Design, Synthesis and Kinetic Study of Coumarin-Based Mutual Prodrug of 5-Fluorouracil and Dichloroacetic acid

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Abstract

On the basis of known coumarin-based prodrug system, a novel coumarin-based mutual prodrug of 5-fluorouracil and dichloroacetic acid was designed, synthesized and evaluated as a promising oral chemotherapeutic agent basing on *in vitro* stability study in HCl buffer (pH 1.2) and in phosphate buffer (pH 7.4), as well as *in vitro* release study in human serum. The chemical structure of prodrug was confirmed by analyzing its FTIR, ^{1}H NMR, ^{13}C NMR and MS-ESI spectra. The results of *in vitro* kinetic study indicated that the prodrug was significantly stable in HCl and in phosphate buffers, and was hydrolyzed in human serum followed pseudo first order kinetics.

Keywords: Coumarin-based prodrug, 5-fluorouracil, Dichloroacetate, kinetics.

تصميم، تصنيع ودراسة حركية بادئ الدواء التبادلي المرتكز على الكومارين لـ ٥- فلوروبراسيل وثنائي الكلور حامض الخليك

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الخلاصة

اعتمادا على نظام بادئ الدواء المرتكز على الكومارين، تم تصميم وتصنيع بادئ دواء تبادلي غير مألوف مكون من ٥- فلوروبراسيل وثنائي الكلور حامض الخليك، وتقيمه كعلاج كيميائي فموي واعد استنادا الى دراسة الاستقرارية خارج جسم الكائن الحي في محلول حامض الهايدروكلوريك البفري (أس هيدوجيني ٢,١) ومحلول الفوسفيت البفري (أس هيدوجيني ٤,٧)، بالاضافة الى دراسة التحرر في مصل الدم البشري خارج جسم الكائن الحي.

تم التأكد من الشكل الكيميائي لبادئ الدواء من خلال تحليل اطيافه للأشعة تحت الحمراء، الرنين النووي المغناطيسي للهيدروجين وللكربون بالاضافة لطيف الكتلة. لقد أشارت نتائج الدراسة الحركية خارج جسم الكائن الحي الى استقرارية بادئ الدواء التبادلي بشكل كبير في محلول حامض الهايدروكلوريك البفري ومحلول الفوسفيت البفري وتحلله في مصل الدم البشري متبعا حركيات الرتبة الزائفة الأولى.

الكلمات المفتاحية : الدواء المرتكز على الكومارين ،٥-- فلوروبراسيل ، ثنائي الكلور حامض الخليك ، الحركية.

Introduction

The classical antimetabolite, 5-fluorouracil (5-FU), exerts its antitumor effect by inhibiting thymidylate synthase[(1)] and by misincorporating into DNA or RNA to inhibit its normal function[(2)]. It is widely used since its discovery in 1957 as a single agent in treatment of colorectal cancer and as a part of various regimens in treatment of breast, head, neck and upper gastrointestinal tumors[(3)].

However, 5-FU suffers from many drawbacks; first, it can cause many toxic effects as gastrointestinal disorders, myelosuppression, oral mucositis and cardiotoxicity[(4)].Secondly, its efficiency is limited by the short plasma half-life after intravenous administration due to the activity of dihydropyrimidine dehydrogenase (DPD), and irregular absorption with unpredictable plasma level after oral administration[(5)]. Thirdly, the resistance of some tumors to chemotherapeutic effect of 5-FU; such resistance is a result of high expression of thymidine phosphorylase or low reserves of reduced folates[(6)].

To tackle these problems, many approaches have been developed and evaluated; the most important one is the use of prodrug strategy to yield 5-FU prodrugs that can avoid certain routes of degradation[7] or can target the tumor cells[(8)].

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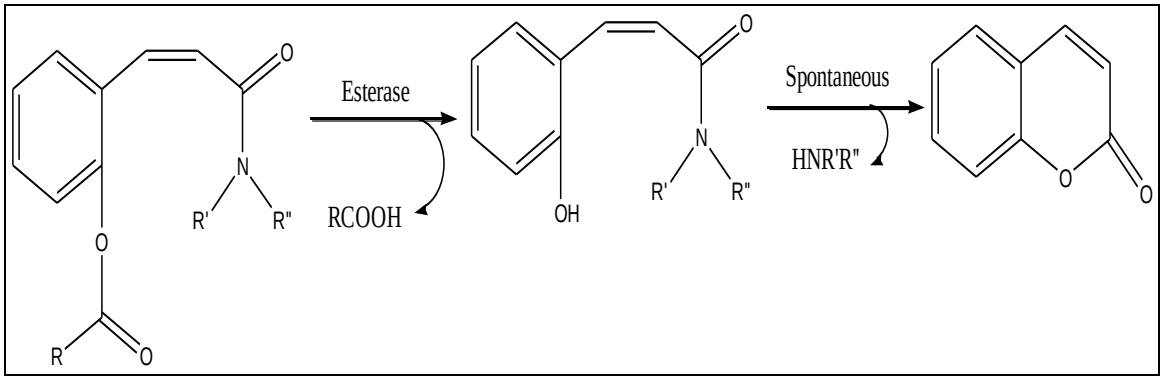
Received: 3/11/2015

Accepted: 2/1/2016

There is no doubt that prodrug strategies play a critical role in the development of drug delivery and drug discovery; one of these strategies is a coumarin-based prodrug system that is important for preparing esterase-sensitive prodrugs of alcohols, amines and peptides[9]. This system (Scheme 1) has several advantages such as the facile lactonization when an acyl group (R) is hydrolyzed by esterase and the safety profile of the final product, coumarin[10]. To date, this system is successfully used to prepare several prodrugs of opioid peptide[11], non-peptide analgesic[12] and peptidomimetic[13].



Scheme(1): The illustration of a coumarin-based prodrug system for amine.

Metabolic abnormity is a phenotypic trait of cancer cells. It is observed that cancer cells generally utilize glycolysis for energy production rather than oxidative phosphorylation. Thus, pyruvate is converted to lactate through anaerobic metabolism rather than its conversion to acetyl-CoA by action of pyruvate dehydrogenase (PDH) in aerobic glucose metabolism, this is called Warburg effect. Pyruvate dehydrogenase kinase (PDK) can inactivate PDH in many glycolytic phenotypes including cancer and switch the metabolism from anaerobic glycolysis to aerobic oxidation which is proved to be detrimental to tumor growth[14-16].

Dichloroacetate (DCA) has been known for many years as an experimental drug for treating certain metabolic disorders (e.g. inborn errors of metabolism) and as a promising agent in cancer therapy[17]. DCA is a well-known inhibitor of mitochondrial PDK; thus it can reverse Warburg effect, restore mitochondrial function, induce apoptosis and diminish the growth advantage of highly glycolytic tumors[18].

It is approved that the co-administration of DCA with 5-FU potentiates the antitumor activity of 5-FU in treatment of different types of cancer[19]. In addition, there are many reports indicated the beneficial effect of DCA in the treatment of glioblastoma, metastatic carcinomas, ovarian cancer, colorectal cancer, endometrial cancer, lung cancer and breast cancer[20- 22].

The aim of this work was to synthesize a novel coumarin - based mutual prodrug of 5-FU and DCA starting from coumarin and to evaluate it as a promising oral chemotherapeutic agent by monitoring its *in vitro* stability in HCl buffer (pH 1.2) and in phosphate buffer (pH 7.4), as well as its *in vitro* release in human serum**.**

Experimental

Materials

All chemicals and solvents used in this work were purchased from commercial sources and used without further purification. Coumarin was purchased from Sigma-Aldrich, DCA and $LiAlH_4$ from Tokyo Chemical Industry (TCI) and the others from Fluka.

Instruments

The instruments used for structure elucidation of the prodrug were: Bruker Avance DRX-400 MHz (Germany) to scan NMR spectra that were expressed in part per million upfield to TMS as an internal standard, Shimadzu LCMS-2020 Single Quadrupole Liquid Chromatograph Mass Spectrometer (Japan) with electrospray ionization source to measure the mass spectrum. The LCMS and NMR spectra were performed in Japan via Japan Food Research laboratories (JFRL). Bruker-Alpha ATR-FTIR spectrophotometer (Germany) to record the IR spectrum that performed in College of Pharmacy/University of Mosul.

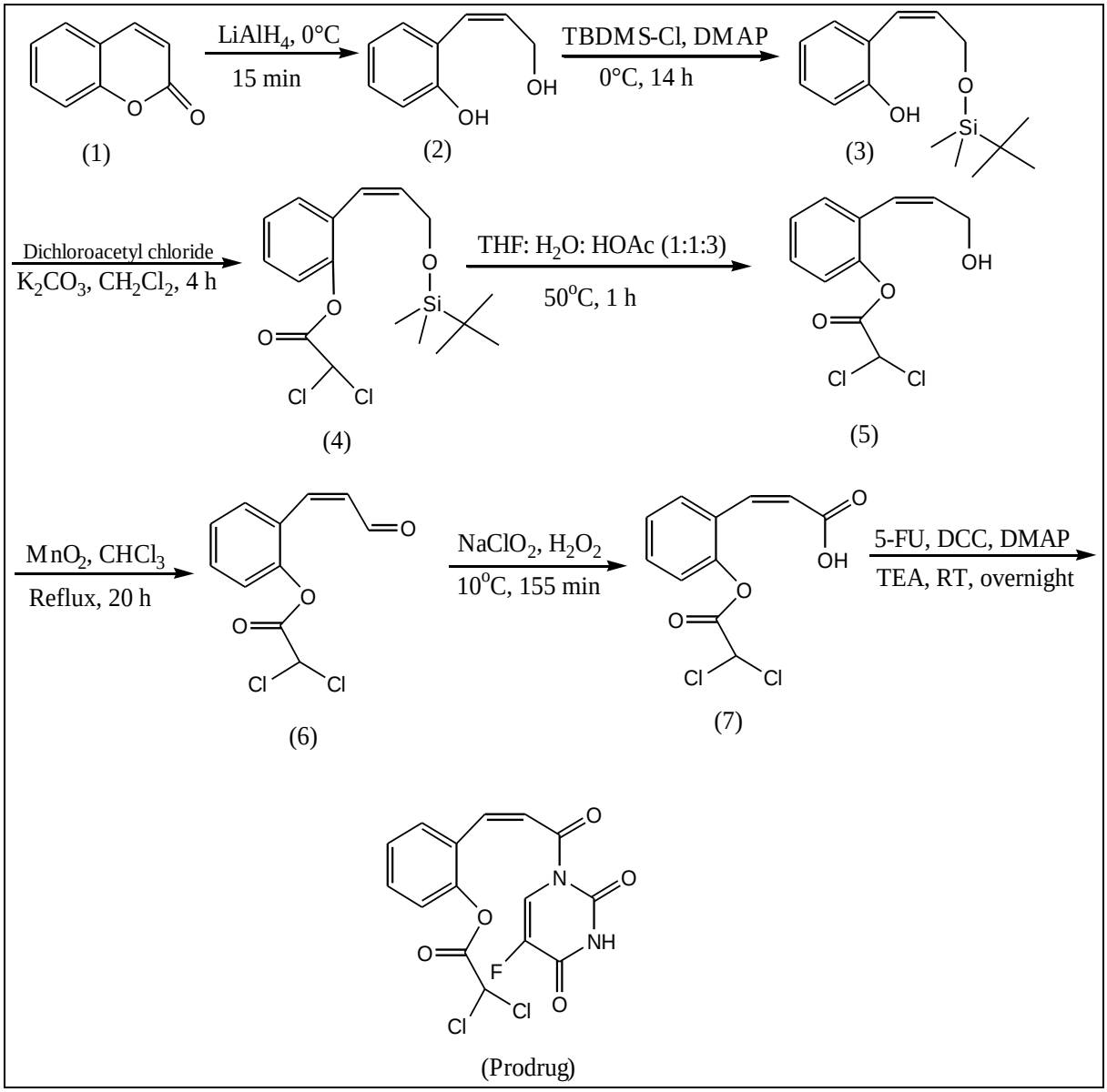
Melting points were determined, using open capillary method, on an electrochemical CIA 9300 melting point apparatus (UK) and were uncorrected. The instrument used to identify UV spectra and to follow kinetic study was Carrywinn UV Varian UV/Visible spectrophotometer.

The purity of compounds and the completion of reactions were checked by thin layer

chromatography (TLC) using precoated silica gel plates (60G F254, Merck) and the spots on chromatograms were localized via UV light (at 366 nm). Structures, the calculated molecular formula and molecular weights were carried out by using Chemdraw Ultra 2010.

Synthesis

Dichloroacetyl chloride [23]**.** Freshly distilled thionyl chloride (10 ml) was added dropwise to a cold solution of dichloroacetic acid (2.1 ml, 25 mmol) in 10 ml dry ether with continuous stirring under anhydrous condition. The mixture was heated at 40°C for 30 minutes and then refluxed for two hours. The solvent and the excess of thionyl chloride were removed under reduced pressure. The product was obtained by distillation at 108°C as slightly yellowish liquid with 82% of yield and λ_{max} (ethanol) = 244 nm.



Scheme (2): Synthetic pathway of coumarin-based mutual prodrug.

(Z)-2-(3-hydroxypropenyl)phenol (2).

A solution of 1 (3.65 g, 25 mmol) in 50 ml dry ether was placed in an ice bath and treated with a solution of 1.0 M of pure $LiAlH_4$ in dry ether (1.9 g of $LiAlH_4$ dissolved in 50 ml, 50 mmol). After stirring for 15 min, 5 % HCl (25 ml) was added to the reaction at 0°C. Then the solution was adjusted to pH 5 with 1M HCl and extracted with ether (3×50 ml). The ether layer was dried over Na_2SO_4, filtered and evaporated. The residue was dissolved in

ethanol, filtrated and evaporated to afford the desired product.

(Z)-2-(3-(*tert-butyldimethylsilyloxy)propenyl) phenol (3)*.

A solution of compound 2 (3.43 g, 22.8 mmol) in 40 ml dry THF was placed in an ice bath. To this solution, *tert*-butyldimethylsilyl (TBDMS) chloride (3.79 g, 25 mmol) dissolved in 35 ml dry THF was added at 0°C. Then N,N-dimethylaminopyridin (DMAP) (4.18 g, 34 mmol) in 40 ml dry THF was added in a dropwise manner. After stirring for 14 hours at 0°C, the solution was filtered and evaporated to remove the THF. The residue was re-dissolved in ethyl acetate (50 ml) and washed with 1 M HCl (2 × 27 ml), 5 % $NaHCO_3$ (22 ml) and H_2O (22 ml). The ethyl acetate layer was dried over Na_2SO_4, filtered and evaporated. The residue was then crystallized from $CHCl_3$.

(Z)-2-(3-(*tert-butyldimethylsilyloxy)propenyl) phenyl dichloroacetate (4)*

To a solution of compound 3 (2.65 g, 10 mmol) in 25 ml dry CH_2Cl_2 treated with K_2CO_3 (600mg), solution of dichloroacetyl chloride (1 ml, 10 mmol) in 10 ml dry CH_2Cl_2 was added dropwise for 30 minutes. The reaction mixture was refluxed for 4 hours. The progress of reaction was monitored by TLC using ether: ethyl acetate (1:1) mixture as a mobile phase. The reaction mixture was washed with water (2×25 ml); the organic layer was dried over anhydrous Na_2SO_4 and concentrated in rotary evaporator to dryness. The residue was re-dissolved in ethyl acetate, filtrated and evaporated to afford the desired product.

(Z) -2 - (3- hydroxypropenyl)phenyl dichloroacetate (5)

To a solution of compound 4 (3 g, 8 mmol) in THF (20 ml), water (20 ml) was added. This was followed by dropwise addition of acetic acid (60 ml). The mixture was stirred at 50^{O}C for one hour and then evaporated to remove THF, water and acetic acid under reduced pressure. Ethyl acetate (50 ml) was added to the residue, which was washed with 5 % $NaHCO_3$ (2 × 25 ml) and water (2 × 25 ml). The ethyl acetate layer was dried over Na_2SO_4, filtered and evaporated. The desired product was then crystallized from ethanol.

***(Z)-2- (3-oxopropenyl)phenyl dichloroacetate* (6)**

A suspension of 5 (2.09 g, 8 mmol) and MnO_2 (3.48 g, 40 mmol) in $CHCl_3$ (50 ml) was heated at reflux for 20 hours. The hot mixture was filtered, washed with warm $CHCl_3$ (2×25 ml) and the solvent was evaporated under reduced pressure. The residue was re-dissolved in 30 ml acetone, filtered and the filtrate was evaporated to afford the desired product.

(Z)- 3- [2- (dichloroacetyloxy) phenyl] acrylic acid (7)

A solution of sodium chlorite (660 mg, 7.33 mmol) in water (6 ml) was added dropwise very slowly to a stirred mixture of compound 6 (1.04 g, 4 mmol) in ACN (15 ml), sodium dihydrogen phosphate (102 mg, 0.85 mmol) in water (1.65 ml), and 30 % hydrogen peroxide (0.5 ml, 4.17 mmol). During the addition, the reaction temperature was kept at 10^{O}C in a cold water bath and oxygen evolution from the solution was observed visually until the end of the reaction. When oxygen evolution was ended (about 155 minutes from the first addition of sodium chlorite), a small amount of sodium sulfite (0.05 g) was added to destroy the unreacted HOCl and H_2O_2. The solution was acidified with 1 M HCl to pH 2. The mixture was then extracted with ethyl acetate (2×50 ml). The combined ethyl acetate layer was washed with saturated sodium chloride solution (2 × 25 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was re-dissolved in ACN, treated with aqueous solution of 10% $NaHCO_3$ to pH 6.5 and filtered. The filtrate was acidified with 1M HCl to pH 2.5 and the desired product obtained by filtration.

(Z)- 2 [3- (5 – fluorouricyl)-3-oxopropenyl] phenyl dichloroacetate (prodrug)

To a cold solution of 7 (1.1 g, 4 mmol) in 50 ml freshly distilled DMSO, 5-FU (0.52 g, 4 mmol), DCC (1 g, 4.8 mmol), DMAP (40 mg, 0.33 mmol) and triethylamine (0.6 ml , 4 mmol) were sequentially added to the reaction mixture. The solution was stirred overnight at room temperature. After addition of methanol (10 ml) and acetic acid (0.75 ml), the mixture was stirred for one hour and then neutralized with 10% aqueous $NaHCO_3$ solution. The resulting solid was filtered and the filtrate was evaporated by rotary evaporator. The resulted residue was washed with (20 ml×3) distilled water and then dried. The desired product was crystallized from a mixture of chloroform and petroleum ether.

Kinetic study

The synthesized prodrug was subjected to chemical hydrolysis in buffers of physiological pH values, and enzymatic hydrolysis in human serum. These hydrolytic reactions were monitored by double beam UV/Visible spectrophotometer for decreasing in the concentration of prodrug with the time by applying a Beer's law equation, that is:

Absorbance = Ɛ × L × C

Where **Ɛ** is the molar extinction coefficient or called absorbance coefficient.

L is the path length of cell holder (2cm).

C is the concentration.

Stability study in acidic buffer (pH 1.2) and in basic buffer (pH 7.4).

The *in vitro* chemical hydrolysis of the prodrug was studied in (0.1 M) hydrochloric acid buffer (pH 1.2) and in (0.1 M) phosphate buffered saline (pH 7.4). A sample (5µmol) of prodrug was dissolved in 2 ml anhydrous DMSO in a 100ml beaker. To this solution, 48 ml preheated buffer solution was added with gentle stirring to achieve a final concentration of 100 µM. At the end of addition, the time was detected and the resulted solution was kept at a constant temperature (37 ± 1°C) using a water bath. Then, the solution was divided into a set of ten test tubes; each one would contain 5 ml.

At selected time intervals of 30, 60, 90, 120, 150, 180, 210 and 240 minutes, a test tube was removed from a water bath and its content extracted with 2 ml CH_2Cl_2. Aliquot (2 ml) was withdrawn from aqueous layer and estimated at defined λ_{max} on UV/Visible spectrophotometer to determine the remaining concentration of prodrug.

Release study in serum.

The *in vitro* enzymatic hydrolysis of the prodrug was studied in serum; a sample (2.5µmol) of prodrug was dissolved in 2 ml phosphate buffered saline in a 50ml beaker. To this solution, 23 ml preheated serum was added with gentle stirring to achieve a final concentration of 100 µM. At the end of addition, the time was detected and the resulted solution was kept at a constant temperature (37 ± 1°C) using a water bath. Then, the solution was divided into a set of ten test tubes; each one would contain 2.5 ml.

At selected time intervals of 30, 60, 90, 120, 150, 180, 210 and 240 minutes, a test tube was removed from a water bath and its content extracted with 2 ml CH_2Cl_2. Aliquot (2 ml) was withdrawn from aqueous layer and estimated at defined λ_{max} on UV/Visible spectrophotometer to determine the remaining concentration of prodrug.

Results and Discussion

The oral use of 5-FU was forsaken during the past decades because of its irregular absorption[24] and unpredictable plasma level with high intra- and inter-individual variations due to the fickle activity of DPD in the gastrointestinal mucosa[25]. Although the oral use of DCA is well tolerated, but it may cause many gastrointestinal (e.g. nausea, vomiting and heartburn)[26] and central nervous system (e.g. neuropathy, confusion and twitching) related side effects that are highly dose dependent[27].

In an attempt to optimize the drug-like properties of 5-FU and DCA, a novel coumarin-based esterase-sensitive mutual prodrug was designed, synthesized and evaluated depending on *in vitro* stability study in HCl buffer (pH 1.2) and in phosphate buffer (pH 7.4), as well as on *in vitro* release study in human serum.

Synthetic part

The prodrug was synthesized through a sequence of 7 linear steps starting from coumarin; this synthetic pathway (scheme 2) can be considered as a modification to that described by Wang *et al* [28]. The first step involved reduction of coumarin to an open ring diol with lithium aluminum hydride ($LiAlH_4$). Higher temperature than 0°C and/or longer reaction time may lead to reduce the exocyclic double bond whereas the use of commercially available $LiAlH_4$ may lead to lower the yield to high extend.

The following steps involved selective protection of allylic hydroxyl group resulted from the previous step with TBDMS-chloride, and acylation of free phenolic hydroxyl group with dichloroacetyl chloride. When aromatic ester is formed, the TBDMS ether was cleaved under acidic condition to afford allylic hydroxyl group that converted to carboxylic acid group in two steps. Coupling of the free carboxylic acid with 5-FU was carried out by using dicyclohexyl carbodiimide (DCC) as an activating agent.

Table(1): The physicochemical properties of compounds (1-8).

Compound number	Physical appearance	Yield %	M.p. (^{o}C)	R_f chloroform: acetone (4:1)	λ_{max} (nm)e
1	White needle like crystals	-------	68-70	0.696	315
2	White crystals	41	148-151	0.337	286
3	White crystals	76	122-124	0.556	281
4	White needle like crystals	79	86-88	0.601	307
5	White crystals	92	102-105	0.478	304
6	White powder	68	119-122	0.576	316
7	White crystals	89	154-156	0.489	311
prodrug	White needle like crystals	82	136-138	0.589	326

Table (2): The calculated molecular formula, molecular weight and elemental analysis of compounds (1-8).

Comp. No.	Molecular formula	M. Wt.	Elemental analysis						
			%C	*%H*	*%Cl*	*%F*	*%N*	*%O*	*%Si*
1	$C_9H_6O_2$	146.14	73.96	4.14	-----	-----	-----	21.90	-----
2	$C_9H_{10}O_2$	150.17	71.98	6.71	-----	-----	-----	21.31	-----
3	$C_{15}H_{24}O_2Si$	264.44	68.13	9.15	-----	-----	-----	12.10	10.62
4	$C_{17}H_{24}Cl_2O_3Si$	375.36	54.40	6.44	18.89	-----	-----	12.79	7.48
5	$C_{11}H_{10}Cl_2O_3$	216.10	50.60	3.86	27.16	-----	-----	18.38	-----
6	$C_{11}H_8Cl_2O_3$	259.08	50.99	3.11	27.37	-----	-----	18.53	-----
7	$C_{11}H_8Cl_2O_4$	275.08	48.03	2.93	25.78	-----	-----	23.26	-----
Prodrug	$C_{15}H_9Cl_2FN_2O_5$	387.15	46.54	2.34	18.31	4.91	7.24	20.66	-----

The structure of compounds (2-7) was characterized by monitoring the presence and/or absence of specific functional groups using the FTIR spectrophotometer, whereas the prodrug structure was established by analyzing its FTIR, ^{1}H NMR, ^{13}C NMR and mass spectra.

^{1}H-NMR (DMSO-d_6) spectrum of the prodrug (Figure 1) showed the chemical shift of the following protons: δ 11.95 ppm (s, 1H, N***H***), δ 7.97 ppm (d, 1H, -FC=C***H***-N), δ 7.72 ppm (d, 1H, Ar-C***H***), δ 7.30-7.45 ppm (m, 4H, aromatic), δ 6.45 ppm (d, 1H, CH=C***H***-CO), and δ 6.25 ppm (s, 1H, C***H***Cl_2).^{13}C-NMR (DMSO-d_6) spectrum of the prodrug (Figure 2) reported the chemical shift of the following carbons: δ 164.75 ppm (O-***C***O-), δ 160.90 ppm (=CH-***C***O-N), δ 158.08 ppm (CF-***C***O-), δ 154.03 ppm (-***C***-O), δ 150.05 ppm (N-***C***O-NH), δ 143.55 ppm (Ar-***C***H), δ 141.39 ppm (-***C***F), δ 131.88, 127.91, 124.48, 116.93, 116.68 ppm (aromatic), δ 126.09 ppm (FC=***C***H), δ 118.87 ppm (-***C***H-CO-N) and δ 73.53 ppm (-***C***HCl_2).

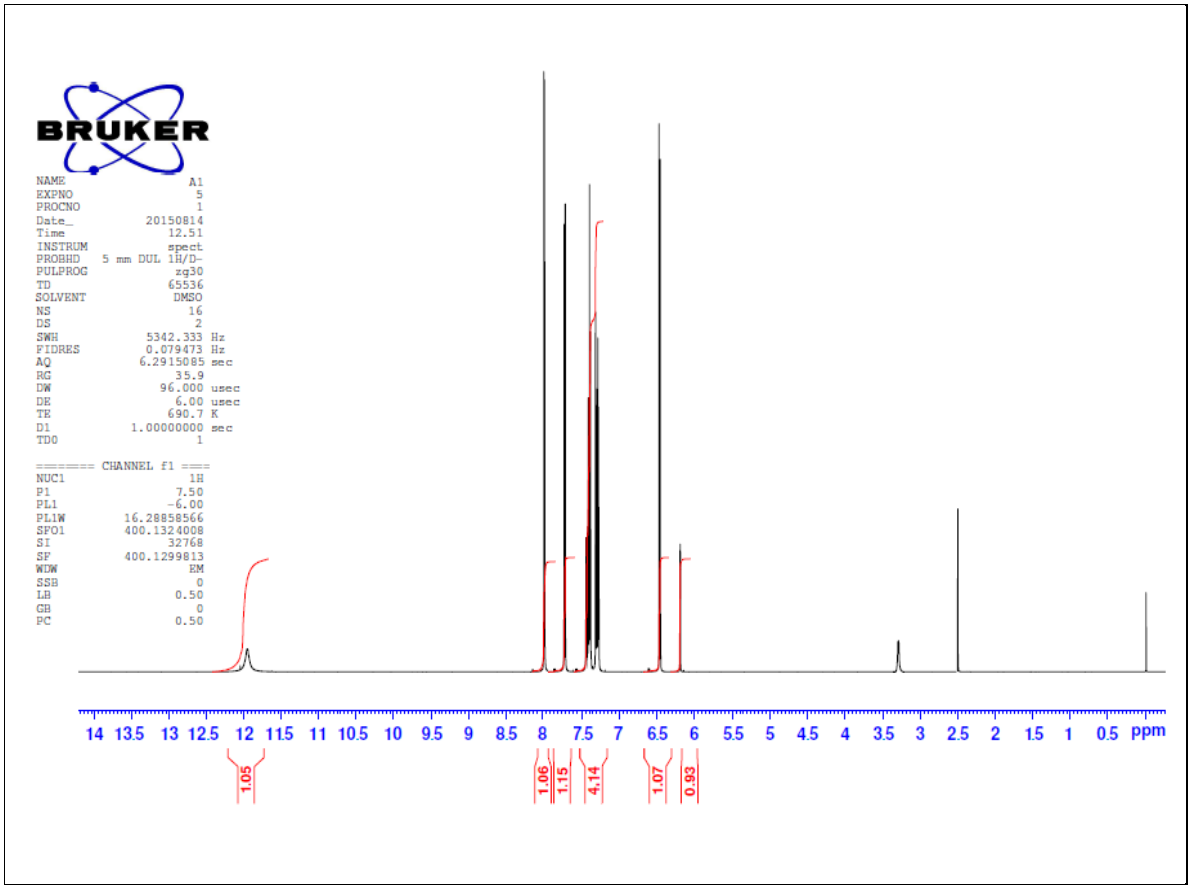


Figure (1): ^{1}H-NMR (DMSO-*d6*) spectrum of the prodrug.

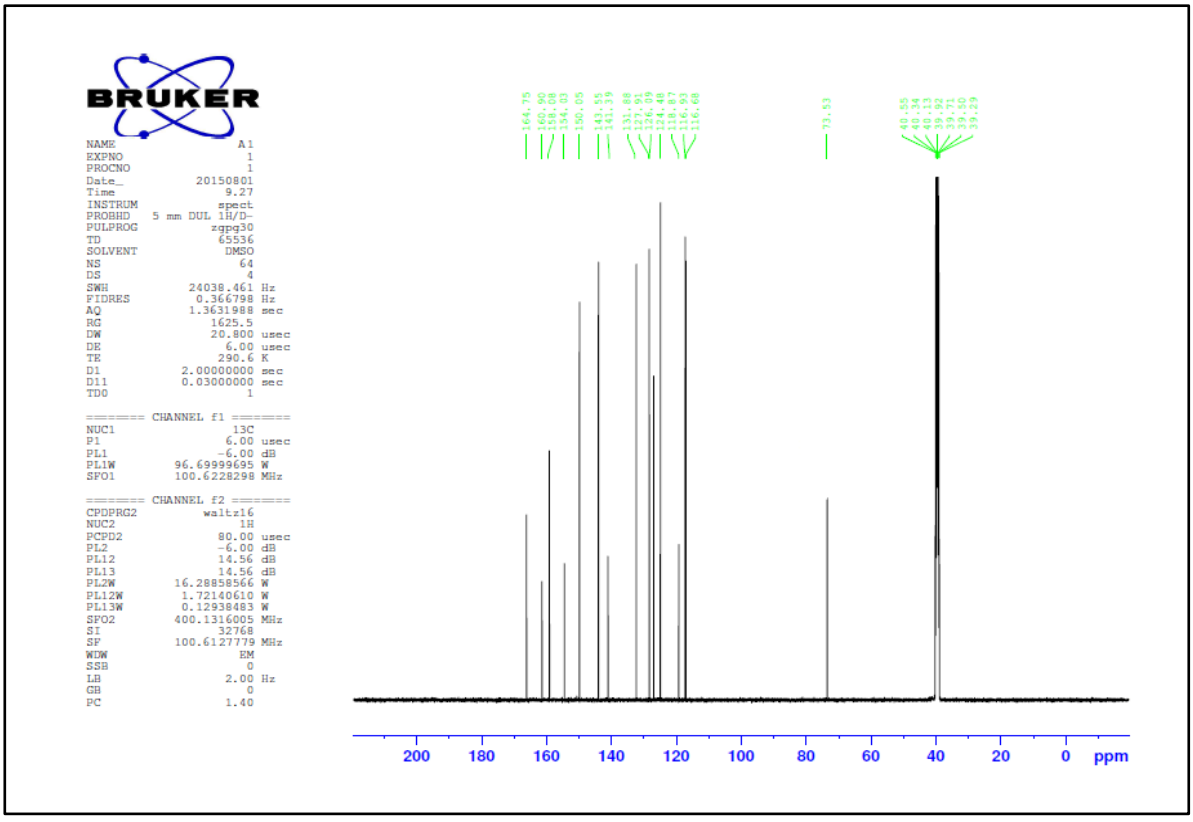


Figure (2): ^{13}C-NMR (DMSO-*d6*) spectrum of the prodrug.

FTIR spectrum of the prodrug revealed the characteristic absorption band for the following functional groups: ν 797 cm^{-1} (C-Cl), ν 1167 cm^{-1} (C-F), ν 1648 cm^{-1} (C=O, 3^{O} amide), ν 1668 cm^{-1} (C=O, 2^{O} amide), ν 1732 cm^{-1} (C=O, ester), ν 2822 cm^{-1} (-CH), ν 3037 cm^{-1} (=CH) and multiple bands for N-H at ν 3114.33, 3153, 3186 cm^{-1}. MS-ESI spectrum (*m/z*) of the prodrug operated in a positive mode (Figure 3) characterized the mass of the following products: 388 $[M+H]^{+}$, 402 [M+Nebulizer gas,CH_3], 410 $[M+Na]^{+}$, 276 [M- O=C=Cl_2], 248 [M-(O=C=Cl_2 & CO)].

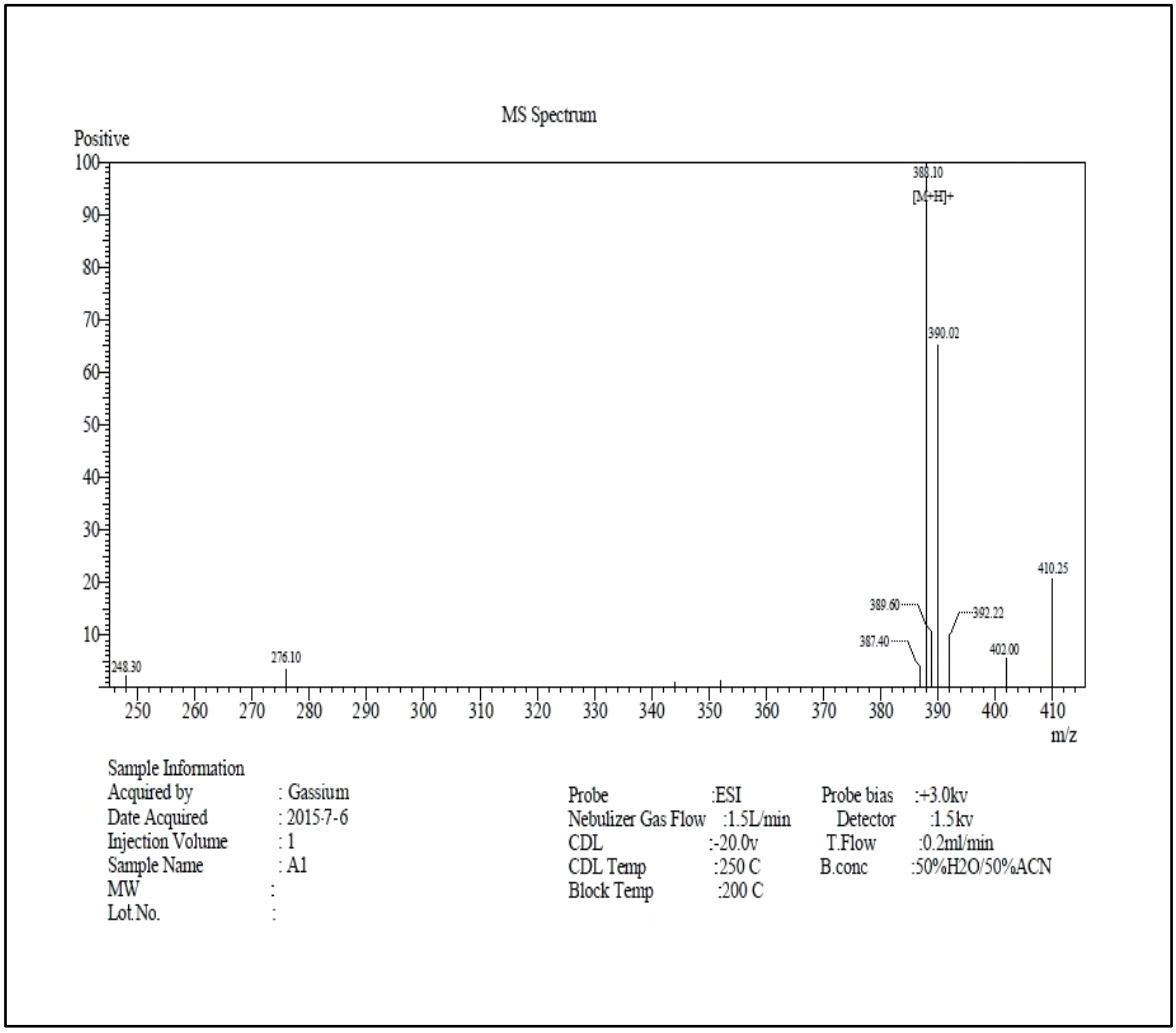


Figure (3): MS-ESI spectrum (*m/z*) of the prodrug operated in a positive mode.

Kinetic part

Under experimental conditions, the stability study in HCl buffer (pH 1.2) and in phosphate buffer (pH 7.4) showed a significant stability of prodrug with half-lives of about 33hr and 18hr respectively. In human serum, the prodrug was liberated 5-FU and DCA followed pseudo first order kinetics (Figure 4) with half-life of about 7hr. Data obtained from the kinetic study (average of three trials) were listed in Table 3, while the kinetic parameters listed in Table 4.

Table (3): Data obtained from kinetic study.

Absorbance	Medium	Time (min.)	x ($M\times10^6$)	a-x ($M\times10^6$)	ln a/a-x
0.0568	pH 1.2	0	0	100.000	0
0.0596	pH 7.4		0	100.000	0
0.0531	Serum		0	100.000	0
0.0562	pH 1.2	30	1.0391	98.9609	0.0104
0.0584	pH 7.4		1.8940	98.0160	0.0200
0.0505	Serum		4.8206	95.1794	0.0494
0.0556	pH 1.2	60	2.0722	97.9278	0.0209
0.0574	pH 7.4		3.7521	96.2479	0.0382
0.0484	Serum		8.8512	91.1488	0.0927
0.0551	pH 1.2	90	3.0282	96.9718	0.0308
0.0561	pH 7.4		5.8725	94.1275	0.0605
0.0458	Serum		13.7848	86.2152	0.1483
0.0544	pH 1.2	120	4.2077	95.7923	0.0430
0.0553	pH 7.4		7.2148	92.7852	0.0749
0.0436	Serum		17.9397	82.0603	0.1977
0.0539	pH 1.2	150	5.1002	94.8998	0.0523
0.0542	pH 7.4		9.1189	90.8811	0.0956
0.0419	Serum		21.0923	78.9077	0.2369
0.0533	pH 1.2	180	6.1620	93.8380	0.0636
0.0533	pH 7.4		10.5705	89.4295	0.1117
0.0391	Serum		26.3653	73.6347	0.3061
0.0528	pH 1.2	210	7.0246	92.9754	0.0728
0.0519	pH 7.4		12.9195	87.0805	0.1383
0.0376	Serum		29.2399	70.7601	0.3459
0.0522	pH 1.2	240	8.0371	91.9629	0.0838
0.0511	pH 7.4		14.1847	85.8153	0.1529
0.0355	Serum		33.1450	66.8550	0.4026

a = conc. of prodrug at zero time and (a-x) = conc. of prodrug remaining for any time.

Table (4): Parameters obtained from kinetic study.

pH1.2	pH 7.4	Serum
$\mathcal{E}$ = 284	$\mathcal{E}$ = 298	$\mathcal{E}$ = 265.5
λ_{max} = 313 nm	λ_{max} = 338 nm	λ_{max} = 332 nm
$t_{1/2}$ = 1991.38 min	$t_{1/2}$ = 1087.91 min	$t_{1/2}$ = 420.80 min
k_{obs} = 0.000348 min^{-1}	k_{obs} = 0.000637 min^{-1}	k_{obs} = 0.001647 min^{-1}

ε = absorbance coefficient and K_{obs} = observed rate constants of hydrolysis.

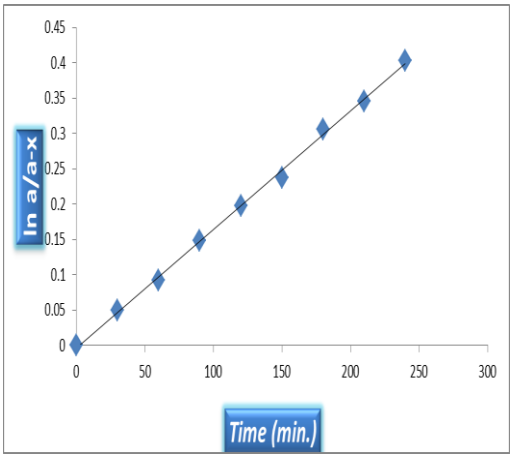


Figure (4): Pseudo first order slope of the *in vitro* hydrolysis of prodrug in human serum.

Conclusion

This work reported the first attempt to utilize a coumarin-based prodrug system to deliver two active moieties which are 5-FU and DCA. Thus, it is believed that the synthesized compound is a first agent belongs to a new prodrug strategy, the coumarin-based mutual prodrug system, and is a promising oral chemotherapeutic agent. Further studies are recommended to establish the result obtained from this work.

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