Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

Prodrug of 5-Fluorouracil and 5-Ethynyluracil: Synthesis, Characterization, and Release Study

Yasser Fakri Mustafa*, Mahmood Khudhayer Oglah, Moath Kahtan Bashir, Eman Tareq Mohammed, and Raghad Riyadh Khalil

Pharmaceutical Chemistry Department, College of Pharmacy, Mosul University-41002, Nineveh, Iraq.

*Yasser Fakri Mustafa. http://orcid.org/0000-0002-0926-7428, +9647701615864.
Dr.yassermustafa@uomosul.edu.iq

Abstract

The oral administration of the standard cytotoxic agent 5-fluorouracil is extensively limited in the last three decades. This limitation has owing to the inconsistent intestinal absorption of this drug because of the mutable activity of the enzyme housed in the intestinal mucosa named dihydropyrimidine dehydrogenase. In this report, a prodrug consists of 5-fluorouracil and 5-ethynyluracil was designed to providethe mutual release of these two active drugs using a lactonization-facilitated release method.The synthesis of the target prodrug was proceeded through seven subsequent steps using coumarin as a precursor. The spectra obtained from different spectrophotometers, including FTIR, ^{1}H-NMR, and ^{13}C-NMR, were confirmed the chemical backbones of the synthetic intermediate compounds and the target prodrug. The chemical stability of the target prodrug was investigated chemically in the HCl- (pH 1.2) and phosphate-(pH 6.8) buffers. Also, the capacity of the prodrug to release its active portions was evaluated utilizinghuman serum. The results gathered from the chemical stability studies indicated that the targeted prodrug hassubstantial stability in the HCl-bufferwith $t_{1/2}$ of 33.18 hours, and in the phosphate-buffered saline with $t_{1/2}$ of 18.14 hours, adapting pseudo-first-order kinetics. Besides, the prodrug can free the two active drugswith $t_{1/2}$of 4.62 hr in human serum adapting zero-order kinetics. The authors concluded that the target prodrug may represent a potential applicant as a mutual prodrug for the oral intake ofthe 5-fluorouracil and 5-ethynyluracil.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

Keywords: Lactonization; 5-Fluorouracil;5-Ethynyluracil;Prodrug; Release study.

Introduction

From the original design and synthesizing in 1957, 5-fluorouracil (FU) has been notarized for treatingseveral phenotypesof cancer [1]. Nevertheless, the chemotherapeutic application of this tumor-fighting drugis being precautioneddue to its high-frequent side effects, inferior targetability, and poor tumor sensitivity because of the developed resistance[2], [3]. To modulate these obstacles, several advances have been investigated such as manipulating the administration programs[4], modifying the metabolic fates[5], designing and synthesizing new fluoro-pyrimidines[6], and applying various prodrug strategies[7].

Inside the viable cell, FU must be transformed via different metabolic pathways and steps into active forms since it is a prodrug [8]. The enzyme named dihydropyrimidine dehydrogenase and housed in the liver (DPDE) accounts for the basisof extracellular destruction of the plurality of the FU dose[7]. The utility of FU as an oral cytotoxic agent has been questioned because of its unpredictable GIT absorption that subsequently results in the fluctuation of the plasma levels with significant intra- and inter-individual versions [9]. These outcomes could attribute to the changeablepotential of DPDE localized in the GIT mucosa[10].

To enhance the oral bioavailability of the FU chemotherapy, interfering withthe negative role of DPDEthrough its inhibition has become a potential target[11]. Although there are many evaluated inhibitory compounds, those analogs to FU exhibited the highest potential as DPDE inactivators[12]–[17], and 5-ethynyluracil (EU)was the best[18].

Through the past 50 years, much interesthas polarizedto the design and synthesis of the lactonization-facilitated release (LFR) prodrugs [19]–[23]. This prodrug phenotype is valuable to enhance the therapeutic potency of many polar drugs by modulating their hydrophilicity [24]or shifting their metabolism to another direction [25]. The LFR prodrug system,as depicted in Scheme-1,revealsmany benefits, such as the eloquent release of the active component (s) when the LFR prodrug assaults via esterase enzyme[26]. Besides, the rate of releasing the active component (s) may be modified

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

through the presence of various functional groups on the coumarin chemical nucleus [27]. Moreover, the last compound, coumarin, acquired since the active component (s) are free from the LFR prodrug is documented to be safe[28].

Scheme-1. The activation two steps of the LFR prodrug system.

This report aims to apply the LFR prodrug system for designing and synthesizing a mutualprodrug. On activation, this LFR prodrug can releaseFUas an oral cytotoxic agent and EU as its metabolic modulator. To satisfy this aim, the chemical stability of the synthesized prodrug and its release profiles were evaluated using two buffer systems, simulating those found in the GIT, and human plasma, respectively.

Experimental

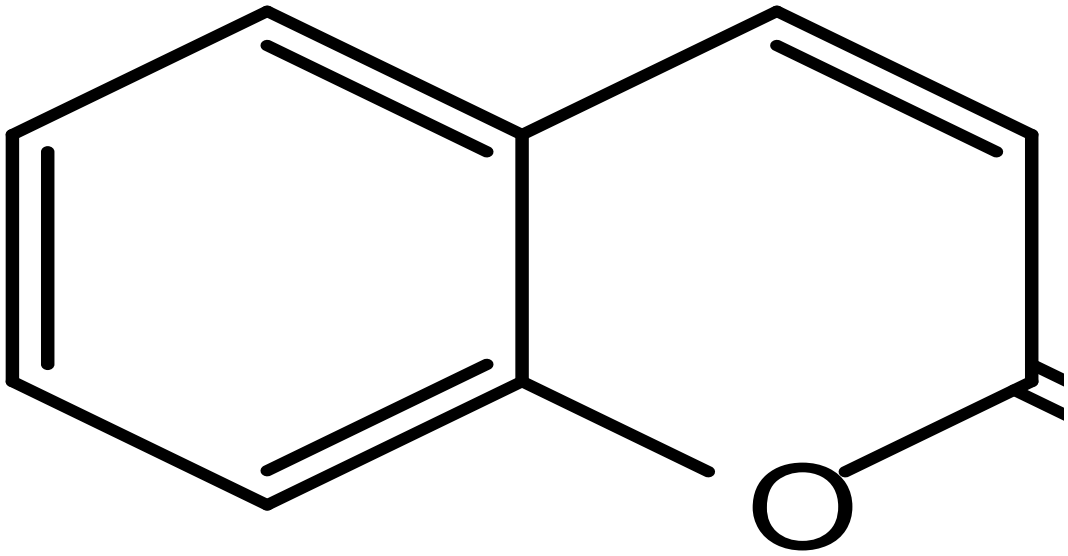
Materials and Methods

The chemicals and solvents utilized for synthesizing the LFR prodrug and its intermediate compounds, and studying the*in vitro* release were acquired from international sources. The instruments used to confirm the chemical structures of thesynthesized products 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 LFR 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 F_{254}, Merck) and the eluent system of chloroform: acetone (4:1) were used as stationary and mobile phases, respectively.

Chemical synthesis

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.



Coumarin

Scheme-2. Synthetic pathway of the LFR prodrug.

*Synthesis of the intermediate**a***

The mixture of formaldehyde (5 ml, 37%) and FU (1.04 g, 8 mmol) in 25 ml H_2O was magnetically agitated at 60°C until a solution formed (~ 45min). The resultant solution was vaporized to dryness under reduced pressure, and the titled product was recrystallized from ethanol[29], [30].

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

*Synthesis of the intermediate **b***

In an ice bath, the solution ofcoumarin (25 mmol, 3.65 g) in 50 ml dry ether was handled with the solution of lithium aluminum hydride(50 mmol, 1.9 g, $LiAlH_4$) 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 Na_2SO_4, filtered, and vaporized. The titled product was recrystallized from EtOH[31].

*Synthesis of the intermediate**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 of4-dimethylaminopyridin (34 mmol, 4.18 g, DMAP) in 40 ml dry THF was dropwise added. The reaction mixture was stirred for 14 hr, filtered, and vaporized to dryness. The crude was dissolved in EtOAc (50 ml) and washed serially with HCl (50 ml, 1N), $NaHCO_3$ (25 ml, 5%), and H_2O (25 ml). The organic layer was dehydrated over Na_2SO_4, filtered, and vaporized. The titled product was recrystallized from $CHCl_3$[31], [32].

*Synthesis of the intermediate **d***

To a mixture of (**c**) (10 mmol, 2.65 g) and(**a**)(10 mmol, 1.60 g) in 50 ml dry $CHCl_3$, malonyl chloride (10 mmol, 1 ml)was added. The reaction mixture was refluxed for 3 hr 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 Na_2SO_4, concentrated under reduced pressure, and eluted in column chromatography by a mixture of $CHCl_3$: EtOH (2:1) affording the titled product[31], [33].

*Synthesis of the intermediate **e***

Intermediate**d** (4 mmol, 1.97 g) was dissolved in a mixture of H_2O (10 ml), THF (10 ml), and AcOH (30 ml). After stirring for 60 min at50^OC, the mixture was vaporized under reduced pressure. The crude was dissolved in 50 ml EtOAc, and the resulted solution was washed with 5 % $NaHCO_3$ (50 ml, 5%)and H_2O (50 ml). The EtOAc layer was dehydrated over Na_2SO_4, filtered, andvaporized under reduced pressure. The target product was recrystallized from EtOH[34], [35].

*Synthesis of the intermediate **f***

Intermediate **e**(4 mmol, 1.51 g) and MnO_2 (20 mmol, 1.74 g) were suspended in $CHCl_3$ (30 ml) and refluxed for 20 hr. The hot mixture was filtered, and the resulted

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

solidwashed with 30 ml warm $CHCl_3$.The collected organic layer was vaporized under reduced pressure, and the crude wasdissolved in 30 ml acetone and filtered. The solution was vaporized affording the target product[36].

Synthesis of the intermediate ***g***

An aqueous solution prepared by dissolving (7.33 mmol, 660 mg) of $NaClO_2$in 10 ml H_2O was added slowly to a stirred mixture of the intermediate **f** (4 mmol, 1.50 g), NaH_2PO_4 (0.85 mmol, 102 mg), and H_2O_2 (30%, 4.17 mmol, 0.5 ml) in 25 ml ACN. Throughout the addition process, the temperature of the reaction was preserved below 10^OC using anice-water bath, and the oxygen bubbles were observed from the reaction mixture. As the formation of the bubblesstopped, Na_2SO_3(0.05 g) was added to devastate the unreacted H_2O_2 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 overNa_2SO_4, filtered, and vaporized. The crude was dissolved in ACN, treated with aqueous $NaHCO_3$ solution to pH 6.5, and filtered. The target product was separated as the filtrate acidified to pH 3 by 1N HCl[37].

Synthesis of the ***LFR 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 hr. Then, the reaction mixture was treated with MeOH (5 ml), and AcOH (0.5 ml), stirred for 60min, andneutralized with aqueous $NaHCO_3$ solution. The precipitate was filtered, the filtrate was vaporized, and the crude was washed with H_2O. The prodrug was crystallized from a mixture of EtOH: $CHCl_3$ (2:1.5)[31].

In vitro kinetic studies

Chemical stability

The chemical stability of the synthesized LFR prodrug was investigated in buffers with two pH values, which are HCl (pH 1.2) buffer and phosphate-buffered saline (pH 6.8)[38], [39]. This study was monitored via UV/Visible spectroscopy for dropping in prodrug concentration versus time utilizing the following mathematical formula of Beer's law[40]:$\boldsymbol{Absorbance = \mathcal{E} \times L \times C.}$

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

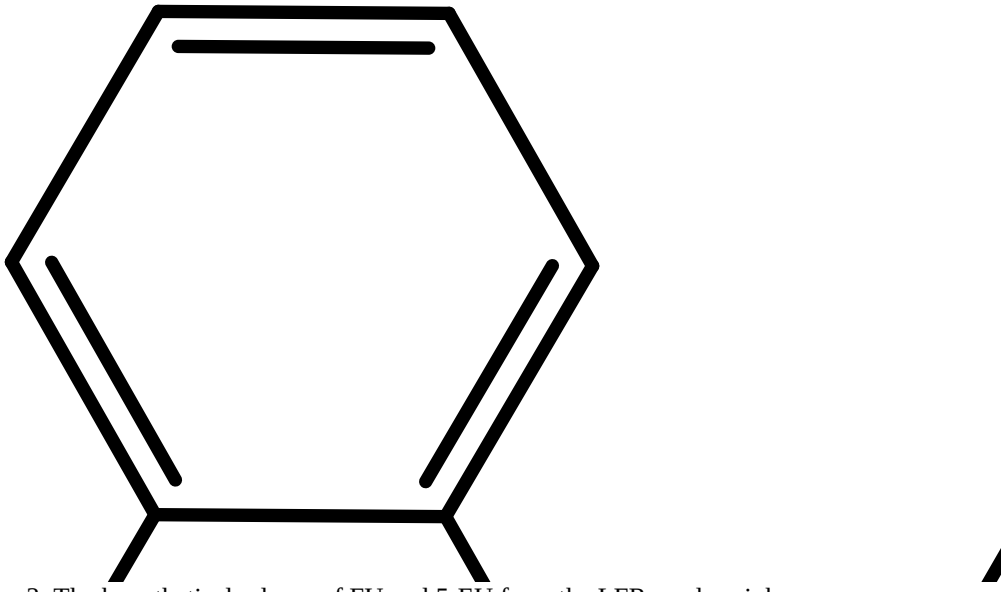
Briefly,a preheated solution of the LFR prodrug (5 μmol) in 2 ml DMSO was mixed with 48 ml preheated buffer solution. The time was begun to record, and the resulted solution was preserved at a 37°Cutilizing a warm water bath. Subsequently, the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

solution was splitinto 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 hr, an individual test tube was elected and its content was mixed with 2 ml CH_2Cl_2. The aqueous aliquot (2 ml) was estimated spectrophotometrically at defined $\lambda_{max}$to detect the residual concentration of the prodrug[41].

Enzymatic hydrolysis

A similar procedure to that followed for investigating the chemical stability was employed to monitor the hydrolysis of the synthesized LFR 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[42]–[44]. 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.



Scheme-3. The hypothetical release of FU and 5-EU from the LFR prodrug inhuman serum.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

Results and Discussion

Rationalization of the prodrug design

The synthesized LFR prodrug was designed in an attempt to optimize the clinical usefulness of 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 FU, minimizes its destruction via DPDE, and provides the opportunity of the mutual effect.In this concern, the values of log P for the FU, EU, and the target prodrug were found to be -0.90, -0.51, and 1.76 respectively. This indicatedthat the target LFR prodrug has better lipophilicity than those of its precursor drugs that may improve the oral bioavailability of FU. Also, the concurrent release of FU and EU may reduce the destruction of FUby DPDE 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 LFR prodrug to release FU and EU concurrently with an acceptable$t_{1/2}$ in a human serum.

Synthetic pathway

The pathway followed for the synthesis of the LFR 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[31]. 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 below0°C andthe 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 EU via DCC affording the target LFR prodrug in the last synthetic steps.

Structural elucidation

The physicochemical properties and the spectral data acquired from the employed instruments for the target LFR prodrug and its reaction intermediates are listed below. These data were confirmed the chemical frameworks of the synthesized products.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

a: White powder; % yield=70; mp=194-196°C; R_f =0.24; $C_5H_5FN_2O_3$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 10.24 (1H, s, N***H***), 7.94 (1H, s, =C***H***), 5.11 (2H, s, C***H***$_2$), 3.56 (1H, s, O***H***) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ= 158.1 (FC-***C***=O), 148.3 (HN-***C***=O), 140.6 (***C***-F), 126.3 (***C***=CF), 73.4 (***C***H_2-OH) ppm.

b: White powder; % yield=39; mp=148-150°C; R_f =0.34; $C_9H_{10}O_2$; λ_{max} (MeOH)=286 nm; FTIR (ν, cm^{-1}, stretching): 3235, 3202 (O-H), 3046 (=C-H), 2918 (-C-H),1642, 1588 (C=C); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 7.62, 7.24, 7.01, 6.83 (4H, m, aromatic), 6.94 (1H, d, ph-C***H***=), 6.08 (1H, q, =C***H***-CH_2OH), 5.52 (1H, s, ph-O***H***), 4.26 (2H, d, HO-C***H***$_2$), 3.62 (1H, s, CH_2-O***H***) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ= 159.3 (Ar ***C***-OH), 130.1, 127.4, 122.3, 119.6 (Ar ***C***), 126.5 (Ar C-***C***H=), 125.0 (=***C***H-CH_2), 115.6 (Ar ***C***-CH=), 53.2 (***C***H_2-OH) ppm.

c: White powder; % yield=73; mp=122-124°C; R_f =0.56; $C_{15}H_{24}O_2Si$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 7.60, 7.25, 7.01, 6.82 (4H, m, aromatic), 6.92 (1H, d, ph-C***H***=), 6.04 (1H, q, =C***H***-CH_2), 5.56 (1H, s, ph-O***H***), 4.48 (2H, d, Si-O-C***H***$_2$), 1.41 (9H, s, C***H***$_3$-C-Si), 0.48 (6H, s, Si-C***H***$_3$) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ= 159.0 (Ar ***C***-OH), 130.6, 128.1, 124.2, 119.8 (Ar ***C***), 126.2 (Ar C-**C**H=), 125.2 (=**C**H-CH_2), 115.1 (Ar ***C***-CH=), 52.4 (***C***H_2-O-Si), 33.2 (O-Si-***C***-), 26.4 (Si-C-***C***H_3), 12.3 (Si-***C***H_3) ppm.

d: White powder; % yield=79; mp=96-98°C; R_f =0.31; $C_{23}H_{29}FN_2O_7Si$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 10.18 (1H, s, N***H***), 8.02 (1H, s, FC=C***H***), 7.82, 7.72, 7.34, 6.27 (4H, m, aromatic), 6.95 (1H, d, ph-C***H***=), 6.08 (1H, q, =C***H***-CH_2), 5.96 (2H, s, N-C***H***$_2$-O), 4.56 (2H, d, Si-O-C***H***$_2$), 3.26 (2H, s, O=C-C***H***$_2$-C=O), 1.40 (9H, s, C***H***$_3$-C-Si), 0.43 (6H, s, Si-C***H***$_3$) ppm; ^{13}C-NMR (DMSO-d_6, 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-***C***H=), 127.2 (FC=***C***H), 124.4 (=***C***H-CH_2), 120.2 (Ar ***C***-CH=), 80.2 (N-***C***H_2-O), 52.4 (***C***H_2-O-Si), 42.4 (O=C-***C***H_2-C=O), 33.1 (O-Si-***C***-), 26.4 (Si-C-***C***H_3), 12.2 (Si-***C***H_3) ppm.

e: White powder; % yield=92; mp=106-108°C; R_f =0.26; $C_{17}H_{15}FN_2O_7$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 10.22 (1H, s, N***H***), 8.00 (1H, s, FC=C***H***), 7.80, 7.73, 7.36, 6.28 (4H, m, aromatic), 6.94 (1H, d, ph-C***H***=), 6.01 (1H, q, =C***H***-CH_2), 5.95 (2H, s, N-C***H***$_2$-O), 4.55 (2H, d, C***H***$_2$-OH), 3.52 (1H, s, O***H***), 3.28 (2H, s, O=C-C***H***$_2$-C=O) ppm;

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

^{13}C-NMR (DMSO-d_6, 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-***C***H=), 127.6 (FC=***C***H), 124.3 (=***C***H-CH_2), 120.0 (Ar ***C***-CH=), 80.0 (N-***C***H_2-O), 52.7 (***C***H_2-OH), 42.5 (O=C-***C***H_2-C=O) ppm.

f: White powder; % yield=66; mp=119-121°C; R_f =0.31; $C_{17}H_{13}FN_2O_7$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 10.16 (1H, s, N***H***), 9.85 (1H, d, C***H***O), 8.02 (1H, s, FC=C***H***), 7.83, 7.74, 7.35, 6.29 (4H, m, aromatic), 6.89 (1H, d, ph-C***H***=), 6.00 (1H, t, =C***H***-CHO), 5.98 (2H, s, N-C***H***$_2$-O), 3.32 (2H, s, O=C-C***H***$_2$-C=O) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ= 188.2 (***C***HO), 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-***C***H=), 126.9 (FC=***C***H), 124.2 (=***C***H-CHO), 120.8 (Ar ***C***-CH=), 80.5 (N-***C***H_2-O), 42.2 (O=C-***C***H_2-C=O) ppm.

g: White powder; % yield=82; mp=134-136°C; R_f =0.27; $C_{17}H_{13}FN_2O_8$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 10.64 (1H, s, COO***H***), 10.18 (1H, s, N***H***), 8.03 (1H, s, FC=C***H***), 7.83, 7.74, 7.35, 6.30 (4H, m, aromatic), 6.88 (1H, d, ph-C***H***=), 6.24 (1H, d, =C***H***-COOH), 5.92 (2H, s, N-C***H***$_2$-O), 3.36 (2H, s, O=C-C***H***$_2$-C=O) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ= 171.4 (***C***OOH), 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-***C***H=), 126.4 (FC=***C***H), 120.7 (Ar ***C***-CH=), 116.2 (=***C***H-COOH), 80.0 (N-***C***H_2-O), 42.5 (O=C-***C***H_2-C=O) ppm.

LFR prodrug: White powder; % yield=78; mp=156-158°C; R_f =0.23; $C_{23}H_{15}FN_4O_9$; 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); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 10.18 (2H, s, N***H***), 8.19 (1H, s, =C***H***-N), 8.06 (1H, s, FC=C***H***), 7.84, 7.71, 7.37, 6.27 (4H, m, aromatic), 6.92 (1H, d, ph-C***H***=), 6.22 (1H, d, =C***H***-CO), 5.94 (2H, s, N-C***H***$_2$-O), 3.36 (2H, s, O=C-C***H***$_2$-C=O), 3.11 (1H, s, ≡C***H***) ppm; ^{13}C-NMR (DMSO-d_6, 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-***C***H=), 126.0 (FC=***C***H), 124.4 (N-***C***H=C-), 122.9 (=***C***H-C=O), 121.1 (Ar ***C***-CH=), 105.4 (=***C***-C≡CH), 83.5 (=C-***C***≡CH), 80.1(=C-C≡***C***H), 78.9 (N-***C***H_2-O), 42.8 (O=C-***C***H_2-C=O) ppm.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

In vitro kinetic studies

Chemical stability

Under experimental conditions, the LFR 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 hr and 18.13 hr, respectively. This stability may be contributed to the steric hindrance around the ester linkages affording great stability versus nucleophilic attack[45]. 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[46].

Although the hydrolysis of the LFR 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[47]. 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[48].

Release study

TheLFR prodrug was able to liberate FU and EU obeying zero-order kinetics with $t_{1/2}$equals to 4.62 hr.This finding revealed that the LFR prodrug could reach the target with a good circulating time and liberate the two active moieties[49]. From the kinetics phenotype, it is concluded that the LFR prodrug can be taken orally in a low-frequency fashion [50]resulting in the improvement of the patient compliance[51].

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 EU and the time.

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

Absorbance	Time (hr)	x ($M\times10^6$)	a-x ($M\times10^6$)	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.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

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

Absorbance	Time (hr)	x ($M\times10^6$)	a-x ($M\times10^6$)	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 (hr)	x ($M\times10^6$)
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
ε = 284 L mol^{-1} cm^{-1}	ε = 298 L mol^{-1} cm^{-1}	ε = 1930.86 L mol^{-1} cm^{-1}
λ_{max} = 281 nm	λ_{max} = 312 nm	λ_{max} = 292 nm
$t_{1/2}$= 33.19 hr	$t_{1/2}$= 18.13hr	$t_{1/2}$ = 4.62 hr
k_{obs}= 5.8×10^6 hr^{-1}	k_{obs}= $10.62\times10^6 hr^{-1}$	k_{obs}= 10.83×10^6 $M.hr^{-1}$

ε = Absorbance coefficient, and K_{obs} = observed rate constant.

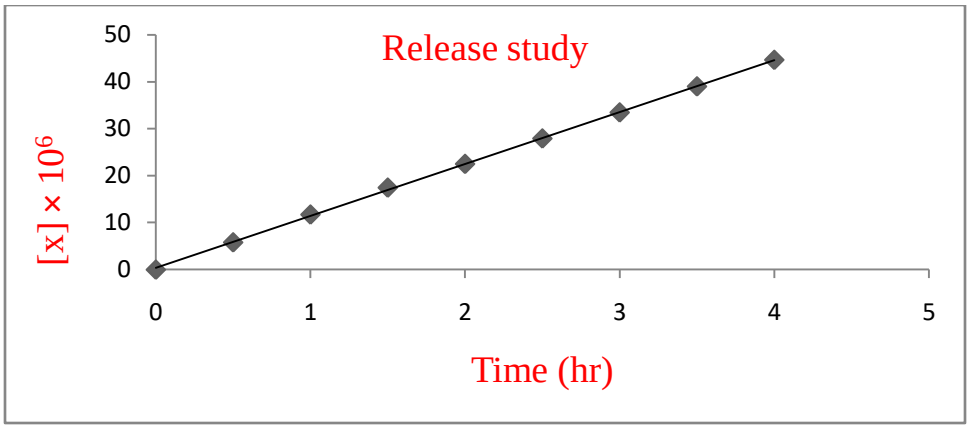


Figure-1. Graphical representation of the relationship between the released concentration of EU and the time in human serum.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

Conclusion

This work concluded that FU and its potent metabolic modulator, EU, could be incorporated into one chemical entity utilizing a LFR 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 LFR prodrug was able to release FU and EU obeying zero-order kinetics with a $t_{1/2}$ of 4.62 hr in a human serum. The value of this $t_{1/2}$allows the LFR prodrug to reach intact to the target eliciting an improvement in the therapeutic efficacy. Accordingly, the synthesized prodrug may represent a potential applicant as an oral form of FU with improved lipophilicity and efficacy to serve better in therapeutics.

Conflict of interest

The authors stated that there is no conflict of interest.

References

[1] Q. N. Cui, Y. C. Hsia, S. C. Lin, R. L. Stamper, J. Rose-Nussbaumer, N. Mehta, T. C. Porco, A. Naseri, and Y. Han, "Effect of mitomycin c and 5-flurouracil adjuvant therapy on the outcomes of Ahmed glaucoma valve implantation," *Clin. Exp. Ophthalmol.*, vol. 45, no. 2, pp. 128–134, 2017, doi: 10.1111/ceo.12811.

[2] D. S. KIM, K. MIN, and S. K. LEE, "Cell Cycle Dysregulation Is Associated With 5-Fluorouracil Resistance in Gastric Cancer Cells," *Anticancer Res.*, vol. 40, no. 6, pp. 3247–3254, 2020, doi: 10.21873/anticanres.14306.

[3] S. Blondy, V. David, M. Verdier, M. Mathonnet, A. Perraud, and N. Christou, "5-Fluorouracil resistance mechanisms in colorectal cancer: From classical pathways to promising processes," *Cancer Sci.*, vol. 111, no. 9, pp. 3142–3154, 2020, doi: 10.1111/cas.14532.

[4] G. Zhu, M. Zhao, Q. Han, Y. Tan, Y. Sun, M. Bouvet, S. R. Singh, J. Ye, and R. M. Hoffman, "Combination of Trabectedin with Oxaliplatinum and 5-Fluorouracil Arrests a Primary Colorectal Cancer in a Patient-derived Orthotopic Xenograft Mouse Model," *Anticancer Res.*, vol. 39, no. 11, pp. 5999–6005, 2019, doi: 10.21873/anticanres.13805.

[5] Y. Nishizawa, R. Ikeda, M. Yamamoto, K. Kawahara, Y. Shinsato, K. Minami, M. Nitta, H. Terazono, S. I. Akiyama, T. Furukawa, and Y. Takeda, "5-aza-2-deoxycytidine enhances the sensitivity of 5-fluorouracil by demethylation of the thymidine phosphorylase promoter," *Anticancer Res.*, vol. 39, no. 8, pp. 4129–4136, 2019, doi: 10.21873/anticanres.13571.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

[6] A. Cavaliere, K. C. Probst, A. D. Westwell, and M. Slusarczyk, "Fluorinated nucleosides as an important class of anticancer and antiviral agents," *Future Med. Chem.*, vol. 9, no. 15, pp. 1809–1833, 2017, doi: 10.4155/fmc-2017-0095.

[7] S. Pasban, H. Raissi, M. Pakdel, and F. Farzad, "Enhance the efficiency of 5-fluorouracil targeted delivery by using a prodrug approach as a novel strategy for prolonged circulation time and improved permeation," *Int. J. Pharm.*, vol. 568, no. July, p. 118491, 2019, doi: 10.1016/j.ijpharm.2019.118491.

[8] S. Mindt, S. Aida, K. Merx, A. Müller, T. Gutting, M. Hedtke, M. Neumaier, and R. D. Hofheinz, "Therapeutic drug monitoring (TDM) of 5-fluorouracil (5-FU): New preanalytic aspects," *Clin. Chem. Lab. Med.*, vol. 57, no. 7, pp. 1012–1016, 2019, doi: 10.1515/cclm-2018-1177.

[9] Y. Ota, A. Nakamura, E. E. Elboray, Y. Itoh, and T. Suzuki, "Design, synthesis, and biological evaluation of a conjugate of 5-fluorouracil and an LSD1 inhibitor," *Chem. Pharm. Bull.*, vol. 67, no. 3, pp. 192–195, 2019, doi: 10.1248/cpb.c18-00577.

[10] S. Giret, C. Théron, A. Gallud, M. Maynadier, M. Gary-Bobo, M. Garcia, M. Wonga Chia Man, and C. Carcel, "A designed 5-fluorouracil-based bridged silsesquioxane as an autonomous acid-triggered drug-delivery system," *Chem. - A Eur. J.*, vol. 19, no. 38, pp. 12806–12814, 2013, doi: 10.1002/chem.201301081.

[11] S. F. W. Zhiwei Wen, Paloma R. Tuttle, A. Hasan Howlader, Anna Vasilyeva, Laura Gonzalez, Antonija Tangar, Ruipeng Lei, Eduardo E. Laverde, Yuan Liu, Jaroslava Miksovska, "Fluorescent 5-Pyrimidine and 8-Purine Nucleosides Modified with an N-Unsubstituted 1,2,3-Triazol-4-yl Moiety," *J Org Chem*, vol. 84, no. 6, pp. 3624–3631, 2015, doi: 0.1021/acs.joc.8b03135.

[12] E. Rivera, J. C. Chang, V. Semiglazov, O. Burdaeva, M. G. Kirby, and T. Spector, "Eniluracil plus 5-fluorouracil and leucovorin: Treatment for metastatic breast cancer patients in whom capecitabine treatment rapidly failed," *Clin. Breast Cancer*, vol. 14, no. 1, pp. 26–30, 2014, doi: 10.1016/j.clbc.2013.08.018.

[13] M. K. Oglah and Y. F. Mustafa, "Curcumin analogs: synthesis and biological activities," *Med. Chem. Res.*, vol. 29, no. 3, pp. 479–486, 2020, doi: 10.1007/s00044-019-02497-0.

[14] M. K. Oglah, Y. F. Mustafa, M. K. Bashir, and M. H. Jasim, "Curcumin and its derivatives: A review of their biological activities," *Syst. Rev. Pharm.*, vol. 11, no. 3, pp. 472–481, 2020, doi: 10.5530/srp.2020.3.60.

[15] M. K. Oglah and Y. F. Mustafa, "Synthesis , antioxidant , and preliminary

antitumor activities of new curcumin analogues," *J. Glob. Pharma Technol.*, vol. 12, no. 2, pp. 854–862, 2020.

[16] M. K. Bashir, Y. F. Mustafa, and M. K. Oglah, "Antitumor, antioxidant, and antibacterial activities of glycosyl-conjugated compounds: A review," *Syst. Rev. Pharm.*, vol. 11, no. 4, pp. 175–187, 2020, doi: 10.31838/srp.2020.4.26.

[17] Y. F. Mustafa, M. K. Bashir, and M. K. Oglah, "Original and innovative advances in the synthetic schemes of coumarin-based derivatives: A review," *Syst. Rev. Pharm.*, vol. 11, no. 6, pp. 598–612, 2020, doi: 10.31838/srp.2020.6.90.

[18] M. Slavíčková, M. Janoušková, A. Šimonová, H. Cahová, M. Kambová, H. Šanderová, L. Krásný, and M. Hocek, "Turning Off Transcription with Bacterial RNA Polymerase through CuAAC Click Reactions of DNA Containing 5-Ethynyluracil," *Chem. - A Eur. J.*, vol. 24, no. 33, pp. 8311–8314, 2018, doi: 10.1002/chem.201801757.

[19] V. Kartsev, A. Geronikaki, S. Bua, A. Nocentini, A. Petrou, B. Lichitsky, M. Frasinyuk, J. Leitans, A. Kazaks, K. Tars, and C. T. Supuran, "Extending the inhibition profiles of coumarin-based compounds against human carbonic anhydrases: Synthesis, biological, and in silico evaluation," *Molecules*, vol. 24, no. 19, pp. 1–12, 2019, doi: 10.3390/molecules24193580.

[20] Y. F. Mustafa, R. R. Khalil, and E. T. Mohammed, "Antimicrobial activity of aqueous extracts acquired from the seeds of two apples ' cultivars," *Syst. Rev. Pharm.*, vol. 11, no. 2, pp. 382–387, 2020, doi: 10.5530/srp.2020.2.56.

[21] Y. F. Mustafa, E. T. Mohammed, and R. R. Khalil, "Antioxidant and antitumor activities of methanolic extracts obtained from Red delicious and Granny Smith apples' seeds," *Syst. Rev. Pharm.*, vol. 11, no. 4, pp. 570–576, 2020, doi: 10.31838/srp.2020.4.84.

[22] E. T. Mohammed and Y. F. Mustafa, "Coumarins from Red Delicious apple seeds : Extraction , phytochemical analysis , and evaluation as antimicrobial agents," *Syst. Rev. Pharm.*, vol. 11, no. 2, pp. 64–70, 2020, doi: 10.5530/srp.2020.2.11.

[23] R. R. Khalil and Y. F. Mustafa, "Phytochemical, antioxidant and antitumor studies of coumarins extracted from Granny Smith apple seeds by different methods," *Syst. Rev. Pharm.*, vol. 11, no. 2, pp. 57–63, 2020, doi: 10.5530/srp.2020.2.10.

[24] Q. Xie, X. Wang, X. Wang, Z. Jiang, and Z. Qiu, "Design , synthesis , and bioavailability evaluation of coumarin-based prodrug of meptazinol," *Bioorg Med Chem Lett*, vol. 15, no. 22, pp. 4953–4956, 2005, doi: 10.1016/j.bmcl.2005.08.016.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

[25] W. Wang, G. Camenisch, D. C. Sane, H. Zhang, E. Hugger, G. L. Wheeler, R. T. Borchardt, and B. Wang, "A coumarin-based prodrug strategy to improve the oral absorption of RGD peptidomimetics," *J. Control. Release*, vol. 65, no. 1–2, pp. 245–251, 2000, doi: 10.1016/s0168-3659(99)00241-2.

[26] B. Wang, H. Zhang, A. Zheng, and W. Wang, "Coumarin-based prodrugs. Part 3: Structural effects on the release kinetics of esterase-sensitive prodrugs of amines," *Bioorganic Med. Chem.*, vol. 6, no. 4, pp. 417–426, 1998, doi: 10.1016/S0968-0896(98)00014-5.

[27] X. Yang, X. Hou, B. Wang, M. Li, and H. Fang, "Density functional theory based quantitative structure-property relationship studies on coumarin-based prodrugs," *Biosci. Trends*, vol. 6, no. 4, pp. 234–240, 2012, doi: 10.5582/bst.2012.v6.5.234.

[28] A. Aspatwar, E. Berrino, S. Bua, F. Carta, S. Parkkila, C. T. Supuran, A. Aspatwar, E. Berrino, S. Bua, and F. Carta, "Toxicity evaluation of sulfamides and coumarins that efficiently inhibit human carbonic anhydrases," *J. Enzyme Inhib. Med. Chem.*, vol. 35, no. 1, pp. 1765–1772, 2020, doi: 10.1080/14756366.2020.1822829.

[29] Y. F. Mustafa, M. K. Oglah, and M. K. Bashir, "Conjugation of sinapic acid analogues with 5- Fluorouracil: Synthesis, preliminary cytotoxicity, and release study," *Syst. Rev. Pharm.*, vol. 11, no. 3, pp. 482–489, 2020, doi: 10.5530/srp.2020.3.61.

[30] A. M. Nejres, H. K. Ali, S. P. Behnam, and Y. F. Mustafa, "Potential effect of ammonium chloride on the optical physical properties of polyvinyl alcohol," *Syst. Rev. Pharm.*, vol. 11, no. 6, pp. 726–732, 2020, doi: 10.31838/srp.2020.6.107.

[31] Y. F. Mustafa and N. A. Al-omari, "Design , Synthesis and Kinetic Study of Coumarin-Based Mutual Prodrug of 5-Fluorouracil and Dichloroacetic acid," *Iraqi J Pharm Sci*, vol. 25, no. 1, pp. 6–16, 2016.

[32] A. M. Nejres, Y. F. Mustafa, and H. S. Aldewachi, "Evaluation of natural asphalt properties treated with egg shell waste and low density polyethylene," *Int. J. Pavement Eng.*, 2020, doi: 10.1080/10298436.2020.1728534.

[33] M. K. Bashir, Y. F. Mustafa, and M. K. Oglah, "Synthesis and antitumor activity of new multifunctional coumarins," *Period. Tche Quim.*, vol. 17, no. 36, pp. 871–883, 2020.

[34] Y. F. Mustafa, "Synthesis, characterization and antibacterial activity of novel heterocycle, coumacine, and two of its derivatives," *Saudi Pharm. J.*, vol. 26, no. 6, pp. 870–875, 2018, doi: 10.1016/j.jsps.2018.03.010.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5671 - 5688
Received 05 March 2021; Accepted 01 April 2021.

[35] Y. F. Mustafa, "Synthesis, characterization and preliminary cytotoxic study of sinapic acid and its analogues," *J. Glob. Pharma Technol.*, vol. 11, no. 9, pp. 1–10, 2019.

[36] Y. F. Mustafa, M. A. Najem, and Z. S. Tawffiq, "Coumarins from Creston apple seeds: Isolation, chemical modification, and cytotoxicity study," *J. Appl. Pharm. Sci.*, vol. 8, no. 8, pp. 049–056, 2018, doi: 10.7324/JAPS.2018.8808.

[37] A. A. J. Mahmood, Y. F. Mustafa, and M. Abdulstaar, "New coumarinic azo-derivatives of metoclopramide and diphenhydramine: Synthesis and in vitro testing for cholinesterase inhibitory effect and protection ability against chlorpyrifos," *Int. Med. J. Malaysia*, vol. 13, no. 1, pp. 3–12, 2014.

[38] N. Hwang, Y. Pei, J. Clement, E. S. Robertson, and Y. Du, "Identification of a 3-β-homoalanine conjugate of brusatol with reduced toxicity in mice," *Bioorg. Med. Chem. Lett.*, vol. 30, no. 23, p. 127553, 2020, doi: 10.1016/j.bmcl.2020.127553.

[39] H. Aldewachi, Y. F. Mustafa, R. Najm, and F. Ammar, "Adulteration of Slimming Products and its Detection Methods," *Syst. Rev. Pharm.*, vol. 11, no. 3, pp. 289–296, 2020, doi: 10.5530/srp.2020.3.33.

[40] G. Chi, W. Man, J. Wang, Y. Song, J. H. Wong, Y. Zhao, T. S. Lau, K. T. Leung, T. H. Chan, H. Wang, J. Kwong, T. B. Ng, and C. C. Wang, "Therapeutic potential of a novel prodrug of green tea extract in induction of apoptosis via ERK / JNK and Akt signaling pathway in human endometrial cancer," *BMC Cancer*, vol. 20, no. 1, pp. 1–14, 2020, doi: https://doi.org/10.1186/s12885-020-07455-3.

[41] M. K. Oglah, M. Kahtan Bashir, Y. Fakri Mustafa, E. T. Mohammed, R. R. Khalil, and Y. F. Mustafa, "Synthesis and biological activities of 3,5-disubstituted-4-hydroxycinnamic acids linked to a functionalized coumarin," *Syst. Rev. Pharm.*, vol. 11, no. 6, pp. 717–725, 2020, doi: 10.31838/srp.2020.6.106.

[42] S. Watanabe, S. Vikingsson, M. Roman, H. Green, R. Kronstrand, and A. Wohlfarth, "In Vitro and In Vivo Metabolite Identification Studies for the New Synthetic Opioids Acetylfentanyl , Acrylfentanyl , Furanylfentanyl , and 4-Fluoro-Isobutyrylfentanyl," *AAPS J*, vol. 19, no. 4, pp. 1102–1122, 2017, doi: 10.1208/s12248-017-0070-z.

[43] Y. F. Mustafa and N. T. Abdulaziz, "Biological potentials of hymecromone-based derivatives : A systematic review," *Syst. Rev. Pharm.*, vol. 11, no. 11, pp. 438–452, 2020, doi: 10.31838/srp.2020.11.65.

[44] Y. F. Mustafaa, N. T. Abdulazizaa, and M. H. Jasima, "4-Methylumbelliferone and its derived compounds: A brief review of their cytotoxicity," *Egypt. J. Chem.*, vol.

64, no. 4, pp. 1807–1816, 2021, doi: 10.21608/EJCHEM.2021.59702.3281.

[45] M. Li, Z. Liang, X. Sun, T. Gong, and Z. Zhang, "A polymeric prodrug of 5-fluorouracil-1-acetic acid using a multi-hydroxyl polyethylene glycol derivative as the drug carrier.," *PLoS One*, vol. 9, no. 11, p. e112888, 2014, doi: 10.1371/journal.pone.0112888.

[46] D. Zhao, H. Zhang, W. Tao, W. Wei, J. Sun, and Z. He, "A rapid albumin-binding 5-fluorouracil prodrug with a prolonged circulation time and enhanced antitumor activity," *Biomater. Sci.*, vol. 5, no. 3, pp. 502–510, 2017, doi: 10.1039/c6bm00884d.

[47] W. Stroberg and S. Schnell, "On the validity and errors of the pseudo-first-order kinetics in ligand – receptor binding," *Math. Biosci.*, vol. 287, no. 5, pp. 1–9, 2016, doi: 10.1016/j.mbs.2016.09.010.

[48] K. Anzo, M. Harada, and T. Okada, "Enhanced Kinetics of Pseudo First-Order Hydrolysis in Liquid Phase Coexistent with Ice," *J Phys Chem A*, vol. 117, no. 41, pp. 10619–10625, 2013, doi: https://doi.org/10.1021/jp409126p.

[49] L. Zhang, J. Alfano, and D. Race, "Zero-order release of poorly water-soluble drug from polymeric films made via aqueous slurry casting," *Eur J Pharm Sci*, vol. 117, no. 5, pp. 245–254, 2019, doi: 10.1016/j.ejps.2018.02.029.Zero-order.

[50] M. Laracuente, M. H. Yu, and K. J. Mchugh, "Zero-order drug delivery: State of the art and future prospects," *J. Control. Release*, vol. 327, no. 9, pp. 834–856, 2020, doi: 10.1016/j.jconrel.2020.09.020.

[51] N. Wen, Y. Dong, R. Song, W. Zhang, C. Sun, X. Zhuang, Y. Guan, Q. Meng, and Y. Zhang, "Zero-Order Release of Gossypol Improves Its Antifertility E ff ect and Reduces Its Side E ff ects Simultaneously," *Biomacromolecules*, vol. 11, no. 6, pp. 1918–1925, 2018, doi: 10.1021/acs.biomac.7b01648.