



Synthesis and Kinetic Study of Novel Coumarin- Based Mutual Prodrug of 5-fluorouracil and 5-ethynyluracil

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Abstract: *The oral use of 5-fluorouracil is being deserted in the last decades due to its atypical intestinal absorption, which is primarily attributed to the mutable activity of dihydropyrimidine dehydrogenase located in the gastrointestinal mucosa. In this work, a coumarin-based prodrug system was utilized to synthesize a novel mutual prodrug of 5-fluorouracil and 5-ethynyluracil. This prodrug was designed to afford a concurrent release of these two active drugs resulting in the improvement of the therapeutic efficacy of both. The synthetic pathway involved 7 linear steps starting from coumarin. The chemical structures of the intermediates and prodrug were established by analyzing their FTIR, ¹H-NMR, and ¹³C-NMR spectra. The in vitro chemical stability of the synthesized prodrug was tested in the HCl buffer (pH 1.2) and phosphate-buffered saline (pH 6.8), while its ability to release the active moieties was studied in human serum. The outcomes of these in vitro studies revealed that the prodrug showed a significant stability in the HCl buffer and phosphate-buffered saline with half-lives of 33.19 h and 18.13 hr respectively, obeying pseudo-first-order kinetics. Also, the prodrug was able to release the two active components in human serum with a half-life of 4.62 h obeying zero-order kinetics. It is concluded that the synthesized prodrug represents a promising oral prodrug of 5-fluorouracil and 5-ethynyluracil to serve better in therapeutics.*

Keywords: *Coumarin-based prodrug, 5-fluorouracil, 5-ethynyluracil, kinetics*

1. Introduction

Since its ingenious design and synthesis in 1957, 5-fluorouracil (5-FU) has been authenticated for the management of many tumor phenotypes [1]. However, the functionality of this cytotoxic drug in the chemotherapy is being doubted because of its severe adverse effects, poor targetability, and mounting tumor resistance [2, 3]. To handle these limitations, many approaches have been applied such as the modulation of the administration schedules [4], alteration of the metabolic pathways [5], development of new fluorinated pyrimidines [6], and synthesis of prodrugs [7].

5-FU itself is a prodrug that must be activated inside the cell via several metabolic steps and pathways [8]. The hepatic dihydropyrimidine dehydrogenase (HDPD) represents the cornerstone in the extracellular deactivation of the majority of 5-FU dose [7]. As an oral antitumor agent, the use of 5-FU was deserted in the last decades due to its atypical intestinal absorption that results in irregular plasma levels with marked inter- and intra-individual variations [9]. This is primarily attributed to the mutable activity of HDPD located in the gastrointestinal mucosa [10].

To avoid the negative impact of HDPH on the 5-FU bioavailability, its inhibition has been nominated as a potential target for improving the 5-FU chemotherapy [11]. Through many investigated compounds, uracil analogues substituted at position 5 were shown as the potent HDPD inactivators [12], among them, 5-ethynyluracil (5-EU) is the best one [13].

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In the past four decades, great attention has been directed to the design and synthesis of coumarin-based prodrugs in an attempt to enhance the therapeutic efficacy of many drugs [14]. This type of prodrug is utilized for alcohol- and amine-containing compounds to improve their lipophilicity [15] and minimize their metabolic inactivation [16]. Coumarin-based prodrug system as shown in Scheme 1 offers several advantages such as the facile release of the active agent (s) as the prodrug attacks by esterase enzyme [17]. Also, the release rate can be modulated by the presence of different substitutions on the coumarin backbone [18], and the final product from the release process, coumarin, is safe [19].

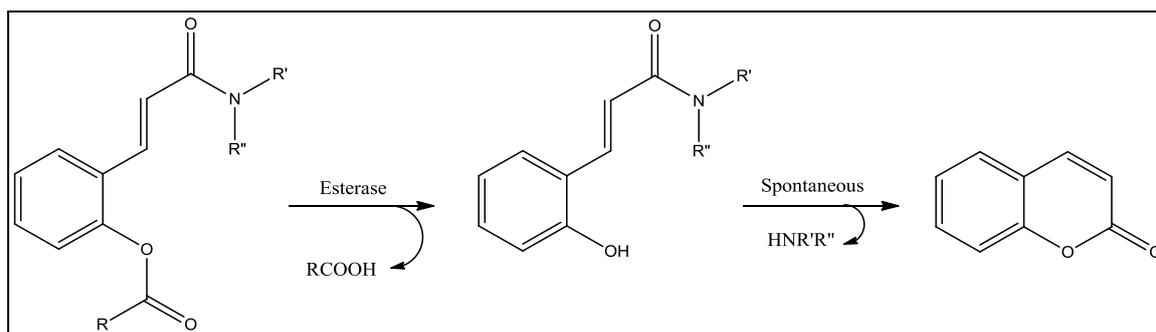


Figure 1. The activation steps of coumarin-based prodrug system

This study aims to employ the coumarin-based prodrug system for synthesizing a mutual oral prodrug. Upon activation, this prodrug may release two active moieties, which are 5-FU and 5-EU. Following synthesis, the prodrug was evaluated for its chemical stability and release profile.

2. Materials and methods

The chemicals and solvents utilized for synthesizing the prodrug and studying its in vitro release were purchased from international sources. The instruments used to confirm the chemical structure of this prodrug were Shimadzu LCMS-2020 with an electrospray ionization source to scan the mass spectrum, Bruker Avance DRX-400 MHz to identify the NMR spectra, and Bruker-Alpha ATR-FTIR to screen the IR spectrum. The equipment employed to specify the UV spectra of the synthesized prodrug and its reaction intermediates was Varian UV/Visible spectroscopy. Also, the same instrument was used to follow the in vitro stability and release studies. Thin-layer chromatography (TLC) was used to check the progress of the reactions and purity of the products. The precoated silica gel plates (60G F254, Merck) and the eluent system of chloroform: acetone (4:1) were used as stationary and mobile phases respectively.

2.1. Chemical synthesis

The synthetic strategy followed for the synthesis of the coumarin-based prodrug is illustrated in Scheme 2.

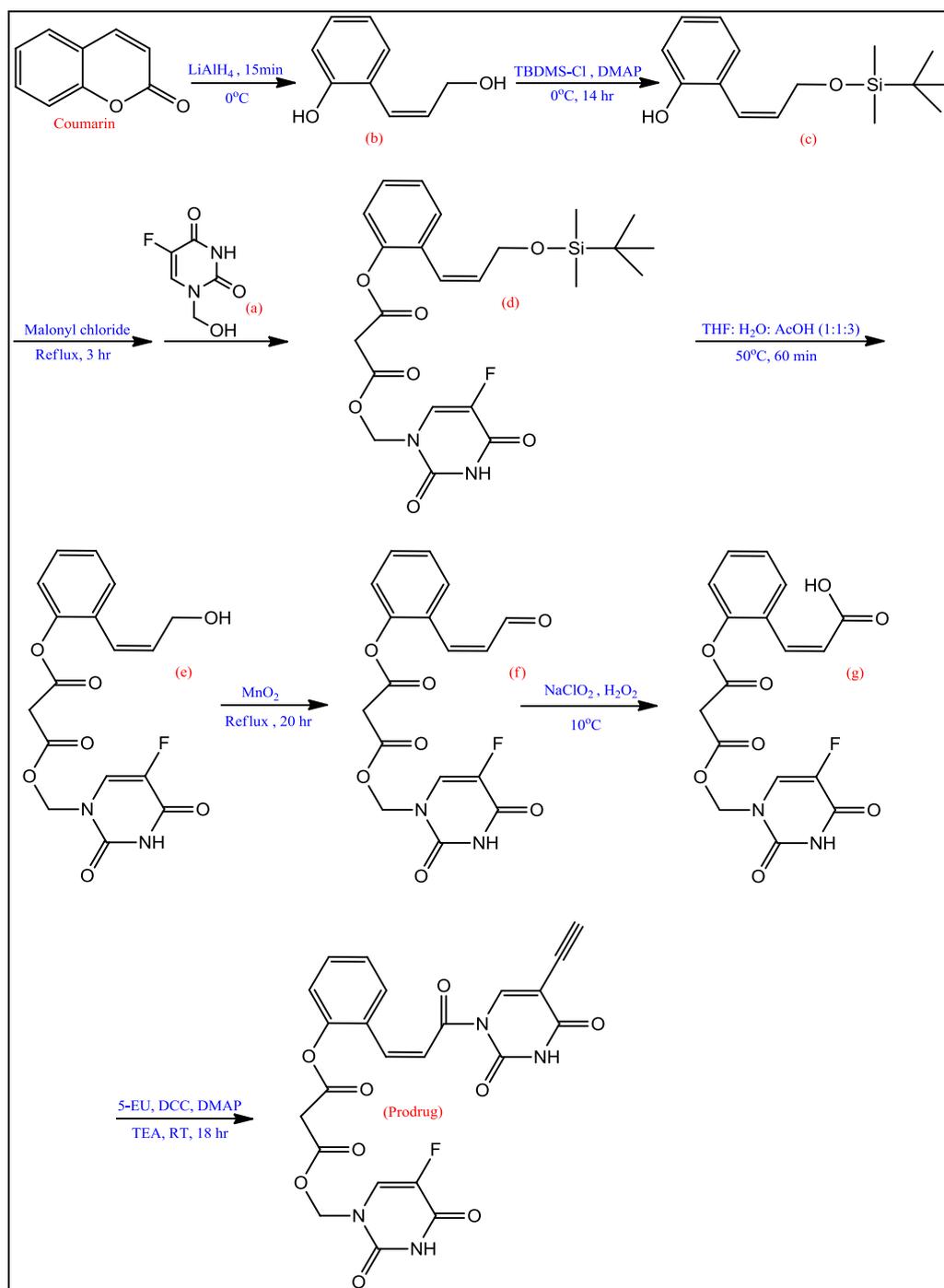


Figure 2. Synthetic pathway of the prodrug

2.2.1. Synthesis of 1-hydroxymethyl-5-fluorouracil (a)

The mixture of formaldehyde (5 mL, 37%) and 5-FU (1.04 g, 8 mmol) in 25 mL H₂O was magnetically agitated at 60°C until a solution formed (~ 45 min). The resultant solution was vaporized to dryness under reduced pressure, and the titled product was recrystallized from ethanol [20].

(a): White powder; % yield=70; mp=194-196°C; R_f =0.24; C₅H₅FN₂O₃; λ_{max} (MeOH)=290 nm; FTIR (ν, cm⁻¹, stretching): 3420 (O-H), 3012 (=C-H), 2891 (-C-H), 1673 (C=O), 1052 (C-F); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.24 (1H, s, NH), 7.94 (1H, s, =CH), 5.11 (2H, s, CH₂), 3.56 (1H, s, OH) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 158.1 (FC-C=O), 148.3 (HN-C=O), 140.6 (C-F), 126.3 (C=CF), 73.4 (CH₂-OH) ppm.



2.2.2. Synthesis of (Z)-2-(3-hydroxypropenyl)phenol (b)

In an ice bath, the solution of coumarin (25 mmol, 3.65 g) in 50 mL dry ether was handled with the solution of lithium aluminum hydride (50 mmol, 1.9 g, LiAlH₄) in 50 mL dry ether. The resultant mixture was stirred for 15 min and subsequently treated with HCl (27 mL, 5%) affording solution of pH 5. The crude was extracted by ether (3×50 mL), and the organic layer was dehydrated over Na₂SO₄, filtered, and vaporized. The titled product was recrystallized from EtOH [21].

(b): White powder; % yield=39; mp=148-150°C; R_f=0.34; C₉H₁₀O₂; λ_{max} (MeOH)=286 nm; FTIR (ν, cm⁻¹, stretching): 3235, 3202 (O-H), 3046 (=C-H), 2918 (-C-H), 1642, 1588 (C=C); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 7.62, 7.24, 7.01, 6.83 (4H, m, aromatic), 6.94 (1H, d, ph-CH=), 6.08 (1H, q, =CH-CH₂OH), 5.52 (1H, s, ph-OH), 4.26 (2H, d, HO-CH₂), 3.62 (1H, s, CH₂-OH) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 159.3 (Ar C-OH), 130.1, 127.4, 122.3, 119.6 (Ar C), 126.5 (Ar C-CH=), 125.0 (=CH-CH₂), 115.6 (Ar C-CH=), 53.2 (CH₂-OH) ppm.

2.2.3. Synthesis of (Z)-2-(3-(tert-butyldimethylsilyloxy)propenyl)phenol (c)

In an ice bath, the solution of (b) (22.8 mmol, 3.43 g) in 40 mL dry THF was handled with the solution of *tert*-butyldimethylsilyl chloride (25 mmol, 3.79 g, TBDMS-Cl) in 35 mL dry THF. To the resultant mixture, a solution of 4-dimethylaminopyridin (34 mmol, 4.18 g, DMAP) in 40 mL dry THF was dropwise added. The reaction mixture was stirred for 14 h, filtered, and vaporized to dryness. The crude was dissolved in EtOAc (50 mL) and washed serially with HCl (50 mL, 1N), NaHCO₃ (25 mL, 5%), and H₂O (25 mL). The organic layer was dehydrated over Na₂SO₄, filtered, and vaporized. The titled product was recrystallized from CHCl₃ [21].

(c): White powder; % yield=73; mp=122-124°C; R_f=0.56; C₁₅H₂₄O₂Si; λ_{max} (MeOH)=281 nm; FTIR (ν, cm⁻¹, stretching): 3412 (O-H), 3052 (=C-H), 2911, 2865 (-C-H), 1644, 1587 (C=C), 942 (Si-O); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 7.60, 7.25, 7.01, 6.82 (4H, m, aromatic), 6.92 (1H, d, ph-CH=), 6.04 (1H, q, =CH-CH₂), 5.56 (1H, s, ph-OH), 4.48 (2H, d, Si-O-CH₂), 1.41 (9H, s, CH₃-C-Si), 0.48 (6H, s, Si-CH₃) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 159.0 (Ar C-OH), 130.6, 128.1, 124.2, 119.8 (Ar C), 126.2 (Ar C-CH=), 125.2 (=CH-CH₂), 115.1 (Ar C-CH=), 52.4 (CH₂-O-Si), 33.2 (O-Si-C-), 26.4 (Si-C-CH₃), 12.3 (Si-CH₃) ppm.

2.2.4. Synthesis of (Z)-2-(3-((tert-butyldimethylsilyloxy)prop-1-en-1-yl)phenyl ((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl) malonate (d)

To a mixture of (c) (10 mmol, 2.65 g) and (a) (10 mmol, 1.60 g) in 50 mL dry CHCl₃, malonyl chloride (10 mmol, 1 mL) was added. The reaction mixture was refluxed for 3 h with constant stirring. The reaction advancement was followed by TLC using a mixture of EtOAc: ether as an eluent. The reaction mixture was washed with water (5×50 mL), and the organic layer was dehydrated over anhydrous Na₂SO₄, concentrated under reduced pressure, and eluted in column chromatography by a mixture of CHCl₃: EtOH (2:1) affording the titled product [21].

(d): White powder; % yield=79; mp=96-98°C; R_f=0.31; C₂₃H₂₉FN₂O₇Si; λ_{max} (MeOH)=307 nm; FTIR (ν, cm⁻¹, stretching): 3019 (=C-H), 2914, 2859 (-C-H), 1722 (C=O, ester), 1673 (C=O, amide), 1641, 1588 (C=C), 1058 (C-F), 942 (Si-O); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.18 (1H, s, NH), 8.02 (1H, s, FC=CH), 7.82, 7.72, 7.34, 6.27 (4H, m, aromatic), 6.95 (1H, d, ph-CH=), 6.08 (1H, q, =CH-CH₂), 5.96 (2H, s, N-CH₂-O), 4.56 (2H, d, Si-O-CH₂), 3.26 (2H, s, O=C-CH₂-C=O), 1.40 (9H, s, CH₃-C-Si), 0.43 (6H, s, Si-CH₃) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 168.6, 167.4 (O=C-O), 157.8 (FC-C=O), 150.2 (N-C=O), 148.1 (Ar C-O-), 142.8 (C-F), 130.1, 128.4, 124.1, 118.3 (Ar C), 129.2 (Ar C-CH=), 127.2 (FC=CH), 124.4 (=CH-CH₂), 120.2 (Ar C-CH=), 80.2 (N-CH₂-O), 52.4 (CH₂-O-Si), 42.4 (O=C-CH₂-C=O), 33.1 (O-Si-C-), 26.4 (Si-C-CH₃), 12.2 (Si-CH₃) ppm.

2.2.5. Synthesis of (Z)-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl (2-(3-hydrox-



yprop-1-en-1-yl)phenyl) malonate (e)

Derivative (d) (4 mmol, 1.97 g) was dissolved in a mixture of H₂O (10 mL), THF (10 mL), and AcOH (30 mL). After stirring for 60 min at 50°C, the mixture was vaporized under reduced pressure. The crude was dissolved in 50 mL EtOAc, and the resulted solution was washed with 5 % NaHCO₃ (50 mL, 5%) and H₂O (50 mL). The EtOAc layer was dehydrated over Na₂SO₄, filtered, and vaporized under reduced pressure. The target product was recrystallized from EtOH [22].

(e): White powder; % yield=92; mp=106-108°C; R_f =0.26; C₁₇H₁₅FN₂O₇; λ_{max} (MeOH)=302 nm; FTIR (ν, cm⁻¹, stretching): 3186 (O-H), 3025 (=C-H), 2914, 2873 (-C-H), 1725 (C=O, ester), 1670 (C=O, amide), 1644, 1582 (C=C), 1046 (C-F); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.22 (1H, s, NH), 8.00 (1H, s, FC=CH), 7.80, 7.73, 7.36, 6.28 (4H, m, aromatic), 6.94 (1H, d, ph-CH=), 6.01 (1H, q, =CH-CH₂), 5.95 (2H, s, N-CH₂-O), 4.55 (2H, d, CH₂-OH), 3.52 (1H, s, OH), 3.28 (2H, s, O=C-CH₂-C=O) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 168.2, 167.6 (O=C-O), 157.5 (FC-C=O), 150.7 (N-C=O), 148.3 (Ar C-O-), 142.9 (C-F), 130.0, 128.5, 124.8, 118.1 (Ar C), 129.4 (Ar C-CH=), 127.6 (FC=CH), 124.3 (=CH-CH₂), 120.0 (Ar C-CH=), 80.0 (N-CH₂-O), 52.7 (CH₂-OH), 42.5 (O=C-CH₂-C=O) ppm.

2.2.6. Synthesis of (Z)-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl (2-(3-oxoprop-1-en-1-yl)phenyl) malonate (f)

Derivative (e) (4 mmol, 1.51 g) and MnO₂ (20 mmol, 1.74 g) were suspended in CHCl₃ (30 mL) and refluxed for 20 h. The hot mixture was filtered, and the resulted solid washed with 30 mL warm CHCl₃. The collected organic layer was vaporized under reduced pressure, and the crude was dissolved in 30 mL acetone and filtered. The solution was vaporized affording the target product [21].

(f): White powder; % yield=66; mp=119-121°C; R_f =0.31; C₁₇H₁₃FN₂O₇; λ_{max} (MeOH)=318 nm; FTIR (ν, cm⁻¹, stretching): 3017 (=C-H), 2907, 2880 (-C-H), 1723 (C=O, ester), 1704 (C=O, CHO), 1667 (C=O, amide), 1644, 1581 (C=C), 1050 (C-F); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.16 (1H, s, NH), 9.85 (1H, d, CHO), 8.02 (1H, s, FC=CH), 7.83, 7.74, 7.35, 6.29 (4H, m, aromatic), 6.89 (1H, d, ph-CH=), 6.00 (1H, t, =CH-CHO), 5.98 (2H, s, N-CH₂-O), 3.32 (2H, s, O=C-CH₂-C=O) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 188.2 (CHO), 167.8, 165.9 (O=C-O), 158.2 (FC-C=O), 150.7 (N-C=O), 146.4 (Ar C-O-), 141.0 (C-F), 130.2, 128.5, 124.2, 118.8 (Ar C), 129.6 (Ar C-CH=), 126.9 (FC=CH), 124.2 (=CH-CHO), 120.8 (Ar C-CH=), 80.5 (N-CH₂-O), 42.2 (O=C-CH₂-C=O) ppm.

2.2.7. Synthesis of (Z)-3-(2-((3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methoxy)-3-oxo-propanoyl)oxy)phenyl)acrylic acid (g)

An aqueous solution prepared by dissolving (7.33 mmol, 660 mg) of NaClO₂ in 10 mL H₂O was added slowly to a stirred mixture of derivative (f) (4 mmol, 1.50 g), NaH₂PO₄ (0.85 mmol, 102 mg), and H₂O₂ (30%, 4.17 mmol, 0.5 mL) in 25 mL ACN. Throughout the addition process, the temperature of the reaction was preserved below 10°C using an ice-water bath, and the oxygen bubbles were observed from the reaction mixture. As the formation of the bubbles stopped, Na₂SO₃ (0.05 g) was added to devastate the unreacted H₂O₂ and HOCl. By using 1N HCl, the reaction mixture was acidified to pH 2 and then extracted with 50 mL EtOAc. The organic layer was washed with 25 mL brine, dehydrated over Na₂SO₄, filtered, and vaporized. The crude was dissolved in ACN, treated with aqueous NaHCO₃ solution to pH 6.5, and filtered. The target product was separated as the filtrate acidified to pH 3 by 1N HCl [21].

(g): White powder; % yield=82; mp=134-136°C; R_f =0.27; C₁₇H₁₃FN₂O₈; λ_{max} (MeOH)=323 nm; FTIR (ν, cm⁻¹, stretching): 3057 (=C-H), 3002 (OH), 1740 (C=O, COOH), 1722 (C=O, ester), 1666 (C=O, amide), 1644, 1580 (C=C), 1046 (C-F); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.64 (1H, s, COOH), 10.18 (1H, s, NH), 8.03 (1H, s, FC=CH), 7.83, 7.74, 7.35, 6.30 (4H, m, aromatic), 6.88 (1H, d, ph-CH=), 6.24 (1H, d, =CH-COOH), 5.92 (2H, s, N-CH₂-O), 3.36 (2H, s, O=C-CH₂-C=O) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 171.4 (COOH), 168.2, 166.4 (O=C-O), 158.6 (FC-C=O), 150.6 (N-C=O), 146.8 (Ar C-O-), 141.1 (C-F), 130.5, 128.5, 124.5, 118.8 (Ar C), 129.9 (Ar C-CH=), 126.4 (FC=CH), 120.7 (Ar C-CH=), 116.2 (=CH-COOH), 80.0 (N-CH₂-O), 42.5 (O=C-CH₂-C=O) ppm.



2.2.8. Synthesis of (Z)-2-(3-(5-ethynyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-oxoprop-1-en-1-yl)phenyl ((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl) malonate (Prodrug)

To a solution of (g) (2 mmol, 0.78 g) in 50 mL freshly distilled DMSO placed in an ice-water bath, 5-EU (2 mmol, 0.27 g), DCC (2.4 mmol, 0.5 g), DMAP (0.17 mmol, 20 mg), and TEA (2 mmol, 0.3 mL) were added serially. The mixture was stirred at room temperature for 18 h. Then, the reaction mixture was treated with MeOH (5 mL), and AcOH (0.5 mL), stirred for 60 min, and neutralized with aqueous NaHCO₃ solution. The precipitate was filtered, the filtrate was vaporized, and the crude was washed with H₂O. The prodrug was crystallized from a mixture of EtOH: CHCl₃ (2:1.5) [21].

(Prodrug): White powder; % yield=78; mp=156-158°C; R_f=0.23; C₂₃H₁₅FN₄O₉; λ_{max} (MeOH)=320 nm; FTIR (ν, cm⁻¹, stretching): 3062 (=C-H), 2925, 2891 (-C-H), 2190 (C≡C), 1725 (C=O, ester), 1668 (C=O, amide), 1648, 1580 (C=C), 1043 (C-F); ¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.18 (2H, s, NH), 8.19 (1H, s, =CH-N), 8.06 (1H, s, FC=CH), 7.84, 7.71, 7.37, 6.27 (4H, m, aromatic), 6.92 (1H, d, ph-CH=), 6.22 (1H, d, =CH-CO), 5.94 (2H, s, N-CH₂-O), 3.36 (2H, s, O=C-CH₂-C=O), 3.11 (1H, s, ≡CH) ppm; ¹³C-NMR (DMSO-d₆, 100 MHz): δ= 168.5, 166.4 (O=C-O), 162.6 (=C-C=O), 160.2 (O=C-N-C=O), 158.6 (FC-C=O), 152.4, 150.7 (N-C=O), 147.2 (Ar C-O-), 142.6 (C-F), 130.2, 128.5, 124.6, 118.5 (Ar C), 129.1 (Ar C-CH=), 126.0 (FC=CH), 124.4 (N-CH=C-), 122.9 (=CH-C=O), 121.1 (Ar C-CH=), 105.4 (=C-C≡CH), 83.5 (=C-C≡CH), 80.1(=C-C≡CH), 78.9 (N-CH₂-O), 42.8 (O=C-CH₂-C=O) ppm.

2.3. In vitro kinetic studies

2.3.1. Chemical stability

The chemical stability of the synthesized prodrug was investigated in buffers with two pH values, which are HCl (pH 1.2) buffer and phosphate-buffered saline (pH 6.8) [23]. This study was monitored via UV/Visible spectroscopy for dropping in prodrug concentration versus time utilizing the following mathematical formula of Beer's law [24]: Absorbance = ε × L × C.

C represents the prodrug concentration, L represents the path length (2 cm) of the cell holder, and ε represents the absorbance coefficient.

Briefly, a preheated solution of prodrug (5 μmol) in 2 mL DMSO was mixed with 48 mL preheated buffer solution. The time was begun to record, and the resulted solution was preserved at a 37°C utilizing a warm water bath. Subsequently, the solution was split into a group of 10 test tubes; each one contains 5 mL. For the time interval of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, or 4 h, an individual test tube was elected and its content was mixed with 2 mL CH₂Cl₂. The aqueous aliquot (2 mL) was estimated spectrophotometrically at defined λ_{max} to detect the residual concentration of the prodrug [25].

2.3.2. Enzymatic hydrolysis

A similar procedure to that followed for investigating the chemical stability was employed to monitor the hydrolysis of the synthesized prodrug in a human serum. The exceptions are the replacement of the buffer solution with the serum, and that the study was conducted by following the increase in the concentration of 5-EU versus time [26]. The concentration of 5-EU was monitored since this agent is the final product released from the mutual prodrug under the influence of esterase enzyme as shown in Scheme 3.

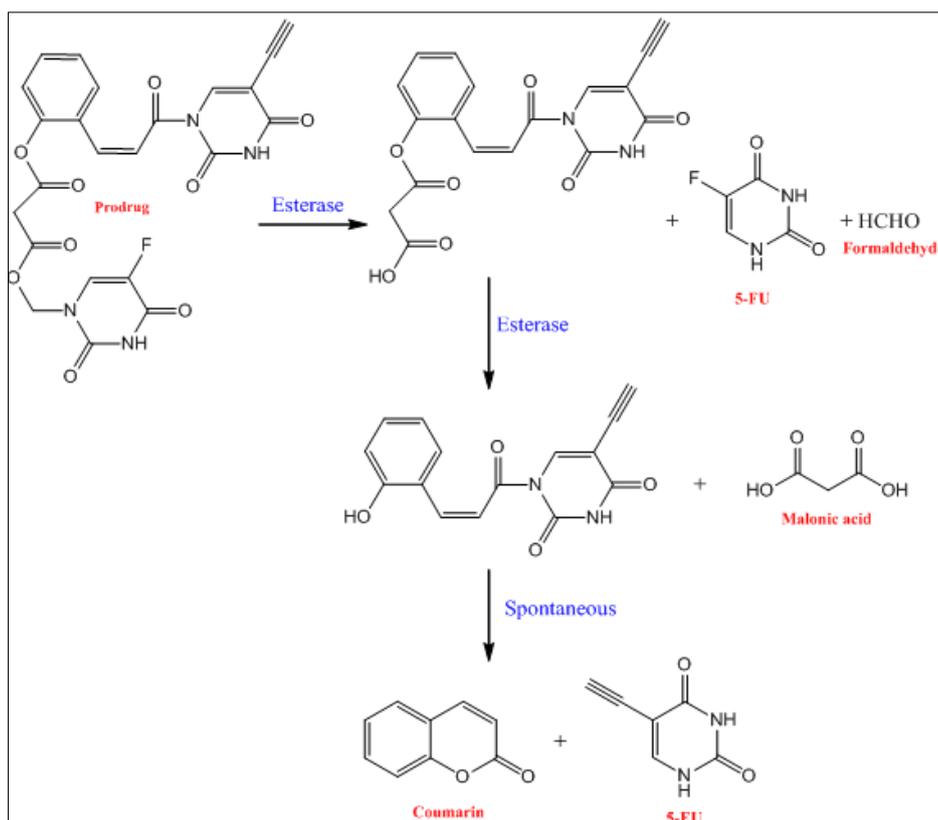


Figure 3. The hypothetical release of 5-FU and 5-EU from the prodrug in human serum

3. Results and discussions

3.1. Rationalization of the prodrug design

The synthesized mutual prodrug was designed in an attempt to optimize the clinical usefulness of 5-FU as an oral drug. This aim was attained by meeting three issues. The first is the selection of the prodrug type that enhances the lipophilicity of 5-FU, minimizes its destruction via HDPD, and provides the opportunity of the mutual effect. In this concern, the values of log P for the 5-FU, 5-EU, and the target prodrug were found to be -0.90, -0.51, and 1.76 respectively. This indicates that the target prodrug has better lipophilicity than those of its precursor drugs which may improve the oral bioavailability of 5-FU. Also, the concurrent release of 5-FU and 5-EU may reduce the destruction of 5-FU by HDPD affording a mutual action.

The second issue is the investigation of the prodrug stability in media with pH values simulating those found in the gastrointestinal tract. The last one is the ability of the synthesized prodrug to release 5-FU and 5-EU concurrently with an acceptable half-life ($t_{1/2}$) in a human serum.

3.2 Synthetic pathway

The pathway followed for the synthesis of the coumarin-based prodrug involved a linear sequence of 7 steps as shown in Scheme 2, and represents a simple variation to that reported by Mustafa and Al-Omari [21]. This variation involved the utilization of malonyl linkage to connect the phenolic hydroxyl group of the carrier molecule with that of compound (a). Coumarin was reduced under highly-controlled conditions into an open ring diol by LiAlH_4 . The temperature was kept below 0°C and the reaction time under 15 min to avoid the reduction of the exocyclic double bond, and the catalyst was in high purity to minimize the side reactions. The resulted allylic hydroxyl group was selectively protected as silyl ether utilizing TBDMS-Cl in the second step. In the following step, the phenolic hydroxyl group has participated with the previously prepared compound (a) in the formation of diester linkage using malonyl chloride as an anchor. In the fourth step, the allylic hydroxyl group was deprotected by an acid and



subsequently oxidized into allylic aldehyde by a selective oxidizing agent, MnO_2 , in the following step. NaClO_2 and H_2O_2 were involved in the oxidation of the allylic aldehyde to allylic carboxylic acid, which was coupled with 5-EU via DCC affording the target prodrug in the last synthetic steps.

3.3 In vitro kinetic studies

3.3.1 Chemical stability

Under experimental conditions, the prodrug exhibited a considerable chemical stability in the HCl buffer and phosphate-buffered saline obeying pseudo-first-order kinetics with half-lives of 33.19 h and 18.13 h, respectively. This stability may be contributed to the steric hindrance around the ester linkages affording great stability versus nucleophilic attack [27]. Also, this finding revealed that the prodrug may be passed intact through the media with a pH range simulating to that found in the gastrointestinal tract [28].

Although the hydrolysis of the prodrug in the utilized buffers depends on two factors including the concentrations of the prodrug and attacking agent, the kinetics was reported to be pseudo-first-order [29]. This is due to that the concentration of the attacking agent is extremely high in comparison with that of the prodrug leading to omit its influence on the kinetics of hydrolysis [30].

3.3.2. Release study

The prodrug was able to liberate 5-FU and 5-EU obeying zero-order kinetics with half-life equals to 4.62 h. This finding revealed that the prodrug could reach the target with a good circulating time and liberate the two active moieties [31]. From the kinetics phenotype, it is concluded that the prodrug can be taken orally in a low-frequency fashion [32] resulting in the improvement of the patient compliance [33].

The outcomes of the in vitro kinetic studies are listed in Tables 1-3, while the resulted kinetic parameters are displayed in Table 4. Figure 1 showed the graphical representation of the relationship between the released concentration of 5-EU and the time.

Table 1. Kinetic outcomes acquired from the stability study in HCl (pH 1.2) buffer

Absorbance	Time (hr)	x ($\text{M} \times 10^6$)	a-x ($\text{M} \times 10^6$)	ln a/a-x
0.1328	0.0	0.0000	100.0000	0.0000
0.1316	0.5	0.9411	99.0589	0.0095
0.1303	1.0	1.8742	98.1258	0.0189
0.1288	1.5	3.0120	96.9880	0.0306
0.1282	2.0	3.4639	96.5361	0.0353
0.1267	2.5	4.6256	95.3744	0.0474
0.1258	3.0	5.2711	94.7289	0.0542
0.1240	3.5	6.6265	93.3735	0.0686
0.1231	4.0	7.2786	92.7214	0.0756

a = Prodrug concentration at zero time that equals to 100 M, and $(a-x)$ = Residual concentration of prodrug at defined time.

Table 2. Kinetic outcomes acquired from the stability study in phosphate-buffered saline (pH 6.8)

Absorbance	Time (hr)	x ($\text{M} \times 10^6$)	a-x ($\text{M} \times 10^6$)	ln a/a-x
0.1301	0.0	0.0000	100.0000	0.0000
0.1280	0.5	1.6329	98.3671	0.0165
0.1259	1.0	3.2414	96.7586	0.0330
0.1240	1.5	4.6887	95.3113	0.0480
0.1215	2.0	6.6103	93.3897	0.0684
0.1198	2.5	7.9020	92.0980	0.0823
0.1177	3.0	9.5311	90.4689	0.1002
0.1162	3.5	10.6841	89.3159	0.1130
0.1140	4.0	12.3422	87.6578	0.1317

Table 3. Kinetic outcomes acquired from the in vitro release study in human serum

Absorbance	Time (h)	x (M×10 ⁶)
0.0000	0.0	00.0000
0.0954	0.5	5.8080
0.0989	1.0	11.7207
0.1032	1.5	17.4477
0.1079	2.0	22.4918
0.1118	2.5	27.9017
0.1167	3.0	33.4394
0.1216	3.5	38.9771
0.1267	4.0	44.6184

Table 4. Kinetic parameters acquired from in vitro kinetic studies

HCl (pH 1.2) buffer	phosphate-buffered saline (pH 6.8)	Serum
$\epsilon = 284 \text{ L mol}^{-1} \text{ cm}^{-1}$	$\epsilon = 298 \text{ L mol}^{-1} \text{ cm}^{-1}$	$\epsilon = 1930.86 \text{ L mol}^{-1} \text{ cm}^{-1}$
$\lambda_{\text{max}} = 281 \text{ nm}$	$\lambda_{\text{max}} = 312 \text{ nm}$	$\lambda_{\text{max}} = 292 \text{ nm}$
$t_{1/2} = 33.19 \text{ h}$	$t_{1/2} = 18.13 \text{ h}$	$t_{1/2} = 4.62 \text{ h}$
$k_{\text{obs}} = 5.8 \times 10^6 \text{ h}^{-1}$	$k_{\text{obs}} = 10.62 \times 10^6 \text{ h}^{-1}$	$k_{\text{obs}} = 10.83 \times 10^6 \text{ M.h}^{-1}$

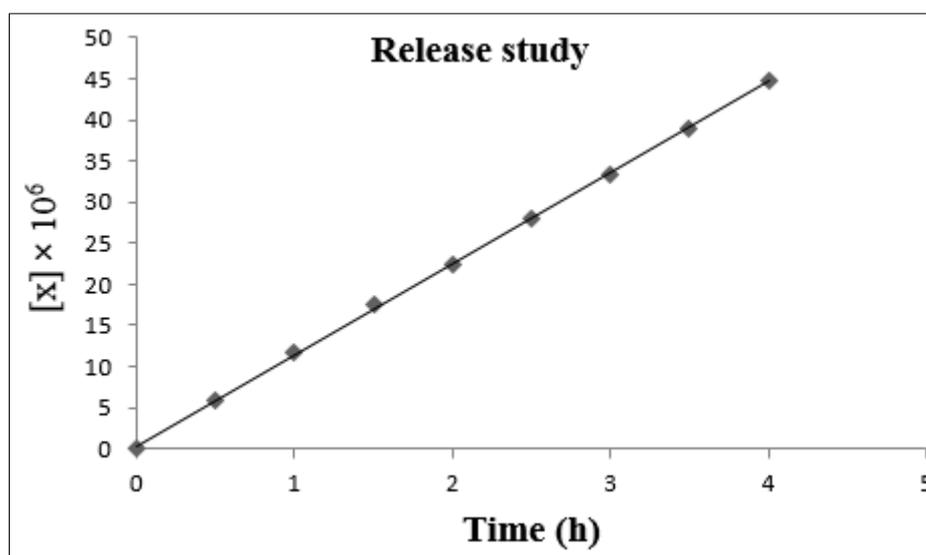


Figure 4. Graphical representation of the relationship between the released concentration of 5-EU and the time in human serum

4. Conclusions

This work concluded that 5-FU and its potent metabolic modulator, 5-EU, could be incorporated into one chemical entity utilizing a coumarin-based prodrug system. Based on the in vitro kinetic studies, the synthesized mutual prodrug was stable in the media of pH values simulating those found in the gastrointestinal tract. Also, the prodrug was able to release 5-FU and 5-EU obeying zero-order kinetics with a half-life of 4.62 h in a human serum. The value of this half-life allows the prodrug to reach intact to the target eliciting an improvement in the therapeutic efficacy. Accordingly, the synthesized prodrug may represent a potential candidate as an oral form of 5-FU with improved lipophilicity and efficacy to serve better in therapeutics.

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