom of the glass flask [27,28].

Under anhydrous circumstances, a blend of (9.6 mmol) monofunctionalized phenol and 1 mL pyridine in 50 mL of dry ether was poured into the flask containing the acyl-chloride modified version of **MSY1**. The reaction mixture was vortexed under the same dry circumstances for 30 minutes at At, and refluxed for a timeframe indicated by the litmus paper changing color as mentioned before. The mixture was treated with 50 mL of distilled water, and the lipophilic layer was isolated, dried, and vaporized. The purification of the synthesized disubstituted anisolodipyrone-derived ester compound was performed by re-crystallizing from a propanone and CH_2Cl_2 (1:2) blend [29,30].

MSY2: Yellowish powder, yield 82%, M.P 168-170°C, λ_{max} 560 nm, R_f value 0.71. IR (cm^{-1}): 3094 (alkene-lactone C-H), 2919 and 2822 (aliphatic C-H), 1729 (pyrone C=O), 1708 (aliphatic C=O), 1663 (cis alkene C=C), 1594 (aromatic C=C). ^{1}H-NMR (ppm): 7.01 (1H, d, *J*= 6 Hz, H-3b, H-5b), 6.75 (4H, d, *J*= 6 Hz, H-2b, H-6b), 6.70 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 4.12 (3H, s, 5-OCH_3), 3.77 (6H, s, 4b-OCH_3), and 3.12 (4H, s, H-1a). ^{13}C-NMR (ppm): 169.6 (C-2a), 162.2 (C-2, C-8), 156.4 (C-4b), 154.4 (C-5), 153.1 (C-4, C-6), 151.8 (C-12, C-14), 144.6 (C-1b), 120.2 (C-2b, C-6b), 115.7 (C-3b, C-5b), 112.2 (C-3, C-7), 111.4 (C-11, C-13), 109.3 (C-10), 65.1 (5-OCH_3), 50.1 (4b-OCH_3), and 28.3 (C, C-1a).

MSY3: Yellowish powder, yield 78%, M.P 165-167°C, λ_{max} 561 nm, R_f value 0.63. IR (cm^{-1}): 3088 (alkene-lactone C-H), 2910 and 2821 (aliphatic C-H), 1732 (pyrone C=O), 1711 (aliphatic C=O), 1666 (cis alkene C=C), and 1600 (aromatic C=C). ^{1}H-NMR (ppm): 7.26 (4H, d, *J*= 6 Hz, H-3b, H-5b), 7.24 (4H, d, *J*= 6 Hz, H-2b, H-6b), 7.03 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.77 (3H, s, 5-OCH_3), 3.12 (4H, s, H-1a), and 2.75 (6H, s, 4b-CH_3). ^{13}C-NMR (ppm): 169.6 (C-2a), 162.2 (C-2, C-8), 154.4

(C-5), 153.0 (C-4, C-6), 151.7 (C-12, C-14), 149.3 (C-1b), 134.3 (C-4b), 122.0 (C-3b, C-5b), 119.1 (C-2b, C-6b), 115.8 (C-3, C-7), 111.4 (C-11, C-13), 109.4 (C-10), 65.2 (5-OCH_3), 27.5 (C-1a), and 24.2 (4b-CH_3).

MSY4: Yellowish powder, yield 56%, M.P 182-184°C, λ_{max} 553 nm, R_f value 0.60. IR (cm^{-1}): 3068 (alkene-lactone C-H), 2916 and 2818 (aliphatic C-H), 1730 (pyrone C=O), 1707 (aliphatic C=O), 1669 (cis alkene C=C), 1595 (aromatic C=C), and 1076 (aromatic C-F), ^{1}H-NMR (ppm): 7.27 (4H, d, *J*= 6 Hz, H-3b, H-5b), 7.25 (4H, d, *J*= 6 Hz, H-2b, H-6b), 6.70 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.76 (3H, s, 5-OCH_3), and 3.12 (4H, s, H-1a). ^{13}C-NMR (ppm): 169.5 (C-2a), 162.3 (C-2, C-8), 158.7 (C-4b), 154.4 (C-5), 153.0 (C-4, C-6), 151.8 (C-12, C-14), 147.9 (C-1b), 120.7 (C-2b, C-6b), 115.8 (C-3b, C-5b), 111.4 (C-3, C-7), 109.4 (C-11, C-13), 108.5 (C-10), 65.1 (5-OCH_3), and 27.6 (C-1a).

MSY5: Yellowish powder, yield 60%, M.P 162-164°C, λ_{max} 555 nm, R_f value 0.60. IR (cm^{-1}): 3067 (alkene-lactone C-H), 2920 and 2823 (aliphatic C-H), 1732 (pyrone C=O), 1711 (aliphatic C=O), 1669 (cis alkene C=C), 1593 (aromatic C=C), and 983 (aromatic C-Cl). ^{1}H-NMR (ppm): 7.54 (4H, d, *J*= 6 Hz, H-2b, H-6b), 7.52 (4H, d, *J*= 6 Hz, H-3"b, H-5"b), 6.70 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.77 (3H, s, 5-OCH_3), and 3.12 (4H, s, H-1a). ^{13}C-NMR (ppm): 169.5 (C-2a), 162.2 (C-2, C-8), 154.4 (C-5), 153.0 (C-4, C-6), 151.8 (C-12, C-14), 150.4 (C-1b), 132.0 (C-4b), 122.9 (C-3b, C-5b), 120.5 (C-2b, C-6b), 115.8 (C-3, C-7), 111.4 (C-11, C-13), 109.4 (C-10), 65.2 (5-OCH_3), and 33.2 (C-1a).

MSY6: Yellowish powder, yield 61%, M.P 142-145 °C, λ_{max} 551 nm, R_f value 0.61. IR (cm^{-1}): 3063 (alkene-lactone C-H), 2916 and 2822 (aliphatic C-H), 1729 (pyrone C=O), 1707 (aliphatic C=O), 1666 (cis alkene C=C), 1595 (aromatic C=C), and 901 (aromatic C-Br), ^{1}H-NMR (ppm): 7.79 (4H, d, *J*= 6 Hz, H-3b, H-5b), 6.96 (4H, d, *J*= 6 Hz, H-2b, H-6b), 6.70 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.77 (3H, s, 5-OCH_3), and 3.13 (4H, s, H-1a). ^{13}C-NMR (ppm): 169.0 (C-2a), 162.3 (C-2, C-8), 154.5 (C-5), 153.0 (C-4, C-6), 151.8 (C-12, C-14), 151.4 (C-1b), 123.6 (C-3b, C-5b), 121.3 (C-2b, C-6b), 118.5 (C-4b), 115.9 (C-3, C-7), 111.4 (C-11, C-13), 109.5 (C-10), 65.2 (5-OCH_3), and 33.2 (C-1a).

MSY7: Yellowish powder, yield 53%, M.P 137-140°C, λ_{max} 553 nm, R_f value 0.62. IR (cm^{-1}): 3065 (alkene-lactone C-H), 2918 and 2824 (aliphatic C-H), 1734 (pyrone C=O), 1712 (aliphatic C=O), 1659 (cis alkene C=C), 1590 (aromatic C=C), and 869 (aromatic C-I). ^{1}H-NMR (ppm): 7.86 (1H, d, *J*= 6 Hz, H-3b, H-5b), 6.84 (4H, d, *J*= 6 Hz, H-2b, H-6b), 6.70 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.75 (3H, s, 5-OCH_3), and 3.13 (4H, s, H-1a). ^{13}C-NMR (ppm): 169.5 (C-2a), 162.2 (C-2, C-8), 154.4 (C-5), 153.1 (C-4, C-6), 151.8 (C-12, C-14), 151.3 (C-1b), 129.6 (C-3b, C-5b), 120.7 (C-2b, C-6b), 115.8 (C-3, C-7), 111.5 (C-11, C-13), 109.4 (C-10), 93.1 (C-4b), 65.1 (5-OCH_3), and 33.2 (C-1a).

Investigation of the medicinal activities of the synthesized compounds

Evaluating the activity as a potential antiproliferative candidate

A quantitative measure known as IC_{50} was used to estimate the antiproliferative impact of the novel disubstituted anisolodipyrone building unit (**MSY1**) and its derived ester compounds (**MSY2-MSY7**) *in-vitro*. By using DMSO as a solvent, six diluted concentrations were obtained from each prepared derivative, as well as the standard 5-fluorouracil (5-FU), with orders of 12.5, 25, 50, 100, 200, and 400 g/mL. In a 96-well plate, the tested cells from a specific tumor line were divided over a growth-enriched medium to obtain 10000 cells per well. After 24 hours of proliferation, every well was individually exposed to one of the previously prepared diluted concentrations. Following 72 hours of exposure, the medium was removed, and the cells' viability was estimated by using an MTT-visual probe solution. From the latter,

28 µL of 3.27 mM was added to each well, followed by incubating the cells at 37°C for 1.5 hours. A microplate reader calibrated at 492 nm was operated to measure the absorbance of the exposed and control wells, symbolized as Ae and Ac, respectively. The antiproliferative activity was represented as a growth inhibition percent (GI%), which was calculated by applying the following equation [31, 32]: GI% = (Ac-Ae) / Ac × 100.

The IC_{50} score was calculated by plotting the GI% values versus the logarithmic concentrations in non-linear regression. The IC_{50} represents the concentration at which 5-FU or prepared derivatives inhibit cellular growth by half. Each tested derivative assessed underwent this method in triplicate.

Inspecting the activity as an antimicrobial candidate

Activity against aerobic gram-negative bacteria

A broth-dilution assay was applied to determine the activity of the novel disubstituted anisolodipyrone building unit (**MSY1**) and its derived ester compounds (**MSY2-MSY7**) against pathogenic aerobic gram-negative bacteria *in-vitro*. Briefly, 7.5 mg of each tested derivative, as well as the standard ciprofloxacin (CPF), was dissolved in 5 mL of methyl sulfoxide (DMSO) to make the mother solution. After that, by using an aqueous diluent, a set of 13 twofold dilutions with concentrations ranging from 0.25 to 1024 g/mL were obtained. In a marked test tube, 3 mL of Mueller-Hinton broth, which is a growth-enriched medium, was added, followed by pipetting 0.2 mL of bio-inoculant diluted to 0.5 McFarland and 1 mL of the tested concentration. The bacterial growth was checked by eye observation after one day of incubation at 37°C. From the concentration that demonstrated nearly zero growth of bacteria, the second set of dilutions were prepared with concentrations based on 4, 1, 0.5, and 0.05 to specify the minimum inhibitory concentration (MIC). Each tested derivative assessed underwent this method in triplicate [33].

Activity against anaerobic bacteria

The procedure applied to estimate the activity against pathogenic aerobic bacteria was similar to that of valuing the activity against those growing anaerobically, with minor differences. The latter included the usage of metronidazole (MNZ) as a standard and Brucella-agar fortified with 5% blood of sheep as a growth-enriched medium. Also, the incubation of the anaerobic bacteria was performed for 48 hours at 37°C in a system supported with an anaerobe marker and a palladium metal as a promoter under anaerobic milieu conditions [nitrogen gas (80%), hydrogen gas (10%), and carbon dioxide gas (10%)]. This methodology was employed for each experimental variant in three replications [34].

Activity against filamentous fungi

The procedure applied to estimate the activity against filamentous fungi was slightly different from that of valuing the activity against the bacteria grown aerobically. The differences included the incubation of filamentous fungus for 48 hours at 30°C. Besides, the standard and the growth-enriched medium used were nystatin (NYS) and Sabouraud-dextrose broth, respectively. The minimum fungal inhibitory concentration has been abbreviated as MFC instead of MIC in the case of pathogenic bacteria [35].

Quantifying the activity as a glucose-regulating candidate

The capacity of the synthesized novel disubstituted anisolodipyrone building unit (**MSY1**) and its derived ester compounds (**MSY2-MSY7**) to recede Y-enzyme and abate P-enzyme was quantified *in-vitro*. These two enzymes are important in the process of

blood glucose level control. From the mother solution (2 mg/mL) for each tested derivative as well as the standard acarbose (AC), different dilutions were attained by using methyl alcohol as a solvent with concentrations of 1000, 800, 400, 200, 100, 50, and 25 µg/mL. A spectrophotometer was operated to measure the absorbance of the tested and control samples which were symbolized as At and Ac, respectively. The glucose-regulating activity was represented as a suppressing percent (S%), which was calculated by applying the following equation [36]: S% = (Ac-At) / Ac × 100.

The SC_{50} score was calculated by plotting the S% values versus the logarithmic concentrations in non-linear regression. The SC_{50} represents the concentration at which AC or prepared derivatives suppress the enzymatic activity by half. This technique was conducted to each tested derivative in replicates.

Y-enzyme receding assay

In the beginning, 20 µL of the tested dilution was mixed with 20 µL of Y-enzyme (0.1 unit/mL) in phosphate-buffered solution (pH 6.8, K_3PO_4) to make the first mixture. The second mixture was created by solubilizing the standard substrate named chemically as 4-nitrophenylglucopyranoside in K_3PO_4 to achieve a concentration of 375 µg/mL. Then, 40 µL of the first mixture was combined with 40 µL of the second mixture and incubated for half an hour at 37 °C. To cease the interaction, 80 µL of K_3PO_4 bearing disodium salt of carbonic acid (0.2 M) was pipetted into the previous combination. The resulting solution's ability to recede the Y-enzyme was measured colorimetrically at 405 nm. The control solution was prepared with the same previous steps, with the exception that the tested dilution was replaced with K_3PO_4 [37].

P-enzyme abating assay

First, 20 µL of the tested dilution was mixed with 20 µL of P-enzyme (2 units/mL) in K_3PO_4 to make the first mixture. The second mixture was created by dispersing the standard substrate named starch in K_3PO_4 to achieve a 2 mL of 500 µg/mL concentration. Then, 40 µL of the first mixture was combined with 40 µL of the second mixture and incubated for 10 min at At. To cease the interaction, 2 mL of aqueous natriumhydroxid solution (0.4 M) bearing anhydrous Seignette salt (12%) and 3,5-dinitro-2-hydroxybenzoic acid (1%) was pipetted into the previous combination. After that, for 15 min, the resulting solution was heated in a boiling water bath, followed by readjusting to 10 mL with distilled water and cooling to At in an ice bath. The resulting solution's ability to abate the P-enzyme was measured colorimetrically at 540 nm. The control solution was prepared with the same previous steps, with the exception that the tested dilution was replaced with K_3PO_4 [37].

Results and discussion

Synthetic pathway

Initially, two attempts were made to prepare the **MSY1**, utilizing strong inorganic acid as a promoter. In the first one, the authors interacted ketone di-α-carboxylic acid with 3,5-dihydroxyanisole in a ratio of 2:1. In the second attempt, ketone di-α-carboxylic acid was interacted with 3,5-dihydroxyanisole in a ratio that ranged from 2:1 to 4:1. In both instances, the resulting product was an anisolopyrone rather than a disubstituted anisolodipyrone. This can be attributed to the weak nucleophilic attacking capacity of the phenolic-OH in the formed intermediate because of the elongated conjugation.

In addition, rather than strong inorganic acid, a variety of promoters were used such as organic, inorganic, homogeneous, and inhomogeneous. However, the same outcome was obtained. Finally, the novel disubstituted anisolodipyrone precursor (**MSY1**) was synthesized by using [Msim]HSO_4, a green reusable promoter.

Medicinal activities of the synthesized compounds

The activity as antiproliferative candidates

The synthesized novel disubstituted anisolodipyrone building unit (**MSY1**) and its disubstituted anisolodipyrone-derived ester compounds (**MSY2**-**MSY7**) were tested as antiproliferative candidates against six cell lines derived from various tumor types, as reported in Table 1. These cell lines were "MCF-7, HeLa, SKG, AMN3, SK-OV-3, and KYSE-30". The universal scientific codes and origins of these lines are: "86012803 and Caucasian breast adenocarcinoma", "93021013 and epithelioid cervix carcinoma", "C27676 and human papillomavirus-related cervical squamous cell carcinoma", "CVCL-M395 and murine mammary adenocarcinoma", "91091004 and Caucasian ovary adenocarcinoma", and "94072011 and squamous cell carcinoma abstracted from Asian esophageal", consequently [33,34].

TABLE 1 The results of investigating **MSY1-MSY7** as antiproliferative candidates

Compound symbol	IC_{50} (μM) ± SD (*n*=3) for the employed tumor/normal cell lines						
	MCF-7	HeLa	SKG	AMN3	SK-OV-3	KYSE-30	RWPE-1
			(Tumor-derived)				(Normal)
5-FU	12.42±0.99	13.37 ±1.05	22.12 ±0.98	24.89 ±1.12	22.43 ±1.16	30.72 ±1.02	34.79±0.96
MSY1	91.16±1.09	62.09 ±1.06	99.03±1.02	63.69±0.94	64.54±0.99	68.92±1.04	40.56±1.03
MSY2	28.52±0.98	24.41 ±0.98	46.11±1.12	54.1±1.03	57.80±1.08	50.02±0.96	55.34±0.98
MSY3	34.20±1.01	41.08 ±0.92	42.02±1.01	67.42±0.95	39.97±1.04	53.66±1.12	48.26±1.13
MSY4	13.10±1.01	13.39 ±1.09	31.05±1.10	28.33±0.92	24.89±0.96	41.36±0.94	112.68±1.04
MSY5	22.48±1.05	16.27 ±1.06	31.19±1.11	30.52±1.11	28.77±0.94	41.62±1.01	57.92±0.96
MSY6	84.13±1.06	53.25 ±1.01	82.58±1.06	62.94±1.02	57.12±1.06	57.32±1.12	44.33±1.02
MSY7	88.82±1.02	53.93 ±0.91	79.38±1.01	69.07±1.04	63.06±0.97	60.11±0.92	40.81±0.94

Ranking the obtained IC_{50} values of the synthesized novel disubstituted anisolodipyrone building unit (**MSY1**) and its disubstituted anisolodipyrone-derived ester compounds (**MSY2**-**MSY7**) concerning each cell line derived from a specific type of tumor is involved in Table 2.

TABLE 2 The rank of antiproliferative activity concerning the synthesized **MSY1-MSY7** versus the investigated tumor-derived cell lines

Activity rank	MCF-7	SK-OV-3	HeLa	SKG	AMN3	KYSE-30
1	**MSY4**	**MSY4**	**MSY4**	**MSY5**	**MSY4**	**MSY4**
2	**MSY5**	**MSY5**	**MSY5**	**MSY4**	**MSY5**	**MSY5**
3	**MSY2**	**MSY3**	**MSY2**	**MSY3**	**MSY2**	**MSY2**
4	**MSY3**	**MSY6**	**MSY3**	**MSY2**	**MSY6**	**MSY3**
5	**MSY6**	**MSY2**	**MSY6**	**MSY7**	**MSY1**	**MSY6**
6	**MSY7**	**MSY7**	**MSY7**	**MSY6**	**MSY3**	**MSY7**
7	**MSY1**	**MSY1**	**MSY1**	**MSY1**	**MSY7**	**MSY1**

Observing the two above tables led to a multitude of points about the antiproliferative activity of the synthesized **MSY1** and its derived ester compounds against the test cell lines that were derived from various tumor types. Firstly, **MSY4** and **MSY5**, which are fluoro- and chloro-containing derivatives, were the most effective ones against all cell lines which were tested. This is partly a result of the electron-attracting ability of the fluoride and chloride monomers, which renders the derivatives

more active. Second, the antiproliferative effects of the synthesized novel disubstituted anisolodipyrone building unit (**MSY1**) and its disubstituted anisolodipyrone-derived ester compounds (**MSY2**-**MSY7**) were lower than those of the standard drug, 5-FU [35].

The cytotoxicity of the synthesized **MSY1** and its derived ester compounds was tested using RWPE-1 as a normal cell epitome "human normal prostate epithelial cells". On this epitome, the novel disubstituted anisolodipyrone building unit (**MSY1**) and its disubstituted anisolodipyrone-derived ester compounds (**MSY2**-**MSY7**) were shown to be safer than 5-FU [35].

The activity as antimicrobial candidates

In this study, the activity of the synthesized derivatives to present as inhibitor for overgrowth of pathogenic aerobic bacteria, anaerobic bacteria, and fungi antibacterial was assessed by using the well-known broth-dilution methodology.

Aerobic bacteria

In this investigation, the following pathogenic aerobic to the gram negative category were involved: "*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Haemophilus influenza, Escherichia coli, Salmonella typhi,* and *Shigella dysenteriae"*. The specific ATCC codes and the abbreviations utilized for these bacteria in this study are: "27853 and P-aeruginosa", "700603 and K-pneumonia", "49247 and H-influenza", "25922 and E-coli","6539 and S-typhi", and "13313 and S-dysenteriae", correspondingly. Besides that, the estimated risk of the synthesized derivatives has been probed on "*Escherichia coli*" strain (BAA-1427, E-coli).

As indicated in the following table, the synthesized **MSY1** and its derived ester compounds have a lower bacterial inhibition effect on strains of bacteria than CPF. In addition, these derivatives, in the order listed, impede microbial activity: **MSY5**, **MSY2**, **MSY3**, **MSY4**, **MSY6**, **MSY7**, and **MSY1**. Furthermore, **MSY5** has more activity within and between our derivatives. This could be due to the chloride functional group as one of the most powerful electron-attacking spares, leading to the formation of a particularly active moiety [38].

Another two facts emerged from observing the impact of the synthesized derivatives on the experimental normal flora bacterial strain to determine their cellular safety profiles. The first is that the synthesized **MSY1** and its derived ester compounds are safer for the BAA-1427 strain than CPF. The second is the estimated risk of our compounds is listed in the incoming descending rank: **MSY1**, **MSY5**, **MSY6**, **MSY4**, **MSY7**, **MSY3**, and **MSY2**.

TABLE 3 The growth inhibitory effect of **MSY1-MSY7** compounds and the standard versus the aerobic bacterial strains involved

Abbreviations of the involved aerobic bacteria	Measured pathogenic term	Investigated compounds							
		CPF	MSY 1	MSY 2	MSY 3	MSY 4	MSY 5	MSY 6	MSY 7
P-aeruginosa	MIC	0.75	14.00	5.00	6.00	6.50	2.05	9.00	9.00
K-pneumonia	MIC	0.40	12.00	4.50	6.50	6.00	2.15	8.00	8.50
H-influenzae	MIC	0.60	13.00	4.00	8.00	6.50	1.80	7.50	9.00
E-coli	MIC	0.85	18.00	4.50	7.50	8.50	1.65	10.00	9.50
S-typhi	MIC	0.80	16.00	6.00	7.00	8.50	2.25	10.50	11.50
S-dysenteriae	MIC	0.55	15.00	5.50	6.50	7.00	2.05	9.00	11.00
E-coli (normal)	MIC	0.90	18.00	9.00	12.00	11.00	14.00	15.00	15.00

Anaerobic pathogenic bacteria

In this microbiological evaluation, the utilized pathogenic anaerobic bacterial strains were: "*Bacteroides fragilis, Clostridium perfringens, Fusobacterium necrophorum,* and *Prevotella melaninogenica"*. The respective ATCC identifiers and acronyms were used for these bacteria in this study: "25285 and B-fragilis", "13124 and C-perfringens", "25286 and F-necrophorum", and "25845 and P-melaninogenica", correspondingly.

Table 4 represents that the synthesized derivatives have far more lower activity compared with the metronidazole, MNZ. In addition, our derivatives inhibited an anaerobic bacterial growth in a similar manner. Finally, their growth inhibitory effects against the test pathogenic microorganisms are listed in the incoming sequence: **MSY2, MSY3, MSY5, MSY4, MSY7, MSY6,** and **MSY1**.

TABLE 4 The growth inhibitory effect of **MSY1**-**MSY7** compounds and the standard versus the anaerobic bacteria employed

Abbreviations of the involved anaerobic bacteria	Measured pathogenic term	Investigated compounds							
		MNZ	MSY1	MSY2	MSY3	MSY4	MSY5	MSY6	MSY7
B-fragilis	MIC	3.00	60.00	12.00	18.00	36.00	40.00	52.00	42.00
C-perfringens	MIC	0.75	52.00	9.00	20.00	38.00	32.00	48.00	38.00
F-necrophorum	MIC	1.75	36.00	11.00	22.00	38.00	34.00	48.00	56.00
P-melaninogenica	MIC	0.75	52.00	12.00	22.00	48.00	38.00	40.00	60.00

Filamentous fungi

The novel disubstituted anisolodipyrone building unit (**MSY1**) and its disubstituted anisolodipyrone-derived ester compounds (**MSY2**-**MSY7**) were assessed *in vitro* against the following pathologic fungal microorganisms: *Aspergillus niger* and *Candida albicans*. The corresponding ATCC serial numbers and the utilized symbols are "16888 and A-niger" and "10231 and C-albicans).

As depicted in Table 5, numerous pivotal comments are decided to make. The most notable difference is that, when compared with nystatin (NYS), the synthesized derivatives, including **MSY1**, **MSY4**, and **MSY5**, have a very powerful fungal inhibition impact. **MSY6** and **MSY7**, on the other hand, show almost no effectiveness against the experimental fungal strains. This could be because the bromide and iodide monomers in these two derivatives have a lower electron-attracting functionality than other substituents, attempting to make the corresponding derivative less active [39]. Finally, our derivatives can suppress the fungal growth in the incoming rank: **MSY1**, **MSY4**, **MSY5**, **MSY3**, **MSY2**, **MSY7**, and **MSY6**.

TABLE 5 The growth inhibitory effect of **MSY1**-**MSY7** compounds and the standard versus two investigated fungi

Abbreviations of the involved filamentous fungi	Measured pathogenic term	Investigated compounds							
		NYS	MSY1	MSY2	MSY3	MSY4	MSY5	MSY6	MSY7
C-albicans	MFC	4.00	1.50	7.00	6.00	1.90	1.90	24.00	16.00
A-niger	MFC	8.00	1.85	12.00	18.00	2.15	2.10	40.00	30.00

The activity as glucose-regulating candidates

Based on the findings of Table 6, some important measurements were recorded. For starters, the synthesized derivatives may inhibit both the Y-enzyme and P-enzyme. Second, our molecules had a lower glucose-regulating effect than the standard, acarbose (AC). Third, of these novel chemical compounds, **MSY2** and **MSY3** have the most potent inhibiting characteristics. This could be credited to the OCH_3 and CH_3 monomers found in these derivatives, correspondingly [40]. The novel derivatives' glucose-regulating characteristics are listed in the following order: **MSY2, MSY3, MSY1, MSY4, MSY5, MSY6,** and **MSY7**.

TABLE 6 The glucose-regulating potential of the standard and **MSY1-MSY7** compounds

Compound symbol	Assays and their corresponding results			
AC	**Y-enzyme receding capacity SC_{50}±SD**	283.04±0.88	**P-enzyme abating capacity SC_{50}±SD**	263.26±0.92
MSY1		396.58±1.04		348.07±0.97
MSY2		364.23±0.98		326.32±0.90
MSY3		366.68±0.95		334.01±0.95
MSY4		402.85±0.96		352.52±1.01
MSY5		413.12±0.98		352.94±1.06
MSY6		421.87±1.02		370.11±0.92
MSY7		423.84±1.06		372.25±0.86

Conclusion

A novel chemical nucleus known as disubstituted anisolodipyrone and symbolized as **MSY1** was successfully created. By esterifying with different monofunctionalized phenols, **MSY1** was transformed to a panel of disubstituted anisolodipyrone-derived ester compounds, marked as **MSY2-MSY7**. The results of investigating the antiproliferative, anti-gram-negative bacteria, anti-anaerobic bacteria, antifungal, cellular safety on normal cells and bacteria, and glucose-regulating properties of the synthesized derivatives revealed interesting conclusions. **MSY4** has the potential to be an effective antiproliferative candidate with a wide-ranged spectrum of effectiveness. In addition, the **MSY5** evidenced as a potent and promising antimicrobial agent. On the other side, **MSY2** has the strongest suppressive properties among the synthesized compounds regarding the investigated Y- and P-enzyme. The findings of the study indicated that our novel compounds could be viewed as medically validated platforms for the invention of the novel potent bioactive candidates.

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