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Design and Synthesis of azo Derivatives of 5-Fluorouacil for Targeting Colon Cancer

Mohammad Abdul Amir Ulaiwy [1], Mohammed Hassan Mohammed[1]
[1] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq
Corresponding Author; College of Pharmacy, Baghdad University, Baghdad, Iraq.
E-mail: dr.mhassanm666666@yahoo.com

Abstract

5-Fluorouracil (5-FU) is a drug that belong to the antimetabolite class of antineoplastic agents, with a broad spectrum of activity against solid tumors, the use of oral 5-FU was abandoned decades ago because of its irregular absorption and rapid degradation. A potential strategy to improve the selective properties of 5-FU is the chemical transformation into reversible derivatives (prodrugs) which are converted to the parent drug by virtue of enzymatic or chemical means. In the present study, three derivatives of 5-fluorouracil have been designed to be synthesized as an azo derivatives of 5-fluorouracil in order to selectively deliver 5-fluorouracil into the cancer cells of the colon. The synthesis of the target compounds were accomplished following multistep reaction procedures. The chemical reactions were followed up and purity of the products was checked by TLC. The structure of the final compounds and their intermediates were characterized and identified by their melting points, infrared spectroscopy and elemental microanalysis. Compounds 2 and 3 were subjected to a stability study in phosphate buffer (pH 1.5 and 7.8) for different time intervals (0 – 240 min) at 37°C.

Keywords: 5-fluorouracil; colorectal cancer; prodrug approach; Colon- specific drug delivery.

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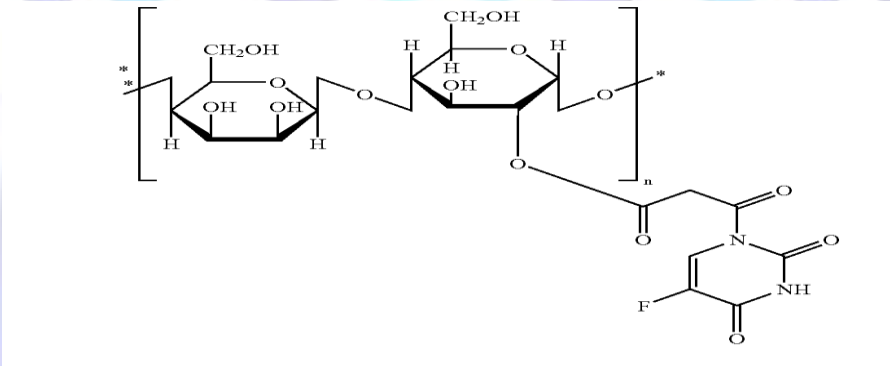
1. Introduction

The aim of most cancer chemotherapeutic drugs currently in clinical use is to kill malignant tumor cells by inhibiting some of the mechanisms implied in cellular division. However, the knowledge of tumor biology has exploded during the past decades and this may pave the way for more active, targeted anticancer drugs [1]. It is obvious that cancer chemotherapy is a very difficult task [2]. One of its main associated problems is the nonspecific toxicity of most anticancer drugs due to their biodistribution throughout the body, which requires the administration of a large total dose to achieve high local concentrations in a tumor. Drug targeting was aimed at preferred drug accumulation in the target cells independently on the method and route of drug administration [3]. One approach that allows improving the selectivity of cytotoxic compounds is the use of prodrugs that are selectively activated in tumor tissues, taking advantage of some unique aspects of tumor physiology, such as selective enzyme expression, hypoxia, and low extracellular pH [4]. Colorectal cancer (CRC) is a type of cancer that starts in the colon or rectum portion of large intestine in the gastrointestinal (GI) tract [5].Occurrence of CRC may be dependent on various factors, namely diet, life style and other environmental parameters, including environmental and food-borne mutagens [6]. It is estimated that chronic inflammation, likely to be caused by a dysregulated intestinal microbiota, contributes to approximately 20% of all CRC cases [7, 8]. 5-Fluorouracil (5-FU) is still a widely used anticancer drug. Since 1957, it has played an important role in the treatment of colon cancer and is used for patients with breast and other cancers, like those of the head and neck [9]. Due to its structure, 5-FU interferes with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell death [10, 11]. .It is administered parenterally because it shows highly variable oral bioavailability due to the unpredictable and incomplete absorption after ingestion of the drug and a marked bioinactivation by dihydropyrimidine dehydrogenase inthe liver and the mucosal membraneof thegastrointestinal tract[12, 13]. Once administrated to a patient, 5-FU tends to distribute randomly through the body with low selectively and concentration in the Lumina of the colorectal tissue, resulted in significant toxicities. If an appropriate large molecular carrier is chemically linked to the 5-FU [1] with specificity for colorectal cancer cells, this will enhance the therapeutic index as well as allow the dosage of the drug to be reduced (if so desired) to minimize toxicities. In addition, with the higher specificity and lower toxicity of such prodrug, the oncologist could also titrate the dosage to the level that a patient could maximally tolerate, thus maximizing the efficacy and the likelihood appositive outcome for the patient. Large molecule carriers include ethylene or acrylic acid polymers, polysaccharides, hydroxyl acid polymers, and amino acid polymers, etc. [14].



[1]

Because of this specific linkage, the 5-FU prodrug cannot be digested or hydrolyzed in the upper gastrointestinal tract and therefore is delivered specifically to the colorectal area. Once arriving at the colorectal area, the 5-FU will be released by hydrolysis of the prodrug by bacterial enzymes in the lower intestinal tract [15]. An association between inflammation and cancer has been suggested for a long time and it is now well-recognized that inflammation is involved in carcinogenesis in several tissues [16]. The development of hepatocellular carcinoma (HCC) caused by hepatitis B virus or hepatitis C virus (HCV) infection , gastric cancer caused by H. pylori infection , and colitis associated cancer caused by ulcerative colitis are representative examples of inflammation-associated carcinogenesis. Various inflammatory mediators are highly expressed in inflammatory tissues, and inflammation-triggered anti-apoptosis activity and cell growth cause cancer development [17]. Inflammatory bowel disease, such as ulcerative colitis and Crohn's disease is a longstanding inflammatory disease of intestine with increased risk for colorectal cancer (CRC). Several molecular events involved in chronic inflammatory process are reported to contribute to multi-step carcinogenesis of CRC in the inflamed colon. They include over-production of free radicals, reactive oxygen and nitrogen species, up-regulation of inflammatory enzymes in arachidonic acid biosynthesis pathway, up-regulation of certain cytokines, and intestinal immune system dysfunction [18].Many compounds belonging to diverse chemical classes have been identified as potential chemopreventive agents, including vitamins and minerals, naturally occurring phytochemicals, and synthetic compounds. Understanding the molecular mechanisms of cancer chemoprevention is not only important for the safe application of these compounds in populations of patients at high risk for cancer, but also allows for further development of novel treatment regimens for cancer patients [19]. The most frequently used chemopreventive agents in UC patients are 5-aminosalicylic acid (5-ASA) compounds (mesalazine and sulfasalazine) as well as ursodeoxycholic acid (UDCA) [20].Mesalazine or 5-aminosalicylic acid (5-ASA) is a drug widely used in IBDs, mainly UC, for the treatment of mild relapses and maintenance of remission. Mesalazine is structurally related to NSAIDs, but unlike these compounds, it is safe and free of serious adverse effects. Epidemiological observations indicate that mesalazine can be also chemopreventive for IBD-associated CRC [21]. Moreover studies conducted in experimental models of carcinogenesis have shown that the drug has many targets in

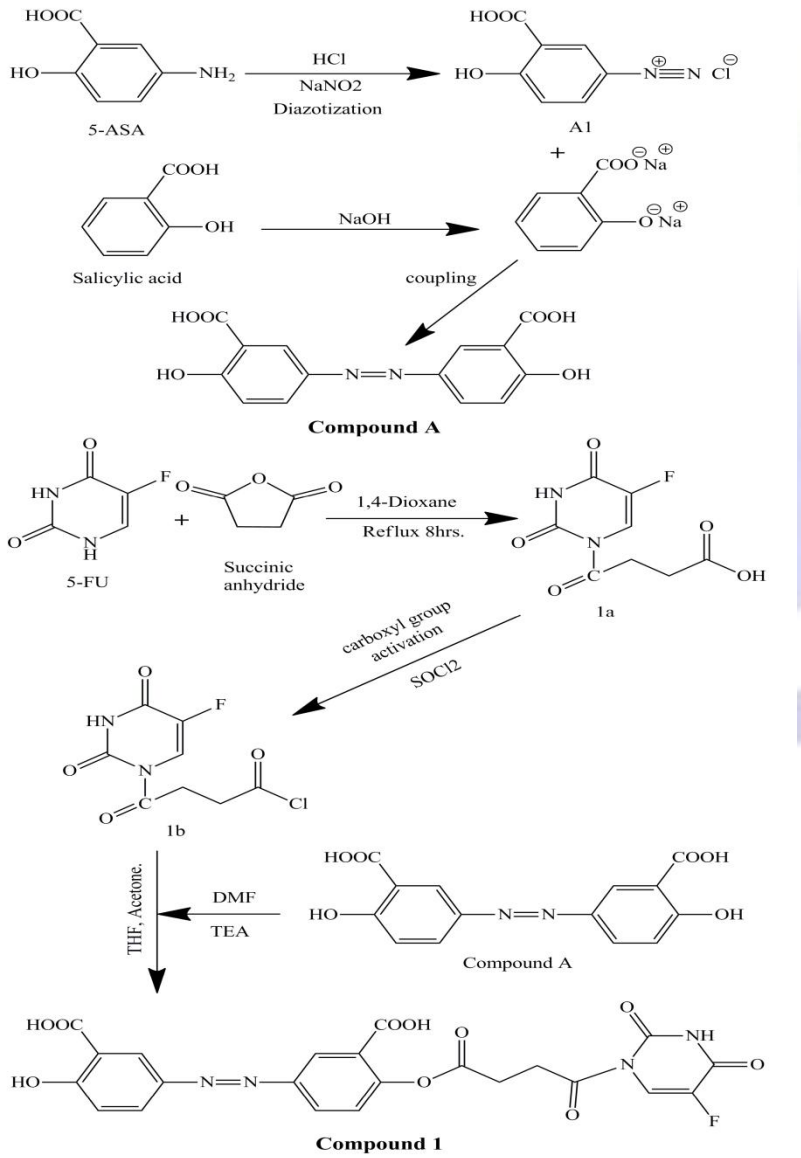
cancer cells and modulates multiple biological pathways that sustain CRC [22]. The colon has recently received great attention as a potential site for the delivery of pharmaceutical moieties. Delivery of drugs via the colon offers numerous therapeutic advantages. The successful delivery of drugs to the colon via the gastrointestinal (GI) tract requires the protection of a drug from being released in the stomach and small intestine. This can be achieved by the use of a special drug delivery system that can protect the drug during its transfer to the colon [23]. Prodrug approach is one of the important approaches for targeting drugs to colon. Colon- specific drug delivery through colon-specific prodrug, activation may be accomplished by the utilization of high activity of certain enzymes at the target site, relative to non-target tissues [24]. In view of these observations, three new derivatives of 5-fluorouracil have been designed, synthesized and characterized.

2. Results and Discussion

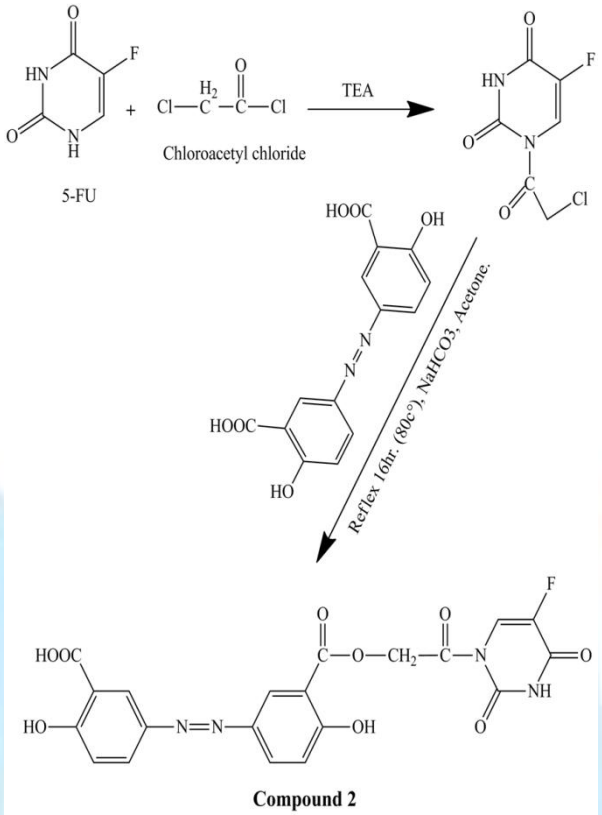
2.1 The chemistry

The designated compounds were synthesized according to schemes (1 to 3). A series of intermediates were synthesized as precursors of the target compounds by several experimental steps. The physical properties of the synthesized compound are showed in table 1. The structure of the final compounds and their intermediates were characterized by elemental microanalysis (table2) and infrared spectroscopy (table

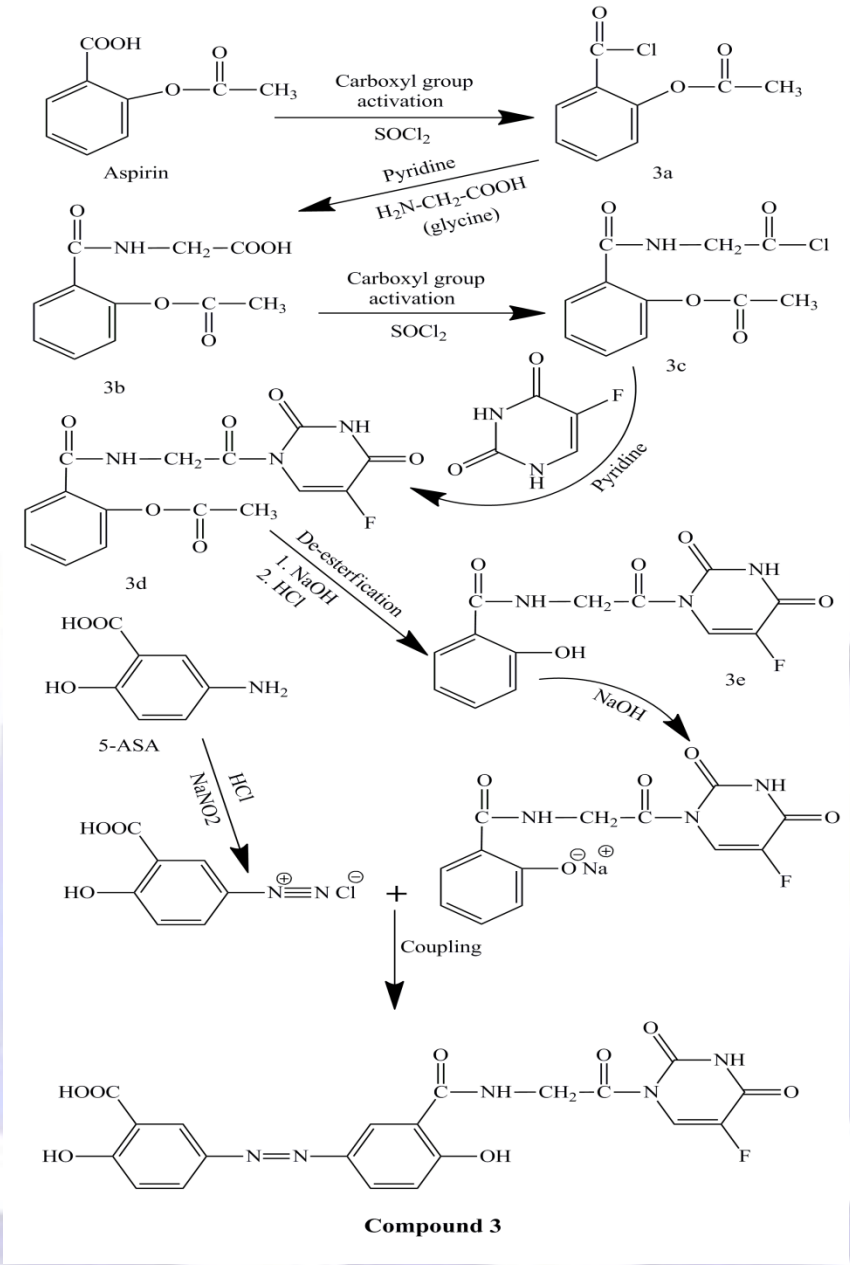
Scheme 1: The synthesis of compound 1.



2: The synthesis of compound 2.



Scheme 3: The synthesis of compound 3.



Table(1): The physical data of the synthesized compounds.

Compound	Physical appearance	%Yield	Melting point (^oC)	R_f value
A	Brown powder	83	146-148	0.37 A* 0.22 B 0.10 C
1a	White powder	28	245-247	0.88 A* 0.56 B
1b	Oily	72	_	_
1	Dark brown powder	63	270 decomposed	0.60 A* 0.53 B 0.357 C
2a	White fluffy powder	73	161-163	0.64 A* 0.58 B
2	Dark red Powder	79	205-207	0.56 A* 0.587 B 0.37 C
3a	Oily	70	_	_
3b	Off white Powder	46	148-150	0.48 A* 0.30 B
3c	Oily	68	_	_
3d	Yellow powder	62	262-265	0.69 A* 0.55 B
3e	White powder	51	285 decomposed	0.58 A* 0.63 B
3	Orange powder	37	178 decomposed	0.20 A* 0.133 B

A*) Chloroform: methanol (9:1)

B) Chloroform: ethyl acetate: methanol (2:2:1).

C) Toluene: ethyl acetate: formic acid (5:4:1).

Table (2): Elemental microanalysis of the final compounds.

Compounds	Chemical formula / Molecular weight	Elemental microanalysis %		
		Element	Calculated	Found
1	$C_{22}H_{15}FN_4O_{10}$ 514.37	C	51.37	52.622
		H	2.94	3.094
		N	10.89	11.233
2	$C_{20}H_{13}FN_4O_9$ 472.34	C	50.86	52.115
		H	2.77	2.911
		N	11.86	12.163
3	$C_{20}H_{14}FN_5O_8$ 471.35	C	50.96	52.187
		H	2.99	3.142
		N	14.86	15.288

Table (3): The characteristic IR bands of synthesized compounds.

Cpd. No.	Characteristic I.R. bands (cm^{-1})
A	(3404, 3240 OH str.* of phenol), (3068 CH ar.* str.), (2600-3034 OH str.of COOH), (1710, 1660 C=O str.of COOH) ,(1587,1512 C=C ar.str.) ,(1483 N=N str.) ,(927,759 CH ar.out of plane).
1a	(3140 NH str.of uracil), (2500-3000 OH str. Of COOH), (3070 CH ar. Str.),(2933&2831 asym.*&sym*.CH_2 str.) ,(1722 C=C-F str. of uracil) ,(1693 C=O str. of COOH) ,(1660 C=O str. of amide) ,(1504 C=C ar.str.) ,(1429 CH bend.of CH_2) ,(1178 C-F str.of uracil).
1	(3240 OHstr.of phenol) ,(3134 NH str. of uracil) ,(3070 CH of benzene) ,(2600-3000 OH str.of COOH) ,(2933,2831 assym.&sym.CH str. of CH_3&CH_2) ,(1772 C=O str. of ester) ,(1720 C=C-F str.of uracil) ,(1670 ,1662 C=O str.of amide) ,(1560 NH bend.) ,(1541 ,1508 C=C ar.str.) ,(1448 N=N str.).
2a	(3148 NH str. of uracil) ,(3073 CH ar.str.) ,(2938,2827 assym. &sym.CH str. of CH_2) ,(1724 C=C-F str. of uracil) ,(1670,1662 C=O str. of amide) ,(1508,1481 C=C ar. Str. overlapped with NH bend. of amide), (1037 C-Cl str.).
2	(3400 OH str. of phenol) ,(3078 NH str. of amide) ,(3051 CH ar. Str.) (2933,2823 assym.&sym.CH str.of CH_2) ,(1714 C=C-F str.of uracil) ,(1683 C=O str.of ester overlapped with C=O str. of COOH) ,(1608 C=O str.of amide) ,(1591 NH bend. f amide) ,(1512,1485 C=C ar.str.) ,(1460 N=N str.)
3b	(3238 NH str. of amide) ,(2500-3000 OH str.of COOH) ,(3061 C=C ar.str.) ,(2918,2862 assym.&sym CH str.of CH_3&CH_2) ,(1747 C=O str. of ester) ,(1658 C=O str. of COOH) ,(1612 C=O str. of amide) ,(1577,1483 C=C ar. str. overlapped with NH bend. Of amide).
3d	(3138 NH str. of amide) ,(3070 CH ar. str.) ,(2931,2831 assym.&sym.CH str. of CH_3&CH_2) ,(1724 C=O str. of ester overlapped with C=C-F str. of uracil) ,(1656 C=O str. of amide) ,(1502,1448 C=C ar.str.&NH bend.of amide) ,(1055 C-F str. of uracil).
3e	(3425 OH str.of phenol) ,(3136 NH str. of amide) ,(3070 C=C ar. str.) ,(2933,2831 assym.&sym. CH str. of CH_2) ,(1722 C=C-F str. of uracil) ,(1672,1660 C=O str.of amide) ,(1506 C=C ar. str.) ,(1089 C-F str. of uracil).
3	(3398 OH str. of phenol) ,(3111 NH str. of amide) ,(3084 CH ar. str.) ,(3034 OH str. of COOH) ,(2948 ,2855 assym.&sym. CHstr.of CH_2) ,(1710 C=O str. of COOH overlapped with C=C-F str. of uracil) ,(1591 C=O str. of amide) ,(1560,1546 C=C ar. str.overlapped with NH bend.of amide) ,(1479 N=N str.) ,(1064 CF str. of uracil).

***(Str. = stretching vibration, ar. = aromatic, assym. = asymmetric, sym.= symmetric)**

2.2.Preliminary hydrolysis study of compounds 2&3 at pH 1.5, pH7.8.

The stability of compounds 2&3 was studied in phosphate buffer solution **(pH 1.5)** and **(pH 7.8)** incubated at 37°C. The rate of hydrolysis was followed spectrophotometrically by recording the decreases in the absorbance of compounds 2&3 accompanying the hydrolysis at their λmax (305nm). The solutions were kept in a water bath at 37°C and samples (3ml) were withdrawn at appropriate time interval (15, 30, 60,120,240 min.) and the absorbances were recorded at the λmax of each compound. The observed pseudo first rate constants were determined from the slopes of the linear plots of log. Concentration versus time. The result are showed in tables (4,5,6 and7) and figures (1, 2,3 and4).

Table (4): The degree of hydrolysis of compound 2 in phosphate buffer (pH 1.5) at different time intervals.

Time (Min.)	Absorbance at 305 nm	Concentration (µg/ml)	Log. concentration
0	0.6062	15.3824	1.1870
15	0.5864	14.8708	1.1723
30	0.5614	14.2248	1.1530
60	0.5275	13.3488	1.1254
120	0.4795	12.1085	1.0830
240	0.4277	10.7700	1.0322

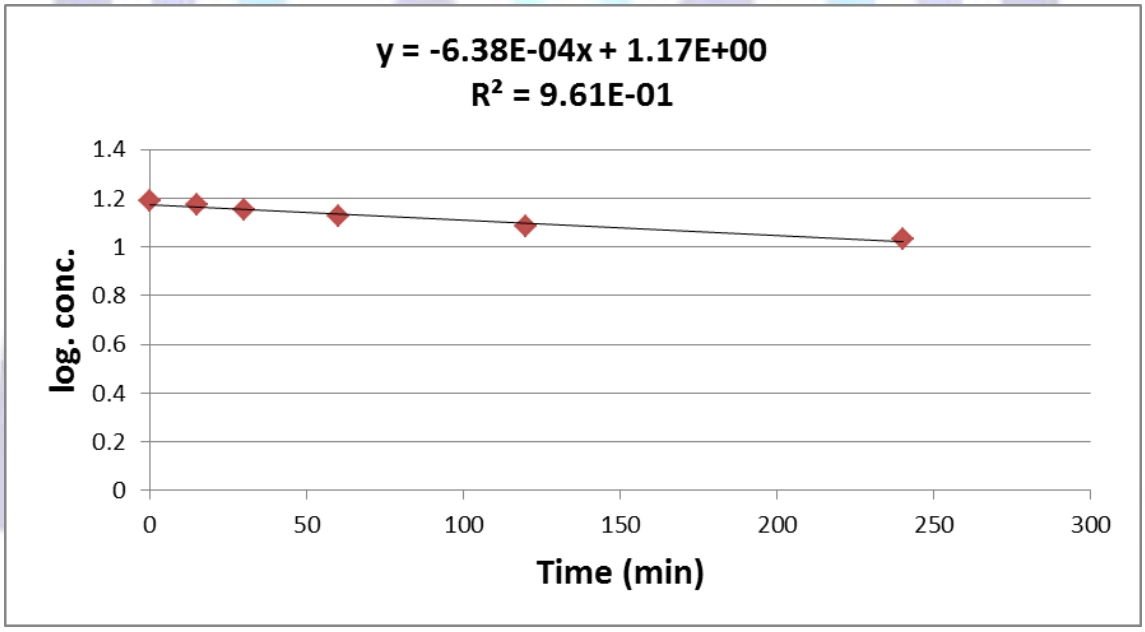


Figure (1). Log concentration of compound 2 incubated in phosphate buffer (pH 1.5) vs. time.

The half-life was calculated using equation (2), which is derived from the pseudo first order kinetic law.

$$t_{1/2} = \frac{0.693}{Kobs} \quad \text{----------eq.2}$$

Therefore; $t_{1/2}$=7.86 hrs.

Table (5): The degree of hydrolysis of compound 2 in phosphatebuffer (pH 7.8) at different time intervals.

Time (Min.)	Absorbance at 305 nm	Concentration (µg/ml)	Log. Concentration
0	0.5988	15.1912	1.1815
15	0.5716	14.4883	1.1610
30	0.5582	14.1421	1.1505
60	0.5011	12.6666	1.1026
120	0.4367	11.0025	1.0415
240	0.3624	9.0826	0.9582

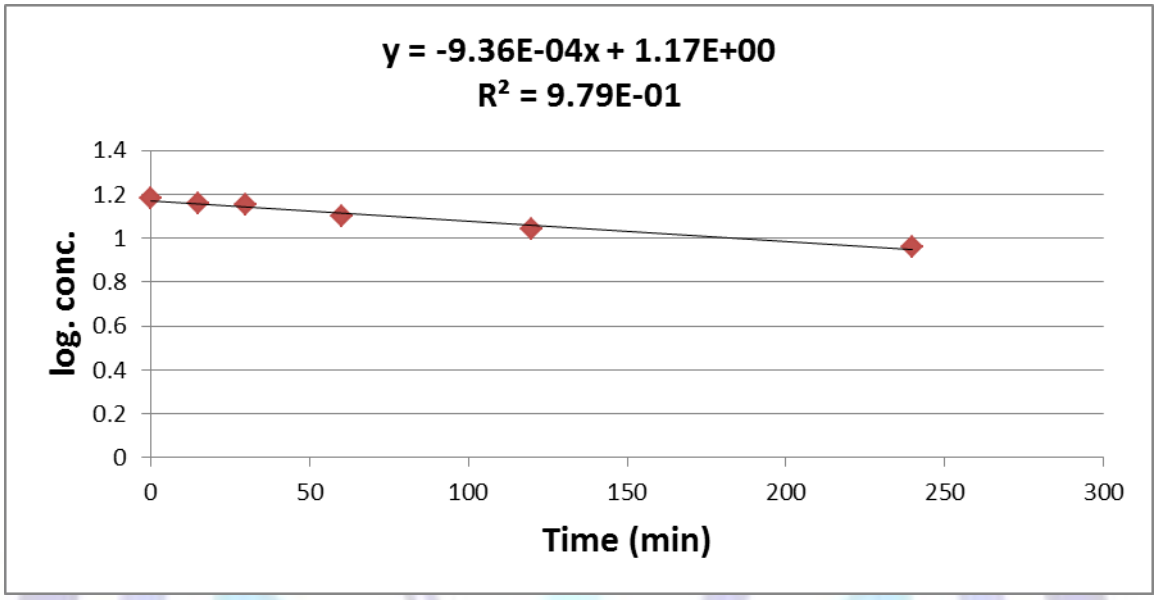


Figure (2): Log concentration of compound 2 incubated in phosphate buffer (pH 7.8) vs. time.

The half-life was calculated using equation (2), which is derived from the pseudo first order kinetic law.

Therefore; $t_{1/2}$=5.35 hrs.

Table (6): The degree of hydrolysis of compound 3 in phosphate buffer (pH 1.5) at different time intervals.

Time (Min.)	Absorbance at 305 nm	Concentration (µg/ml)	Log. Concentration
0	0.5220	15.259	1.1835
15	0.5206	15.218	1.1823
30	0.5125	14.979	1.1754
60	0.5008	14.634	1.1653
120	0.4868	14.221	1.1529
240	0.4577	13.362	1.1258

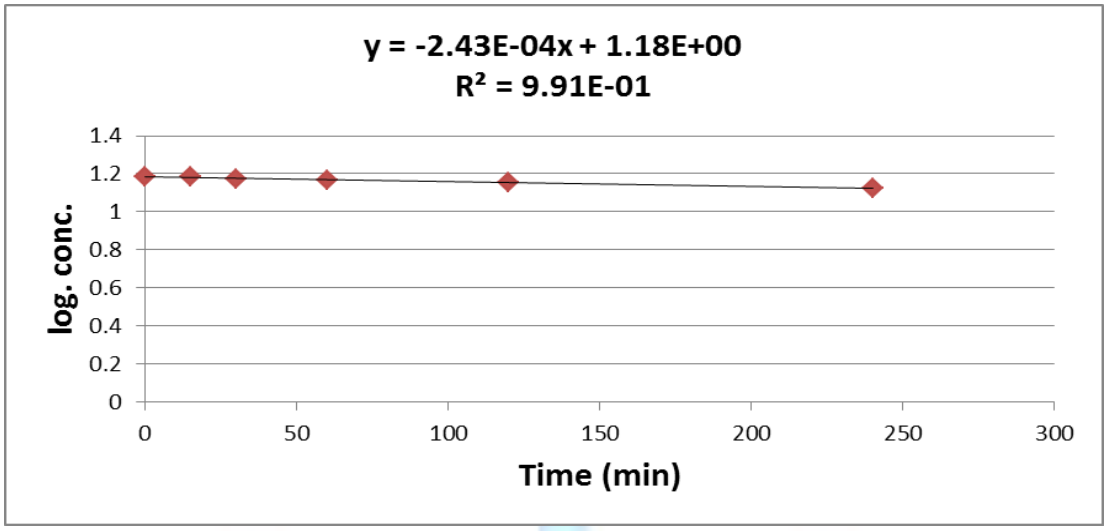


Figure (3): Log concentration of compound 3 incubated in phosphate buffer (pH1.5) vs. time.

The half-life was calculated using equation (2), which is derived from the pseudo first order kinetic law.

Therefore; $t_{1/2}$=20.61 hrs.

Table (7): The degree of hydrolysis of compound 3 in phosphatebuffer (pH 7.8) at different time intervals.

Time (Min.)	Absorbance at 305 nm	Concentration (µg/ml)	Log. Concentration
0	0.4491	13.109	1.1176
15	0.4412	12.876	1.1097
30	0.4326	12.622	1.1011
60	0.4287	12.507	1.0971
120	0.4076	11.884	1.0749
240	0.3873	11.286	1.0525

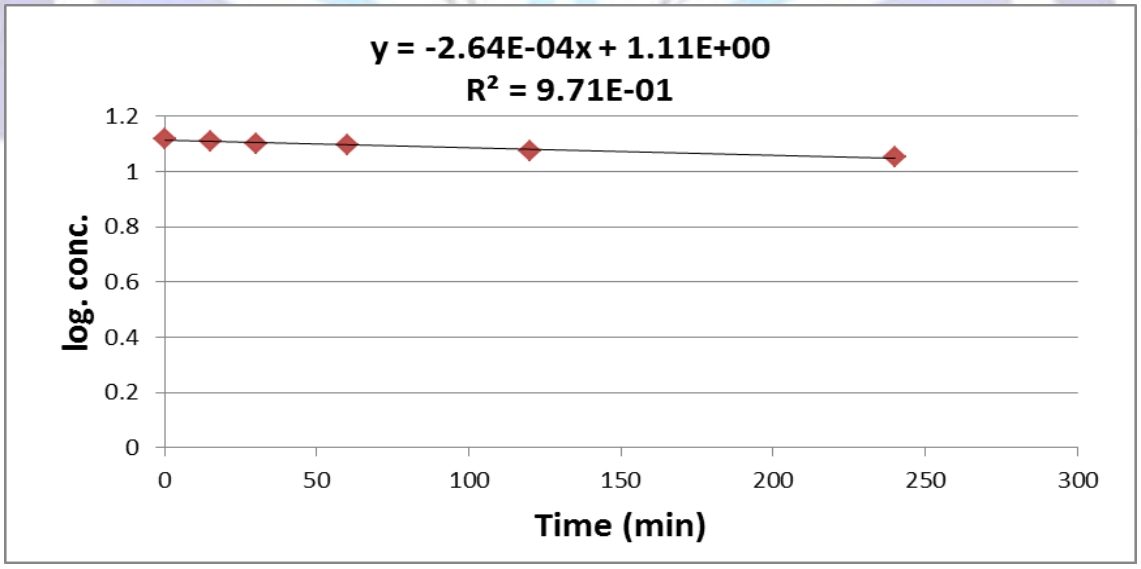


Figure (4): Log concentration of compound 3 incubated in phosphate buffer (pH 7.8) vs. time.

The half-life was calculated using equation (2), which is derived from the pseudo first order kinetic law.

Therefore; $t_{1/2}$=18.97 hrs

3. Experimental

3.1. General

Chemicals used in the synthesis were of analytical grade. The melting points of the compounds and their intermediates (uncorrected) were determined by capillary tube method on Barnstead Electrothermal (USA) and FT-IR spectra were recorded on FT-IR spectrophotometer Shimadzu (Japan) at the college of pharmacy /University of Baghdad. The CHNO analysis was carried out using Euro-vector EA3000A(Italy).Ascending thin layer chromatography(TLC)was run on silica gel 60 F254 pre-coated aluminum sheets, Merck (Germany) to check the purity and the reaction sprogress.The detection of derivatives was done using UV_{254nm} lamp and the chromatograms were eluted by three solvent systems:

A) Chloroform: methanol (9:1) [25].

B) Chloroform: ethyl acetate: methanol (2:2:1).

C) Toluene: ethyl acetate: formic acid (5:4:1).

3.2. Chemical Methods

3.2.1 Synthesis of 3-carboxy-4-hydroxybenzenediazonium chloride (compound A1) [23].

In 500ml conical flask, 5-aminosalicylic acid (3.06g, 20mmol) was dissolved in a mixture of concentrated HCl (20ml), water (50ml) the resulting solution was stirred and cooled in an ice bath to 0C°. Then a cold solution of sodium nitrite (6.9g, 100mmol) in water (15ml) was placed in a dropping funnel and added drop wise into the first solution during 15min.the temperature should not be allowed to rise above 10C°.The last quantity of the sodium nitrite solution was added more slowly and after stirring for 3-4 minutes, a drop of the solution diluted with 4 drops of water was tested with potassium iodide-starch; if no immediate blue color was obtained at point of contact with the paper, a further amount of sodium nitrite solution was added. The testing was continued every 5 minutes until an immediate blue color was obtained. The reaction mixture was further stirred for 20min. in an ice bath at 0-5C°. The diazonium salt formed was used immediately in the next step.

3.2.2 Synthesis of 5, 5'-(diazene-1, 2-diyl)bis(2-hydroxybenzoic acid) (compound A) (Azo bond formation)[23].

Salicylic acid (2.76g, 20mmol) was dissolved in (50ml) of (10%) NaOH solution in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperature was kept below 5C°. The cold diazonium salt solution from the previous step (compound A1) was placed in a dropping funnel, then it was added drop by drop to the cooled, stirred salicylic acid solution, a brown color was developed and a brown crystals soon separated in a good yield, at the end of the addition the mixture was stirred in an ice bath for further 1hr. The product was collected by filtration and washed with water (3x50ml) and dried under vaccum overnight at room temperature.

3.2.3 Synthesis of 4-(5-fluoro-2, 4-dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-4-oxobutanoic acid (Compound 1a) [26].

To a stirring solution of 5-FU (3.9g, 30mmol) in dry dioxane (50ml), succinic anhydride (3g, 30mmol) was added. The mixture was refluxed for 8hours and stirred overnight at room temperature. Solvent was removed; the residue was basified with saturated solution of sodium bicarbonate (30ml), stirred for 15min. and filtered. The filtrate was acidified with concentrated HCl to PH 2 and cooled; a white precipitate produced which was filtered and washed twice with distilled water (50ml). The precipitate was dried to give N-substituted succinamic acid derivative (1a).

3.2.4 Synthesis of4-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-oxobutanoylchloride. (Compound1b).

Compound 1a (1.15g, 5mmol) was suspended in dry chloroform (20ml) in around bottom flask, the mixture was stirred in an ice bath where the temperature should be below 0C°, then thionyl chloride (0.7ml, 10mmol) was added drop wise over a period of 30minutes with continuous stirring. After completing the addition the temperature was allowed to reach room temperature and reflux was then started at about 75-80C° for 3hours. The solvent and the excess thionyl chloride were evaporated by rotary evaporator followed by re-dissolving in chloroform and re-evaporation several times to ensure getting rid of the side products (HCl and SO2 gases), leaving a white oily residue which was used immediately in the next step without further purification.

3.2.5 Synthesis of 5-((3-carboxy-4-((4-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-oxobutanoyl) oxy) phenyl) diazenyl)-2-hydroxybenzoicacid. (Compound1)[27].

Compound 1b (0.9g, 3.62mmol) was dissolved in a mixture (40ml) of dry acetone and THF (2:1) and was added drop wise to compound A (1g, 3.31mmol) which was dissolved in dried DMF (30ml) containing TEA (0.35g, 3.31mmol) with continuous stirring in an ice bath for 3 hours. Stirring was continued at room temperature for further 20 hours. The organic solvents were evaporated under vaccum to give a crude residue which was triturated with acetone several times and then recrystallized from water: acetone (1:5) and was dried in an oven at 40C°.

3.2.6 Synthesis of 1-(2-chloroacetyl)-5-fluoropyrimidine-2, 4(1H, 3H)-dione. (Compound 2a) [28, 29].

A mixture of 5-FU (1.3g, 10mmol) and dry TEA (1.01g, 10mmol) in dry dichloromethane (25ml) was cooled in an ice salt mixture to -10C°. To this reaction mixture, chloroacetyl chloride (0.8ml, 10mmol) in dry chloroform (25ml) was added drop wise through a dropping funnel with constant stirring over a period of 1hour maintaining the temperature constant. The reaction mixture was stirred further for 5hrs. The temperature then was allowed to reach room temperature and reflux was

started at 80C° for 3hours, Solvents were removed and the residue was washed with sodium bicarbonate solution (5% w/v, 30ml) and subsequently with cold water (50ml). The crude product was dried and on recrystallization from ethanol afforded white fluffy powder (compound 2a).

3.2.7 Synthesis of 5-((3-((2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-oxoethoxy)carbonyl)-4-hydroxyphenyl)diazenyl)-2-hydroxybenzoic acid. (Compound 2)[29].

A mixture of the chloroacetylchloride derivative (compound 2a) (2.06g, 10mmol), compound A (3.02g, 10mmol) and sodium bicarbonate (0.84g, 10mmol) in dry acetone (100ml) was refluxed for 15-20hours. After 16hours, the solvent was removed and the residue was triturated with acetone several times then recrystallized from ethanol to give dark red powder (compound 2).

3.2.8 Synthesis of 2-(chlorocabonyl) phenyl acetate. (Compound 3a).

Acetylsalicylic acid (4.5g, 25mmol) was dissolved in dry chloroform (30ml) in around bottom flask; the mixture was stirred in an ice bath where the temperature should be below 0C°. Then thionyl chloride (3.5ml, 50mmol) was added drop wise over a period of 30min. with continuous stirring. After completing the addition the temperature was allowed to reach room temperature and reflux was then started at about 75-80C° for 4hrs. the solvent and the excess thionyl chloride were evaporated with rotary evaporator followed by re-dissolving in chloroform and re-evaporation several times to ensure getting rid of the side products (HCl and SO2 gases) leaving a colorless oily residue (3.48g) which was used immediately in the next step without further purification.

3.2.9 Synthesis of 2-(2-acetoxybenzamido) acetic acid. (Compound 3b) [30].

The whole quantity of compound 3a obtained (3.48g, 17.5mmol) was dissolved in dry chloroform (50ml) and added drop wise to a stirred solution of glycine (1.3g, 17.5mmol) in dry pyridine (50ml) with continuous stirring at ice bath. After the addition was complete, stirring was continued at room temperature for further 3hours. The reaction mixture was then poured into cold water (200ml) followed by addition of few drops of concentrated HCl. The precipitate was collected by filtration, washed with D.W (50ml x2) and recrystallized from ethanol.

3.2.10 Synthesis of 2-((2-chloro-2-oxoethyl) carbamoyl) phenyl acetate.(Compound 3c).

Compound (3b) (2.37g, 10mmol) was dissolved in dry chloroform (30ml) in around bottom flask; the mixture was stirred in an ice bath where the temperature should be below 0C°. Then thionyl chloride (1.4ml, 20mmol) was added drop wise over a period of 30min. with continuous stirring. After completing the addition the temperature was allowed to reach room temperature and then reflux was started at about 75-80C° for 4hrs. the solvent and the excess thionyl chloride were evaporated with rotary evaporator followed by re-dissolving in chloroform and re-evaporation several times to ensure getting rid of the side products (HCl and SO2 gases) leaving a yellowish oily residue (1.73g) which was used immediately in the next step without further purification.

3.2.11 Synthesis of 2-((2-(5-fluoro-2,4-dioxo-3,4-dihydrpyrimidin-1(2H)-yl)-2-oxoethyl) carbamoyl) phenyl acetate. (Compound 3d).

The whole quantity of compound (3c) obtained (1.73g, 6.8mmol) was dissolved in dry chloroform (50ml) and added drop wise to a stirred solution of 5-FU (0.88g, 6.8mmol) in dry pyridine (50ml) with continuous stirring at ice bath. After the addition was complete, stirring was continued at room temperature for further 1hr. and reflux was then started at about 75-80C° for 3hours, Solvents was evaporated and the obtained residue was triturated with petroleum ether (40-60) several times and recrystallized from ethanol and dried to give yellow powder (compound 3d).

3.2.12 Synthesis of N-(2-(5-fluoro-2, 4-dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-2-oxoethyl)-2hydroxybenzamide. (Compound 3e) [31].

To a stirring solution of compound (3d) (1.75g, 5mmol) in a mixture of absolute ethanol : DMF (4:1), kept on 18-22C°, an aqueous solution of 1N NaOH (12.5ml) was added drop wise over a period of 30min. where the stirred reaction mixture was continued for additional 5hrs. then the solvents evaporated, and the residue was dissolved in water and acidified with 1N HCl to get PH 1. After cooling a white precipitate was appeared which was separated by filtration, washed on the filter paper with water and recrystallized from ethanol.

Synthesis of compound 3.

This compound was synthesized by following procedures similar to the procedures followed in the synthesis of compound A.

Synthesis of Compound A1

Compound A1 has been synthesized for preparing compound A and it was prepared again since it was needed in in the synthesis of compound 3.

In 500ml conical flask, 5-ASA (0.77g, 5mmol) was dissolved in a mixture of conc. HCl (5ml) and water (12.5ml). The resulting solution was stirred and cooled in an ice bath to 0C°. Then a cold solution of sodium nitrite (1.73g, 25mmol) in water (10ml) was placed in a dropping funnel and added drop wise into the first solution during 15min. The last quantity of the sodium nitrite solution was added more slowly and after stirring for 3-4 minutes, a drop of the solution diluted with 4 drops of water was tested with potassium iodide-starch; if no immediate blue color was obtained at point of contact with

the paper, a further amount of sodium nitrite solution was added. The testing was continued every 5 minutes until an immediate blue color was obtained. After completing the addition of sodium nitrite solution, the reaction mixture was further stirred for 20min. in an ice bath at 0-5C°. The diazonium salt formed was used immediately in the next step.

3.2.13 Synthesis of 5-((3-((2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-oxoethyl)carbamoyl)-4-hydroxyphenyl)diazenyl)-2-hydroxybenzoic acid. (Compound 3) (Azo bond formation).

Compound (3e) (1.53g, 5mmol) was dissolved in (12.5ml) of 10% NaOH solution in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperature was kept below 5C°. The cold diazonium salt solution from the previous step (compound A1) was placed in a dropping funnel, then it was added drop by drop to the cooled, stirred solution of compound (3e), an orange color was developed and orange crystals soon separated. At the end of the addition the mixture was stirred in an ice bath for further 1 hour. The product was collected by filtration and washed with water (3x50ml) and dried at room temperature overnight.

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