Received: 05 April 2022 | Revised: 06 May 2022 | Accepted: 13 May 2022
DOI: 10.22034/ecc.2022.340784.1459
Eurasian Chemical Communications
SAMI

FULL PAPER

Synthesis and biomedical activities of novel multifunctional benzodipyrone-based derivatives

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In this research, acetone dicarboxylic acid generated from citric acid was employed in the Pechmann coupling reaction to produce a novel multifunctional benzodipyrone precursor (**CSY1**). The latter was reacted with a variety of substituted phenols to produce the six **CSY1**-based derivatives symbolized here as **CSY2-CSY7**. The chemical structures of the precursor and its based derivative were efficiently recognized by examining the acquired spectral charts. Our compounds' cytotoxic, antimicrobial, and hypoglycemic activities were tested *in vitro*. The initial activity was evaluated against six tumorigenic cell lines utilizing an IC_{50} matrix. The antibacterial activity of six aerobic gram-negative bacteria, four anaerobic bacteria, two fungi, and one non-pathogenic bacterial strain was assessed using a broth-dilution test. Furthermore, the hypoglycemic activity on yeast α-glucosidase and porcine α-amylase, two types of blood glucose-controlling enzymes, was calculated. According to the findings of the initial activity, our compounds, notably **CSY4**, displayed a potent-to-moderate wide-range of cytotoxic properties. This action is accompanied by a low risk of normal cell toxicity. Furthermore, these compounds showed promising antibacterial properties, particularly **CSY5** for aerobic gram-negative bacteria, **CSY2** for anaerobic bacteria, and **CSY1** for pathogenic fungi. This promise is supported by our chemicals relative safety when it comes to the bacteria of the normal flora. The compounds had moderate-to-weak inhibitory effects on the blood glucose-controlling enzymes tested, with **CSY2** and **CSY3** having the strongest hypoglycemic potential. Our molecules, according to the results, could provide unique bioactive platforms that might open up new avenues for the identification of novel therapeutically active drugs

KEYWORDS

Synthesis; $[Msim]HSO_4$; benzodipyrone-based derivatives; cytotoxicity; antimicrobial; antidiabetic.

Introduction

Cancer and microbial resistance are two of most critical public health issues. Their prevalence has grown dramatically in practically every region of the planet since 1990 [1]. Despite the fact that many experimental drugs have been developed from both natural [2] and synthetic [3] sources, the effective prevention and treatment of many types of cancer and infection remains a challenge. Consequently, there is an urgent need to synthesize novel chemical entities and explore their biological activities in order to find a viable solution to these two health issues [4].

Coumarin is derived from the French word coumarou, which refers to the Tonka bean seeds of *Dipteryx odorata*, which was one of the primary natural products to be identified in 1820. Coumarin has a pleasant odor, so that it has been utilized in perfumes since 1882 [5,6]. Coumarinic compounds are lactones with a benzene ring fused to an α-pyrone ring, resulting in a conjugated system with a lot of electrons and strong charge-transport properties [7,8]. The coumarin scaffold's simplicity and adaptability make it an appealing starting point for a variety of applications [9,10]. Coumarins are used as fragrances, cosmetics, and industrial additives. Some of its analogues have been employed in tobacco and alcoholic beverages as odor enhancers [11,12]. However, natural products, organic chemistry, and medicinal chemistry [13,14] define their most important role.

Furthermore, several coumarin compounds are being actively explored as medical possibilities for medications with significant pharmacological activity, low toxicity and side effects, less drug resistance, high bioavailability, broad range of therapeutic benefits, and so on, to treat a variety of disorders [15]. Several attempts have been made to create coumarin-based anticoagulants, antioxidants [16], antimicrobial (antiviral, antifungal, and antiparasitic) [17], anticancer [18,19], anti-diabetic, analgesic, anti-neurodegenerative, and anti-diabetic agents. [20], as well as anti-inflammatory drugs [21]. Coumarin compounds also play an essential role in medical chemistry because of their distinctive and adaptable oxygen-containing heterocyclic structure [22]. Furthermore, investigations into coumarins as bioactive agents [23], supramolecular medicinal agents, diagnostic agents, pathologic probes, and biological stains have been conducted [24].

The goal of this research is to create a number of novel multifunctional benzodipyrone-based derivatives with enhanced anticancer, antibacterial, and hypoglycemic properties. The Pechmann reaction was used to synthesize **CSY1** in order to achieve this goal. The latter molecule was utilized as a starting point for coupling with various substituted phenols, yielding six congeners identified as **CSY2-CSY7**. Our chemicals were tested against six tumor cell lines: MCF-7, HeLa, SKG, AMN3, SK-OV-3, and KYSE-30. The antimicrobial properties of these compounds were tested against six aerobic gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Haemophilus influenzae, Escherichia coli, Salmonella typhi, and Shigella dysenteriae*), four anaerobic bacteria (*Bacteroides fragilis, Clostridium perfringens, Fusobacterium necrophorum, and Prevotella melaninogenica*), two fungi (*Candida albicans and Aspergillus niger*), and one non-pathogenic bacterial strain (*Escherichia coli*, BAA-1427). Moreover, the hypoglycemic impact of the synthesized compounds was investigated utilizing yeast α-glucosidase (YG) and porcine α-amylase (PA), two different types of blood glucose-controlling enzymes.

Experimental section

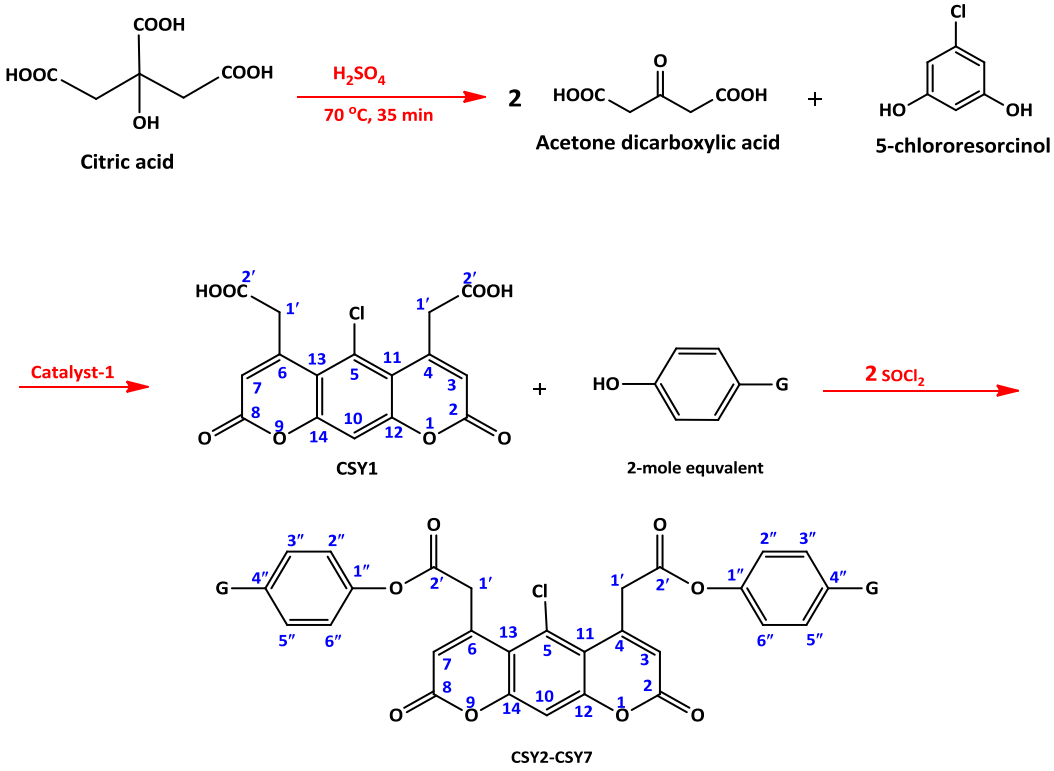
Chemicals and instruments

The chemicals used to synthesize **CSY1** and its derivatives **CSY2-CSY7** were obtain from publicly available worldwide sources and used without further purification. The melting point (M.P) scores of the synthesized compounds were determined using an electrothermal digital CIA-9300 equipment and the USP-dependent capillary technique. Thin-layer chromatography (TLC) was employed to ensure the purity of produced chemical intermediates and compounds, using typical silica gel aluminum-based plates and an eluting combination of $CHCl_3$: acetone (4:1) as an eluting mixture. Instruments employed to scan the UV, FTIR, ^{1}H-, ^{13}C-NMR spectra of the synthesized compounds

included UV- 1600PC UV-Vis, Bruker-α- ATR-FTIR, Bruker Avance DRX-300 (DMSO-d_6) MHz spectrophotometers, respectively. The tumorous-cell lines and pathogenic standard microbes employed in this research were purchased from Sigma-Aldrich Company and prepared for use according to each leaflet instructions.

Synthesis plan

SCHEME 1 depicts the graphic stages used in the synthesis of **CSY1** and its derivatives **CSY2-CSY7**.



CSY1: 2,2'-(5-Chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetic acid
CSY2: G= OCH3; Bis(4-methoxyphenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetate
CSY3: G= CH3 ; Di-p-tolyl 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetate
CSY4: G=F ; Bis(4-fluorophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetate
CSY5: G= Cl ; Bis(4-chlorophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetate
CSY6: G= Br ; Bis(4-bromophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetate
CSY7: G= I ; Bis(4-iodophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-g]chromene-4,6-diyl)diacetate

SCHEME 1 Graphic stages for synthesizing **CSY1** and its based derivatives, **CSY2-CSY7**

Synthesis of acetone dicarboxylic acid

Anhydrous citric acid (0.1 mole, 19.2 g) was agitated in 30 mL concentrated H_2SO_4 for 60 min at 25 °C, then gradually heated to 70 °C (rate of heating regulated by foaming). After 60 min of stirring at this temperature, the carbon monoxide production ended, and a clear solution formed, which was then poured into 250 g of crushed ice water. The obtained ethyl acetate-containing phase was concentrated and dried to generate light yellow acetone dicarboxylic acid with a yield of 40% [25].

Synthesis of the novel multifunctional benzodipyrone precursor (CSY1)

The mixture of 5-chlororesorcinol (5 mmol, 0.72 g), acetone dicarboxylic acid (10 mmol, 1.46 g), and [Msim]HSO_4 as a catalyst (0.096 mmol, 25 mg) was agitated under solvent-free conditions at 40 °C for 30 min. The resultant semi-solid crude was diluted with ethyl acetate (10 mL) when TLC documented

the finishing of the reaction, and the catalyst can be isolated via a decantation. By washing the organic phase with water (25 mL), separating both phases, and evaporating the organic layer, the target **CSY1** compound was acquired and purified by recrystallization from ethanol, affording a creamy powder. After drying, the recovered ionic liquid may be utilized without additional purification in the following run [26].

CSY1: 2,2'-(5-Chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetic acid. Creamy powder, yield= 48%, M.P = 206-208 °C, λ_{max} (Ethanol, nm) = 402 nm, R_f value (chloroform: acetone 4:1) = 0.22. IR (cm^{-1}): 3062 (O-H, carboxylic acid), 3013 (C-H, aryl), 2890 (C-H, alkane), 1732 (CO, lactone), 1690 (CO, COOH), and 1590, 1549 (C=C, aryl). ^{1}H-NMR (ppm): 11.09 (2H, s, COOH), 7.06 (1H, s, H-10), 6.35 (2H, s, H-3,H-7), and 3.12 (4H, s, H-1`, H-1`). ^{13}C-NMR (ppm): 173.14 (C-2`, C-2`), 162.22 (C-2, C-8), 153.02 (C-4, C-6), 151.78 (C-12, C-14), 130.14 (C-5), 127.47 (C-11, C-13), 115.76 (C-10), 113.42 (C-3, C-7), and 30.92 (C-1`, C-1`).

General method for synthesizing CSY1-based derivatives (CSY2-CSY7)

A two-nick round-bottomed flask containing **CSY1** (5 mmol, 1.82 g) in 25 mL of replenished $SOCl_2$ was submerged in a salt-ice bath. A stopper containing blue litmus paper was used to cover the side-nick, while a condenser was used to enclose the center nick. The mixture was gently stirred for 30 min under anhydrous conditions, then for the same amount of time at room temperature (RT), before being refluxed for 3 hrs. The color shift of the litmus paper that replaced every 30 min was used to monitor the progress of the reaction. The excess of $SOCl_2$ was distilled out until the color of the blue litmus paper no longer changed. The acyl-chloride derivative of **CSY1** visually appeared as a white solid substance that remained in the flask's concave [27,28].

A solution of mono-functionalized phenol (9.6 mmol) and pyridine (1 mL) in 50 mL of anhydrous diethyl ether was added in one part at RT to the same flask containing the white residue and stirred under dehydrated conditions for 30 min. The reaction was refluxed for a length of time, as demonstrated by the litmus paper changing color as mentioned above. After the reaction was completed, the organic layer was separated, dried, and vaporized following the addition of 50 mL of H_2O to the reaction mixture. The **CSY1**-based derivative was obtained by recrystallizing from a blend of propanone and CH_2Cl_2 (1:2) [29,30].

CSY2: G= OCH_3; Bis(4-methoxyphenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate. Pale yellow powder, 84%, M.P 160-162 °C, λ_{max} (Ethanol, nm) = 536 nm, R_f value (chloroform: acetone 4:1) = 0.62. IR (cm^{-1}): 3097 (C-H, alkene-lactone), 2918 (C-H, CH_3), 2823 (C-H, CH_2), 1730 (C=O, cyclic lactone ester), 1709 (C=O, side chain ester), 1667 (C=C, lactone), 1596 (C=C, aryl). ^{1}H-NMR (ppm): 7.06, 7.02 (1H, d, H-3", H-5"), 7.00 (1H, s,H-10), 6.75, 6.73 (4H, d, H-2", H-6"), 6.35 (2H, s, H-3, H-7), 4.12 (6H, s, OCH_3), and 3.12 (4H, s, H-1`, H-1`). ^{13}C-NMR (ppm): 169.54 (C-2`, C-2`), 162.20 (C-2, C-8), 156.43 (C-4", C-4"), 153.03 (C-4, C-6), 151.77 (C-12, C-14), 144.56 (C-1", C-1"), 130.14 (C-5), 127.48 (C-11, C-13), 120.12 (C-2", C-6"), 115.75 (C-3", C-5"), 113.43 (C-10), 112.26 (C-3, C-7), 51.1 (OCH_3), and 28.3 (C, C-1`, C-1`).

CSY3: G= CH_3; Di-p-tolyl 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl)diacetate. Pale yellow powder, 80%, M.P 151-153 °C, λ_{max} (Ethanol, nm) = 540 nm, R_f value (chloroform: acetone 4:1) = 0.60. IR (cm^{-1}): 3092 (C-H, alkene-lactone), 2879 (C-H, CH_3), 2822 (C-H, CH_2), 1735 (C=O, cyclic lactone ester), 1710 (C=O, side chain ester), 1670 (C=C, lactone), and 1599 (C=C, aryl). ^{1}H-NMR (ppm): 7.26 (4H, d, H-3", H-5"), 7.24 (4H, d, H-2",H-6"), 7.01 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), 3.12 (4H, s, H-

1`, H-1`), and 2.75 (6H, s, CH_3). ^{13}C-NMR (ppm): 169.52 (C-2`, C-2`), 162.21 (C-2, C-8), 153.01 (C-4, C-6), 151.78 (C-12, C-14), 149.30 (C-1", C-1"), 134.24 (C-4", C-4"),130.10 (C-5), 127.49 (C-3", C-5"), 121.98 (C-11, C-13), 119.03 (C-2", C-6"), 115.76 (C-10), 113.42 (C-3, C-7), 27.51 (C-1`, C-1`), and 24.12 (CH_3).

CSY4: G=F; Bis(4-fluorophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl) diacetate. Pale yellow powder, 55%, M.P 177-179 °C, λ_{max} (Ethanol, nm) = 526 nm, R_f value (chloroform: acetone 4:1) = 0.54. IR (cm^{-1}): 3068 (C-H, alkene-lactone), 2820 (C-H, CH_2), 1731 (C=O, cyclic lactone ester), 1713 (C=O, side chain ester), 1665 (C=C, lactone), 1593 (C=C, aryl), and 1075 (C-F), ^{1}H-NMR (ppm): 7.27 (4H, d, H-3", H-5"), 7.25 (4H, d, H-2",H-6"), 7.00 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), and 3.12 (4H, s, H-1`, H-1`). ^{13}C-NMR (ppm): 169.50 (C-2`, C-2`), 162.24 (C-2, C-8), 158.72 (C-4", C-4"), 153.00 (C-4, C-6), 151.75 (C-12, C-14), 147.92 (C-1", C-1"), 130.08 (C-5), 127.52 (C-11, C-13), 120.73 (C-2", C-6"), 115.75 (C-3", C-5"), 113.44 (C-10), 108.5 (C-3, C-7), and 27.5 (C-1`, C-1`).

CSY5: G= Cl; Bis(4-chlorophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl) diacetate. Pale yellow powder, 61%, M.P 155-157 °C, λ_{max} (Ethanol, nm) = 525 nm, R_f value (chloroform: acetone 4:1) = 0.56. IR (cm^{-1}): 3067 (C-H, alkene-lactone), 2822 (C-H, CH_2), 1729 (C=O, cyclic lactone ester), 1708 (C=O, side chain ester), 1665 (C=C, lactone), 1592 (C=C, aryl), and 980 (C-Cl). ^{1}H-NMR (ppm): 7.54 (4H, d, H-2", H-6"), 7.52 (4H, d, H-3",H-5"), 7.34 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), and 3.12 (4H, s, H-1`, H-1`). ^{13}C-NMR (ppm): 169.53 (C-2`, C-2`), 162.22 (C-2, C-8), 153.04 (C-4, C-6), 151.76 (C-12, C-14), 150.42 (C-1", C-1"), 132.01 (C-4", C-4"), 130.11 (C-3", C-5"), 127.53 (C-5), 122.83 (C-11, C-13), 120.52 (C-2", C-6"), 115.78 (C-10), 113.42 (C-3, C-7), and 33.20 (C-1`, C-1`).

CSY6: G= Br; Bis(4-bromophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl) diacetate. Pale yellow powder, 64 %, M.P 133-135 °C, λ_{max} (Ethanol, nm) = 529 nm, R_f value (chloroform: acetone 4:1) = 0.57. IR (cm^{-1}): 3064 (C-H, alkene-lactone), 2816 (C-H, CH_2), 1730 (C=O, cyclic lactone ester), 1707 (C=O, side chain ester), 1662 (C=C, lactone), 1590 (C=C, aryl), and 898 (C-Br), ^{1}H-NMR (ppm): 7.06 (4H, d, H-3", H-5"), 6.75 (4H, d, H-2",H-6"), 4.12 (1H, s, H-10), 6.35 (2H, s, H-3, H-7), and 3.12 (4H, s, H-1`, H-1`). ^{13}C-NMR (ppm): 169.51 (C-2`, C-2`), 162.26 (C-2, C-8), 153.01 (C-4, C-6), 151.79 (C-12, C-14), 151.33 (C-1", C-1"), 130.16 (C-3", C-5"), 127.52 (C-5), 123.58 (C-11, C-13), 121.28 (C-2", C-6"), 118.49 (C-4", C-4"), 115.80 (C-10), 113.41 (C-3, C-7), and 33.23 (C-1`, C-1`).

CSY7: G= I; Bis(4-iodophenyl) 2,2'-(5-chloro-2,8-dioxo-2,8-dihydropyrano[3,2-*g*]chromene-4,6-diyl) diacetate. Pale yellow powder, 53 %, M.P 130-133 °C, λ_{max} (Ethanol, nm) = 528 nm, R_f value (chloroform: acetone 4:1) = 0.58. IR (cm^{-1}): 3060 (C-H, alkene-lactone), 2821 (C-H, CH_2), 1730 (C=O, cyclic lactone ester), 1708 (C=O, side chain ester), 1659 (C=C, lactone), 1590 (C=C, aryl), and 864 (C-I). ^{1}H-NMR (ppm): 7.84 (1H, d, H-3", H-5"), 7.07 (1H, s, H-10), 6.84 (4H, d, H-2",H-6"), 6.35 (2H, s, H-3, H-7), and 3.13 (4H, s, H-1`, H-1`). ^{13}C-NMR (ppm): 169.50 (C-2`, C-2`), 162.23 (C-2, C-8), 153.02 (C-4, C-6), 151.79 (C-12, C-14), 151.24 (C-1", C-1"),130.12 (C-3", C-5"), (129.61 (C-5), 127.51 (C-11, C-13), 120.72 (C-2", C-6"), 115.83 (C-10), 113.42 (C-3, C-7), 93.01 (C-4", C-4"), and 33.16 (C-1`, C-1 `).

Assessment of biomedical activities

Evaluation of cytotoxic biomedical activity

The *in vitro* biomedical activity of the synthesized novel multifunctional benzodipyrone-based derivatives was determined using the minimum inhibitory concentration (IC_{50}) matrix. Our derivatives, along with the control, 5-fluorouracil (5-FU), were dissolved in DMSO to produce six

concentration levels (400, 200, 100, 50, 25, and 12.5 µg/mL). The tumorous cell lines were divided onto a 96-well plate to achieve 10,000 cells per well, each well was treated separately with varied concentrations of the synthesized novel multifunctional benzodipyrone-based derivatives after 24 hrs. The vitality of the cells was measured after 72 hrs of incubation by removing the medium and incubating the cells for 90 min at 37 °C with 28 µL MTT solution (3.27mM). The absorption spectra of the treated well (As) and control well (Ac) were measured using a microplate reader set at 492 nm. This procedure was performed in triplicate on every synthesized chemical evaluated [31,32]. To calculate the percentage of growth inhibition, the following mathematical equation was used:

$$Growth\ inhibition\ \% = \frac{Ac - As}{Ac} X\ 100$$

Evaluation of antimicrobial biomedical activity

In this part of the study, the activities of the synthesized derivatives as antibacterial and antifungal candidates were evaluated using the well-known broth-dilution technique.

Evaluation of activity towards aerobic gram-negative bacteria

The mother solution was made by combining 7.5 mg of the test compound with 5 mL of methyl sulfoxide. A series of 13 two-fold dilutions with a range of labeled concentrations between 1024 and 0.25 g/mL were then established using autoclaved distilled water as a thinning liquid. As a pre-incubation solution, 3 mL of MHB, 0.2 mL of inoculant diluted to the turbidity of 0.5 McFarland with autoclaved distilled water, and 1 mL of a preset concentration were put in a marked test tube. After a 24-hr incubation period at 37 °C, the growth of bacteria was examined with the naked eye. The previous scientific approach was repeated with diluted quantities based on the values of 4, 1, 0.5, or 0.05, depending on which concentration showed minor bacterial proliferation. The calculated microbiological variable known as minimum inhibitory concentration (MIC) was measured in micrograms per milliliter [33].

Evaluation of activity towards anaerobic bacteria

Despite minor differences, the method utilized to assess the biomedical activity of the multifunctional benzodipyrone-based derivatives against anaerobic pathogenic bacteria was identical to that used to assess the activity against aerobic pathogenic bacteria. The only difference in the microbiological variables is the notation MABC, which stands for minimum anaerobic bactericidal concentration. The differences were in the growth medium, which was Brucella-agar mixed with sheep blood (5%) and the reference drug, Metronidazole (MNZ). In addition, culture was incubated for 48 hrs at 37 °C in a container containing an anaerobic milieu (10 % CO_2, 10 % H_2, and 80 % N_2), an anaerobe marker, and a metal catalyst (palladium) [34].

Evaluation of activity towards pathogenic fungi

The fungicidal activity of the multifunctional benzodipyrone-based derivatives was evaluated using a slightly different method than that utilized to examine their activity against aerobic bacteria. Except for the term MFC, which stands for minimum fungicidal concentration, all of the microbiological variables are the same. The Sabouraud-dextrose broth was used as the growth medium, the reference agent was Nystatin (NYS), and the incubation period was 48 hrs at 30 °C [35].

Evaluation of hypoglycemic biomedical activity

In vitro testing was performed on the suppressive capacity of the synthesized multifunctional benzodipyrone-based derivatives against two phenotypes of the enzyme, porcine α-amylase and yeast α-glucosidase. Both of which are significant in controlling blood glucose levels. The IC_{50} matrix, which is the dose of the synthesized agent necessary to inhibit enzyme activity by 50% under experimental circumstances, was used to describe this capacity. Prior to executing these two tests, different dilutions of the chemical under research were created from the original (2 mg/mL) one. These of 1000, 800, 400, 200, 100, 50, and 25 µM were obtained using MeOH as a solvent [36].

Estimation of the Receding Influence on Yeast α-Glucosidase (YG)

20 µL of the synthesized multifunctional benzodipyrone-based derivative was mixed with the same volume of the reference solution, both containing 0.1 unit/mL of the YG enzyme. The para-nitrophenyl glucopyranoside was solubilized in a K_3PO_4 (pH 6.8) solution to reach the target concentration level of 375 µM. After that, 40 µL of this solution was combined with the compound-enzyme mixture and maintained at 37 °C for 30 min. A K_3PO_4 solution containing 80 µL of carbonic acid disodium salt (0.2 M) was added to the mixture to complete the reaction. The ability of the chemical to decrease enzyme activity was assessed using a colorimetric technique at 405 nm, and the receding percent was calculated using the following equation:

$$\boldsymbol{YG\ receding}\ \% = \boldsymbol{Abs}_{\text{control}} - \boldsymbol{Abs}_{\text{sample}} \div \boldsymbol{Abs}_{\text{control}} \times \mathbf{100}$$

The standard used was acarbose (AC). The reference solution was made in the same way as the examined solution, except using DMSO instead of the synthesized compound [37].

Estimation of the abating influence on porcine α-amylase (PA)

20 µL of the synthesized multifunctional benzodipyrone-based derivative and the same volume of the reference solution, both of which contained 2 units/mL of the PA enzyme, were mixed together. To produce 2 mL of 0.5 mM concentration, the starch substrate was dispersed in K_3PO_4 buffer (pH 6.8). After that, the assessed combination was held at 25 °C for 10 min. After that, 2 mL of a solution of 0.4 M aqueous sodium hydrate, 12% anhydrous L-potassium sodium tartrate, and 1% 2-dinitrocarboxylphenol were added to finish the reaction. The obtained sample was warmed in a water bath for 15 min before being thinned with H_2O to achieve the necessary amount of 10 mL. After that, an ice bath was used to bring the temperature of the mixture to 25 °C. The effectiveness of the chemical combination to reduce enzymatic activity was measured using a colorimetric technique at 540 nm. The percentage of abating was calculated using the following formula:

$$\boldsymbol{PA\ abating}\ \% = \boldsymbol{Abs}_{\text{control}} - \boldsymbol{Abs}_{\text{sample}} \div \boldsymbol{Abs}_{\text{control}} \times \mathbf{100}$$

The standard used was AC. The reference solution was made in the same way as the examined solution, except using DMSO instead of the synthesized compound [37].

Results and discussion

Chemical synthesis

In the first attempt at the synthesis of **CSY1**, the authors employed 2 moles of acetone dicarboxylic acid with 1 mole of 5-chlororesorcinol in the presence of sulfuric acid as a catalyst. In the second one, several moles of acetone dicarboxylic acid ranging between 4 and 8 were separately reacted with 1 mole of 5-chlororesorcinol. The

resultant product in both attempts was a benzopyrone derivative rather than a benzodipyrone. This can be attributed to the weak nucleophilicity power of the hydroxyl group in the formed benzopyrone because of the elongated conjugation. Also, instead of sulfuric acid, various catalysts were employed, including organic, inorganic, homogenous, and heterogeneous. However, the same result appeared. Finally, the novel multifunctional benzodipyrone precursor (**CSY1**) was created utilizing a specific catalyst, which is [Msim]HSO_4.

Biomedical activities

Preliminary cytotoxic biomedical activity

The synthesized novel multifunctional benzodipyrone-based derivatives were tested against six tumorigenic cell lines: 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, squamous cell carcinoma abstracted from Asian esophageal) [33,34]. The acquired outcomes are recorded in Table 1.

The order of IC_{50} values of the synthesized novel multifunctional benzodipyrone-based derivatives concerning each tumorigenic cell line is recorded in Table 2.

TABLE 1 The results of the evaluation of **CSY1-CSY7** as cytotoxic candidates

Compound symbol	IC_{50} (μM) ± SD (n=3)						
	MCF-7	HeLa	SKG	AMN3	SK-OV-3	KYSE-30	RWPE-1
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
CSY1	91.36±0.90	62.17±1.01	100.25±1.09	64.15±1.01	64.82±0.91	69.26±1.09	40.08±0.95
CSY2	28.85±0.93	24.49±1.02	46.38±1.16	54.31±0.98	58.04±1.10	50.37±0.94	54.86±1.01
CSY3	34.67±1.01	41.21±1.00	42.19±0.95	67.59±0.98	40.23±1.02	53.81±1.00	48.02±1.12
CSY4	13.12±1.02	13.56±1.04	31.27±0.93	28.67±0.90	25.25±1.01	41.56±1.08	112.22±1.19
CSY5	22.69±0,98	16.42±1.07	31.41±1.00	30.82±1.02	29.12±1.02	41.79±1.02	57.45±0.95
CSY6	84.24±1.00	53.44±0.94	82.84±1.01	63.26±1.03	57.55±1.07	57.45±1.06	43.91±1.02
CSY7	89.03±1.04	54.17±0.95	79.60±1.05	69.22±1.08	63.27±0.94	60.38±0.96	40.69±1.11

TABLE 2 The order of the anti-tumor activity of **CSY1-CSY7** versus the investigated tumorigenic cell lines

Order of activity	MCF-7	HeLa	SKG	AMN3	SK-OV-3	KYSE-30
1	**CSY4**	**CSY4**	**CSY4**	**CSY4**	**CSY4**	**CSY4**
2	**CSY5**	**CSY5**	**CSY5**	**CSY5**	**CSY5**	**CSY5**
3	**CSY2**	**CSY2**	**CSY3**	**CSY2**	**CSY3**	**CSY9**
4	**CSY3**	**CSY3**	**CSY2**	**CSY6**	**CSY6**	**CSY3**
5	**CSY6**	**CSY6**	**CSY7**	**CSY1**	**CSY2**	**CSY6**
6	**CSY7**	**CSY7**	**CSY6**	**CSY3**	**CSY7**	**CSY7**
7	**CSY1**	**CSY1**	**CSY1**	**CSY7**	**CSY1**	**CSY1**
8	**CSY1**	**CSY1**	**CSY7**	**CSY1**	**CSY7**	**CSY1**

A number of conclusions on the cytotoxic activity of the synthesized multifunctional benzodipyrone-based derivatives against tumorigenic cell lines were drawn from these two tables. To begin with, the fluorinated and chlorinated chemicals (**CSY4** and **CSY5**, respectively) showed the greatest potency against all cell lines examined. This is due to the fluoride and chloride moieties' significant electron-withdrawing ability, which makes the resulting molecule more active. Second, the cytotoxic activity of the multifunctional benzodipyrone-based derivatives was lower than that of 5-FU, the reference [35].

The toxicity of our compounds was tested using RWPE-1 as a model (human normal prostate epithelial cells). The novel multifunctional benzodipyrone-based derivatives were shown to be safer than 5-FU against the test normal cell line [35].

Antimicrobial biomedical activity

The activity of the synthesized benzodipyrone-based derivatives as antibacterial and antifungal candidates was evaluated in this study using the well-known broth-dilution technique.

Aerobic gram-negative bacteria

The pathogenic aerobic gram-negative bacterial strains used in this study were *Pseudomonas aeruginosa* (27853-ATCC, P-aeruginosa), *Klebsiella pneumonia* (700603-ATCC, K-pneumonia), *Haemophilus influenza* (49247-ATCC, H-influenza), *Escherichia coli* (25922-ATCC, E-coli), *Salmonella typhi* (6539-ATCC, S-typhi), and *Shigella dysenteriae* (13313-ATCC, S-dysenteriae). The safety profile of the synthesized derivatives on normal flora bacteria was evaluated on the non-pathological *Escherichia coli* strain (BAA-1427, E-coli).

The initial finding as observed in TABLE 3 is that the synthesized derivatives exhibit a less bacterial growth inhibitory effect on the pathogenic bacterial strains than the reference, CPF. The second is that these derivatives have bacterial growth inhibitory effects in the following order: **CSY5, CSY2, CSY3, CSY4, CSY6, CSY7,** and **CSY1**. Among our derivatives, **CSY5** exhibits the most activity. This might be because the chloride moiety is one of the most potent electron-withdrawing replacements, resulting in the production of a highly active molecule [38].

Another two findings came from determining the safety profile of the synthesized derivatives by examining their effects on the tested normal flora bacterial strain. The first is that, as compared to CPF, they are less toxic to the normal floral *E. coli* strain than CPF. The second is that the order of toxicity concerning these derivatives is in the following sequence, starting with the least toxic: C**SY1, CSY5, CSY6, CSY4, CSY7, CSY3,** and **CSY2**.

TABLE 3 The influence of bacterial growth inhibitory of **CSY1-CSY7** compounds versus gram negative aerobic bacteria

Aerobic gram-negative bacteria	Micro-biological variable	Symbols of the standard and tested synthetic compounds							
		CPF	CSY1	CSY2	CSY3	CSY4	CSY5	CSY6	CSY7
P-aeruginosa ATCC 27853	MIC	0.75	10.00	3.00	4.00	5.00	1.75	7.00	6.00
K-pneumonia ATCC 700603	MIC	0.40	8.00	3.00	3.50	4.00	1.80	5.50	7.00
H-influenzae ATCC 49247	MIC	0.60	8.00	2.50	4.50	4.50	1.55	6.00	6.50
E-coli ATCC 25922	MIC	0.85	15.00	2.00	5.50	5.00	1.30	6.50	7.00
S-typhi ATCC 6539	MIC	0.80	12.00	3.50	4.00	7.00	1.70	8.50	9.00
S-dysenteriae ATCC 13313	MIC	0.55	13.00	4.00	5.00	4.50	1.80	5.50	8.50
E-coli BAA-1427	MIC	0.90	18.00	9.00	12.00	11.00	14.00	15.00	15.00

Anaerobic pathogenic bacteria

In this work, four anaerobic pathogenic bacterial strains were utilized, namely *Bacteroides fragilis* (25285-ATCC, B-fragilis), *Clostridium perfringens* (13124-ATCC, C-perfringens), *Fusobacterium necrophorum* (25286-ATCC, F-necrophorum), and *Prevotella melaninogenica* (25845-ATCC, P-melaninogenica).

The results recorded in Table 4 reveal that the synthesized derivatives have much less activity compared with MNZ, the standard drug. The order of their anaerobic bacterial growth inhibitory effects against the test pathogens is: **CSY2, CSY3, CSY5, CSY4, CSY7, CSY6,** and **CSY1**.

TABLE 4 The influence of bacterial growth inhibitory of **CSY1-CSY7** compounds versus anaerobic bacteria

Anaerobic bacteria	Micro-biological variable	Symbols of the standard and tested synthetic compounds							
		MNZ	CSY1	CSY2	CSY3	CSY4	CSY5	CSY6	CSY7
B-fragilis ATCC 25285	MIC	3.00	42.00	9.00	13.00	28.00	20.00	40.00	28.00
C-perfringens ATCC 13124	MIC	0.75	44.00	7.50	10.00	26.00	20.00	36.00	28.00
F-necrophorum ATCC 25286	MIC	1.75	24.00	7.00	14.00	32.00	22.00	32.00	44.00
P-melaninogenica ATCC 25845	MIC	0.75	36.00	8.50	10.00	32.00	26.00	28.00	42.00

Pathogenic fungi

The fungal growth inhibitory effect of the synthesized multifunctional benzodipyrone-based derivatives was tested against two pathological fungal strains, *Candida albicans* (10231-ATCC, C-albicans), and *Aspergillus niger* (16888-ATCC, A-niger).

Several critical remarks are made, as observed in Table 5. The most notable is that, as compared to NYS, the synthesized derivatives, including **CSY1, CSY4,** and **CSY5**, have a very strong fungal growth inhibitory effect. On the other hand, **CSY6** and **CSY7** exhibit virtually no efficacy against the tested fungal strains. This might be due to the decreased electron withdrawing capabilities of the bromide and iodide moieties in these two compounds compared to other substituents, making the molecule less active [39]. The following is the order in which our derivatives have fungal growth inhibitory effects: **CSY1, CSY4, CSY5, CSY3, CSY2, CSY7,** and **CSY6.**

TABLE 5 The influence of bacterial growth inhibitory of **CSY1-CSY7** compounds versus two investigated fungi

Pathogenic fungi	Micro-biological variable	Symbols of the standard and tested synthetic compounds							
		NYS	CSY1	CSY2	CSY3	CSY4	CSY5	CSY6	CSY7
C-albicans ATCC 10231	MFC	4.00	1.20	4.50	3.50	1.50	1.55	18.00	11.00
A-niger ATCC 16888	MFC	8.00	1.35	10.00	13.00	1.70	1.80	26.00	22.00

Hypoglycemic biomedical activity

Some key observations were made based on Table 6. First, the synthesized multifunctional benzodipyrone derivatives may block both the YG and PA enzymes in the same way. Second, our compounds showed a less hypoglycemic effect than AC, the standard. Third, **CSY2** and **CSY3** have the strongest suppressive properties of these novel chemicals. That could be attributed to the OCH_3 and CH_3 moieties, respectively [40]. The order of hypoglycemic effects of these novel derivatives is **CSY2, CSY3, CSY1, CSY4, CSY5, CSY6,** and **CSY7**.

TABLE 6 The results of investigating the hypoglycemic potential of the synthesized multifunctional benzodipyrone-based derivatives **CSY1-CSY7** and the reference

Compound symbol	Assay and findings			
	YG receding influence RC_{50}±SD		PA abating influence RC_{50}±SD	
AC		283.04±0.88		263.26±0.92
CSY1		396.89±1.05		348.63±.90
CSY2		364.43±0.96		326.67±0.88
CSY3		366.91±0.92		334.24±0.78
CSY4		403.24±0.88		352.78±0.90
CSY5		413.44±0.85		353.32±1.05
CSY6		422.12±0.92		370.43±0.96
CSY7		424.25±0.93		372.56±0.92

Conclusion

This work reports the creation of a novel chemical nucleus, symbolized here as **CSY1**, from which a series of six based derivatives (**CSY1-CSY7**) were synthesized by coupling with various phenol-derived products. The results of investigating the cytotoxic, antibacterial, and hypoglycemic biomedical activities of the synthesized derivatives revealed that **CSY4** can be an effective cytotoxic agent with a broad spectrum of action. Also, the **CSY5** showed promise as an antibacterial agent. Concerning the hypoglycemic effect, **CSY2** has the strongest suppressive properties among the synthesized compounds. Based on the research findings, our compounds can be regarded as bio-medically verified platforms for the discovery of new therapeutically active candidates.

Acknowledgements

The authors are very grateful to the University of Baghdad/College of Pharmacy for their provided facilities, which helped to improve the quality of this work.

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How to cite this article: Salah Hassan Zain Al Abdeen*, Yasser Fakri Mustafa, Shihab Hattab Mutlag. Synthesis and biomedical activities of novel multifunctional benzodipyrone-based derivatives. *Eurasian Chemical Communications*, 2022, 4(10), 938-949. **Link:** http://www.echemcom.com/article_149754.html