Synthesis of 5-Fluorouracil Derivatives as Possible Mutual Prodrugs with Meloxicam and Ibuprofen for Targeting Cancer Tissues

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Abstract

In the present study, five derivatives have been designed to be synthesized as possible mutual prodrugs for 5-Fluorouracil (5-FU) and non steroidal anti-inflammatory drugs (NSAIDs) to selectively deliver the drugs into the cancer cells. The synthesis of the target compounds were accomplished following multistep reaction procedures, the chemical reaction followed up and the purity of the products were checked by TLC. The structure of the final compounds and their intermediates were confirmed by their melting points, infrared spectroscopy and elemental microanalysis, the hydrolysis of compound III was studied using HPLC technique. According to the results mentioned above, compounds (I–V) can be good candidates as possible mutual prodrugs of 5-FU and NSAIDs that can selectively deliver the parent drugs into the cancer cells by the effect of enzymes that elevated in tumor tissues compared with normal tissues.

Key wards: Anticancer, 5-Fluorouracil, NSAIDs, prodrug.

الخلاصة

تم في هذه الدراسة تصميم خمسة مشتقات لتخليقها كمقدمات دوائية ثنائية محتملة للـ (٥- فلورويوراسيل) والادوية المضادة للالتهابات غيرالستيرويدية لايصال الادوية وتحريرها بأنتقائية في الخلايا السرطانية. تم تحضير هذه المركبات باتباع طريقة التفاعل متعدد الخطوات، وتم مراقبة جميع التفاعلات والتأكد من نقاوة المركبات بواسطة كروماتوغرافيا الطبقة الرقيقة، كما تم متابعة المركبات الوسطية والمركبات النهائية والتأكد من تحضيرها من خلال قياس درجات الانصهار والتحليل الطيفي للاشعة تحت الحمراء، والتحليل الدقيق للعناصر. وقد تم دراسة تحلل المركب(III) بأستخدام تقنية الاستشراب السائل عالي الاداء وفقاً للنتائج المبينة اعلاه، يتضح بأن المركبات(I–V) مرشحات كمقدمات دوائية ثنائية محتملة للـ (٥- فلورويوراسيل) والادوية المضادة للالتهابات غيرالستيرويدية لها القدرة على ايصال الادوية بأنتقائية للخلايا السرطانية وبألية تحرير تتضمن تأثير الانزيمات الموجودة بمستوى اعلى في الانسجة السرطانية مقارنة مع الانسجة الطبيعية.

Introduction

5-FU is an antimetabolite of the pyrimidine analogue type, with a broad spectrum of activity against solid tumors of the gastrointestinal tract, pancreas, ovary, liver, brain, breast, etc. [(1)] 5-FU has an unpredictable gastrointestinal absorption and rapid degradation. [(2)] NSAIDs are useful drugs for alleviating pain, fever and inflammation, [(3)] NSAIDs have attracted considerable attention as a new type of antitumor drug. [(4, 5)] Tumor inhibition by NSAIDs may be mediated by distinct cellular processes, these processes involve the ability of NSAIDs to restore apoptosis, induce cell-cycle arrest and inhibit angiogenesis[(6)]. Prodrugs are bioreversible derivatives of drug molecules that undergo an enzymatic and/or chemical transformation in vivo to release the active parent drug. [(7)] Strategies to improve the oral bioavailability and achieve tumor-specific targeting have been the most important developments in prodrug design during the last 5 years [(8)]. Phosphoramidate strategy has been applied to NSAIDs to synthesize novel NSAID derivatives as potential prodrugs for anticancer therapy or chemopreventive applications with less toxic side effects. [(9)] The purpose of this study was to design and synthesize of new compounds (I, II, III, IV, and V) as possible mutual prodrugs for targeting cancer tissue characterized by elevated levels of certain enzymes.

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Materials and Methods

5-FU sodium salt was purchased from EBEWE pharma (Avusturya); Ibuprofen and Meloxicam were purchased from Alsafa Company (Iraq); Phosphorus oxychloride and 2-Chloroethanol were purchased from Fluka (Switzerland); 4-Nitrophenol was purchased from Searle (England); 2-Phenyl ethyl amine was purchased from Riedel-Dehaen (Seelze-Hannover) Germany; Glutathione was purchased from Sigma (USA). All chemicals were reagent grade and obtained from standard commercial sources. Elemental microanalysis were performed using CHN analyzer 1106 Carlo Erba (Italy); Melting points (uncorrected) were measured on Thomas Hoover Electronic melting point apparatus; and are uncorrected; Infra red spectra were recorded as KBr disks on F.T.IR Spectrophotometer (College of Science, University of Al-Mustanseriya); and HPLC analyzer (College of pharmacy, University of Baghdad); Chiller JulaboVC (F30) GMBH (Germany).

Synthesis of compound Ia: *(10)*

To a stirred solution of phosphorus oxychloride (3.88 gm, 25.31 mmol) in dry chloroform (20 ml) at −20 ^{o}C, a solution of 2-phenylethyl amine (3.067 gm, 25.31 mmol) in dry chloroform (20 ml) was added drop wise, then a solution of dry triethylamine (5.12 gm, 50.6 mmol) in dry chloroform (20 ml) was added drop wise to the reaction mixture with continuous stirring at the same temperature. The reaction mixture was stirred for 3.5 hours letting it warm up gradually to 10 ^{o}C, quenched with saturated ammonium chloride solution and the chloroform layer was extracted with distilled water (2 × 20 ml), the chloroform layer was dried over anhydrous sodium sulphate and filtered and the chloroform was evaporated under vacuum to give compound Ia as oily material, the percent yield is (98.60%).

Synthesis of compound Ib: *(11, 12)*

To a stirred solution of compound Ia (1.33 gm, 5.589 mmol) in freshly distilled acetonitrile (20 ml) at −20 ^{o}C, a suspension of sodium 5-fluoro-6-oxo-1,6-dihydropyrimidin-2-olate (0.85 gm, 5.589 mmol) in freshly distilled acetonitrile (20 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature. The mixture was filtered and the solvent of the filtrate was evaporated under vacuum to give compound Ib as oily material with percent yield (34.53%).

Synthesis of compound I: *(11, 12)*

To a stirred solution of compound Ib (0.64 gm, 1.93 mmol) in dry chloroform (20 ml) at −20 ^{o}C, a suspension of (4-hydroxy-2-methyl-N- (5-methyl -1, 3thiazoiyl) benzo1, 2 thiazine -3-carboxamide-1,1-dioxide) (0.678 gm, 1.93 mmol) in dry chloroform (20 ml) and dry pyridine (2 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature. The reaction mixture was filtered and the yellow precipitate was collected and recrystalized from ethanol-petroleum ether to give pale yellow powder of compound I, with percent yield (38.14%), melting point (221^{o}C dec.). The IR data and CHN analysis were listed in tables 1 and 2 respectively.

Synthesis of compound II: *(11, 12)*

To a stirred solution of compound Ib (0.529 gm, 1.595 mmol) in dry chloroform (20 ml) at −20 ^{o}C, a suspension of sodium (S)-2-(4-isobutylphenyl)propanoate (0.364 gm, 1.595 mmol) in dry chloroform (20 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature. The reaction mixture extracted with distilled water (2 ×20 ml), the chloroform layer was dried with anhydrous sodium sulphate and filtered, the chloroform was evaporated under vacuum to yield an oily compound, and the oil residue was triturated several times with petroleum spirit. The obtained solid compound was recrystalized from ethanol-petroleum ether to give white crystals of compound II. The percent yield is (35.25%), melting point 128-130 ^{o}C. The IR data and CHN analysis were listed in tables 1 and 2 respectively.

Synthesis of compound IIIa: *(11, 12)*

To a stirred solution of phosphorus oxychloride (1.903 gm, 12.413 mmol) in freshly distilled acetonitrile (20 ml) at −20 ^{o}C, a suspension of sodium 4-nitrophenolate (2 gm, 12.413 mmol) in freshly distilled acetonitrile (20 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature, then the mixture was filtered and the solvent of the filtrate was evaporated under vacuum to give compound IIIa as oily material, the percent yield is (95.34%).

Synthesis of compound IIIb: *(11, 12)*

To a stirred solution of compound IIIa (2.242 gm, 8.759 mmol) in dry chloroform (20 ml) at −20 ^{o}C, a suspension of sodium (S)-2-(4-isobutylphenyl)propanoate (2 gm, 8.759 mmol) in dry chloroform (20 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature. The mixture washed with distilled water (2× 20 ml), the chloroform layer was dried with anhydrous sodium sulphate and filtered; the chloroform was evaporated under vacuum to give compound IIIb as oily material with percent yield (68.43%).

Synthesis of compound III: [11, 12]

To a stirred solution of compound IIIb (2 gm, 4.697 mmol) in freshly distilled acetonitrile (20 ml) at −20 °C, a suspension of sodium 5-fluoro-6-oxo-1,6-dihydropyrimidin-2-olate (0.714 gm, 4.697 mmol) in freshly distilled acetonitrile (20 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature. The mixture was filtered and the solvent of the filtrate was evaporated under vacuum to yield an oily compound. The oily residue was triturated several times with petroleum spirit. The obtained solid compound was recrystalized from ethanol-petroleum ether to give yellow powder of compound III with percent yield (80.07%), melting point 86-88 °C. The IR data and CHN analysis were listed in tables 1 and 2 respectively.

Synthesis of compound IVa: [11, 12]

To a stirred solution of compound IIIa (1.456 gm, 5.691 mmol) in dry chloroform (20 ml) at −20 °C, a suspension of (4-hydroxy-2-methyl-N-(5-methyl-1,3thiazoiyl)benzo1,2thiazine-3-carboxamide-1,1-dioxide) (2 gm, 5.691 mmol) in dry chloroform (20 ml) and dry pyridine (2 ml) was added drop wise, then the same procedure of compound IIIb was carried out to give compound IVa as oily material, percent yield (38.17%).

Synthesis of compound IV: [11, 12]

To a stirred suspension of compound IVa (0.534 gm, 0.935 mmol) in freshly distilled acetonitrile (20 ml) at −20 °C, a suspension of sodium 5-fluoro-6-oxo-1,6-dihydropyrimidin-2-olate (0.142 gm, 0.935 mmol) in freshly distilled acetonitrile (20 ml) was added drop wise, then the same procedure of synthesis of compound III was carried out to give pale orange powder of compound IV. Percent yield (67.88%), melting point 144-146°C. The IR data and CHN analysis were listed in tables 1 and 2 respectively.

Synthesis of compound Va: [13]

A solution of 2-chloroethanol (0.949 gm, 11.867 mmol) in (5 ml) ethanol was added to a solution of glutathione potassium salt (5 gm, 11.867 mmol) in (15 ml) ethanol and the reaction mixture was heated under reflux for 2 hours, allowed to cool, then acidified with 10% HCL to pH 4. Evaporation of the solvent to afford an oily residue which was triturated with diethyl ether to give white powder of compound Va, percent yield (33.27%), melting point (140 °C dec.).

Synthesis of compound Vb: [14]

To a solution of compound Va (1.9 gm, 5.413 mmol) dissolved in (50 ml) glacial acetic acid, a solution of 30 % hydrogen peroxide (20 ml, 0.176 mol) was added. The mixture was left at room temperature for 12 hours and then evaporated to yield an oily residue. The oily residue was triturated several times with acetone to give white powder of compound Vb. Percent yield (56.05%), melting point (90 °C dec.).

Synthesis of compound Vc: [11, 12]

To a stirred solution of phosphorus oxychloride (0.662 gm, 4.317 mmol) in dry chloroform (20 ml) at −20 °C, a suspension of (4-hydroxy-2-methyl-N-(5-methyl-1,3thiazoiyl)benzo1,2thiazine-3-carboxamide-1,1-dioxide) (1.517 gm, 4.317 mmol) in dry chloroform (20 ml) and dry pyridine (2 ml) was added drop wise, then the reaction mixture was stirred over night at room temperature. Then the mixture washed with distilled water (2 × 20 ml), the chloroform layer was dried with anhydrous sodium sulphate and filtered, the chloroform was evaporated under vacuum to give compound Vc as oily material. Percent yield (32.28%).

Synthesis of compound Vd: [11, 12]

To a stirred solution of compound Vc (0.451 gm, 0.963 mmol) in freshly distilled acetonitrile (20 ml) at −20 °C, a suspension of sodium 5-fluoro-6-oxo-1,6-dihydropyrimidin-2-olate (0.146 gm, 0.963 mmol) in freshly distilled acetonitrile (20 ml) was added drop wise, then the same procedure of compound III was carried out to give compound Vd as oily material. Percent yield (55.80%).

Synthesis of compound V: [11, 12]

To a stirred solution of compound Vd (0.28 gm, 0.498 mmol) in dry chloroform (20 ml) at −20 °C, a suspension of compound Vb (0.190 gm, 0.498 mmol) in dry chloroform (20 ml) was added drop wise, then a solution of triethylamine (0.0504 gm, 0.498 mmol) in dry chloroform(20 ml) was added drop wise into the reaction mixture with continuous stirring at the same temperature, then the same procedure of compound II was carried out to give brown crystals of compound V. Percent yield (40.42%), melting point 124-126 °C. The IR data and CHN analysis were listed in tables 1 and 2 respectively.

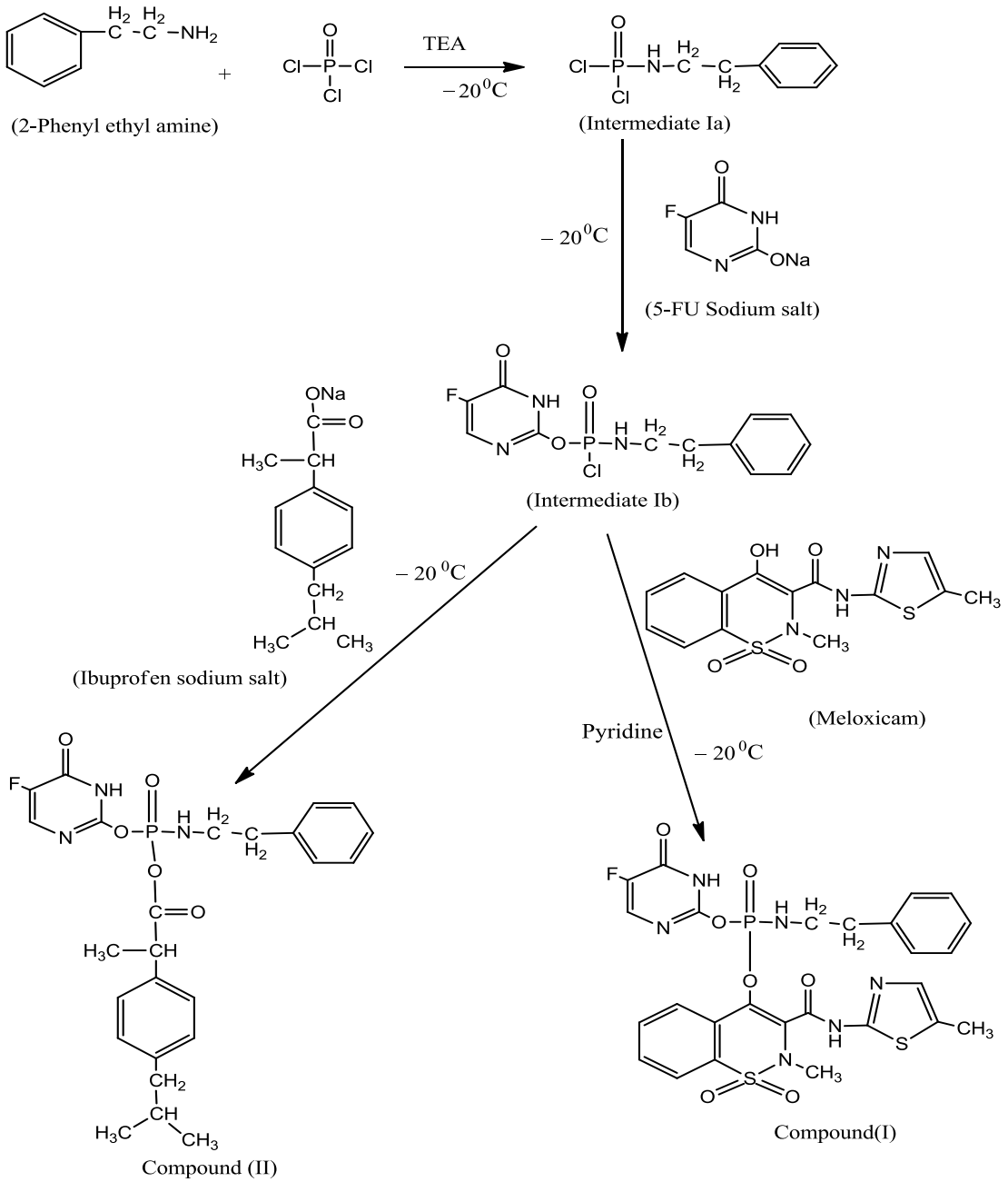
Preliminary hydrolysis of nitro containing compounds

This study was achieved using Zorbax Extend C_{18} column (150mm× 4.6mm, 5µm), with mobile phase consisting of methanol-phosphate buffer pH 6 (20:80 v⁄v), flow rate of 1ml ⁄ min, and column temperature of 30 °C. UV detection was performed at 230 nm [15]. The standard material used was p-aminophenol, a stock solution (10mg⁄10ml) in methanol-phosphate buffer pH 6 (20:80 v⁄v) was prepared, take 0.1ml of stock solution

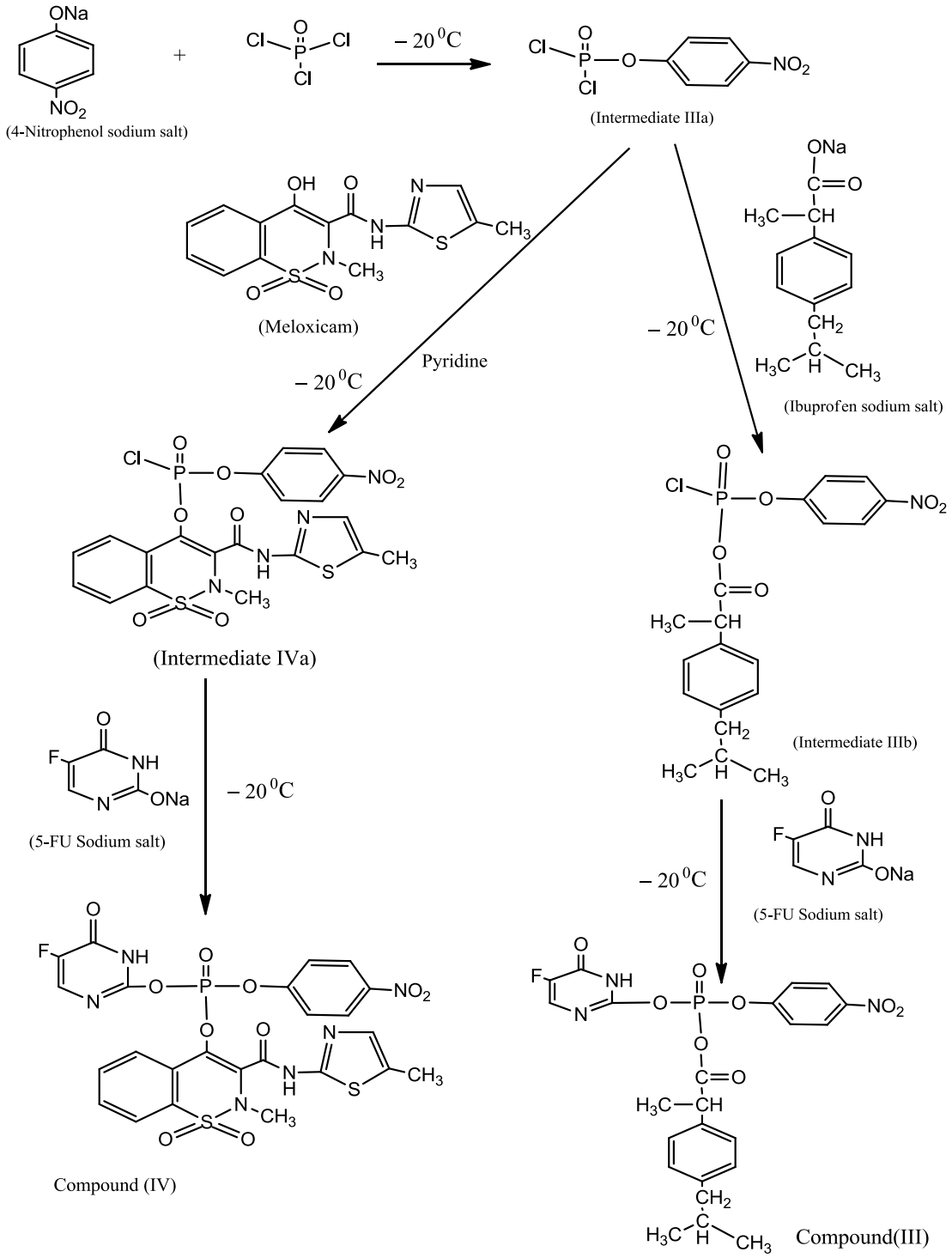
diluted to 10 ml of methanol-phosphate buffer pH 6 (20:80 v⁄v)(0.917µmol, 0.1mg), to be used as such for the hydrolysis procedure.The sample used was compound III (containing nitro phenyl group) and sodium dithionite was added to it as reducing agent, a stock solution of compound III(10mg ⁄ 10ml) in methanol-phosphate buffer pH 6 (20:80 v⁄v) was prepared, take 0.47ml of stock solution diluted to 10 ml of methanol-phosphate buffer pH 6 (20:80 v⁄v) (0.917µmol, 0.47mg). A stock solution of sodium dithionite (10mg ⁄ 10ml) in methanol-phosphate buffer pH 6 (20:80 v⁄v) was prepared, take 0.17ml of stock solution diluted to 10 ml of methanol-phosphate buffer pH 6 (20:80 v⁄v) (0.917µmol, 0.17mg). The mixture of diluted stock solutions of compound III and sodium dithionite was heated on a water bath at 37 ^{o}C for 1 hour [16], to be used as such for the hydrolysis procedure.

Results and Discussion

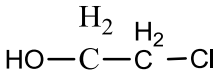
The synthetic pathways for the designed target compounds (I− V) are illustrated in (schemes 1, 2, and 3), while the IR data and CHN analysis were listed in table 1 and 2 respectively.



Scheme (1): Chemical synthesis of compounds I&II.

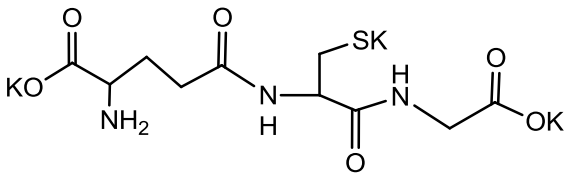


Scheme (2): Chemical synthesis of compounds III&IV.



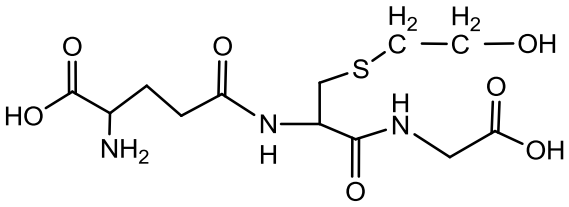
(2-Chloroethanol)

+



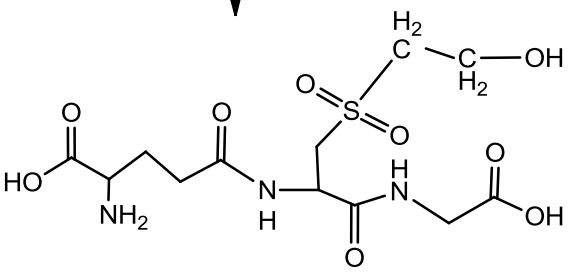
(Glutathione potassium salt)

Reflux

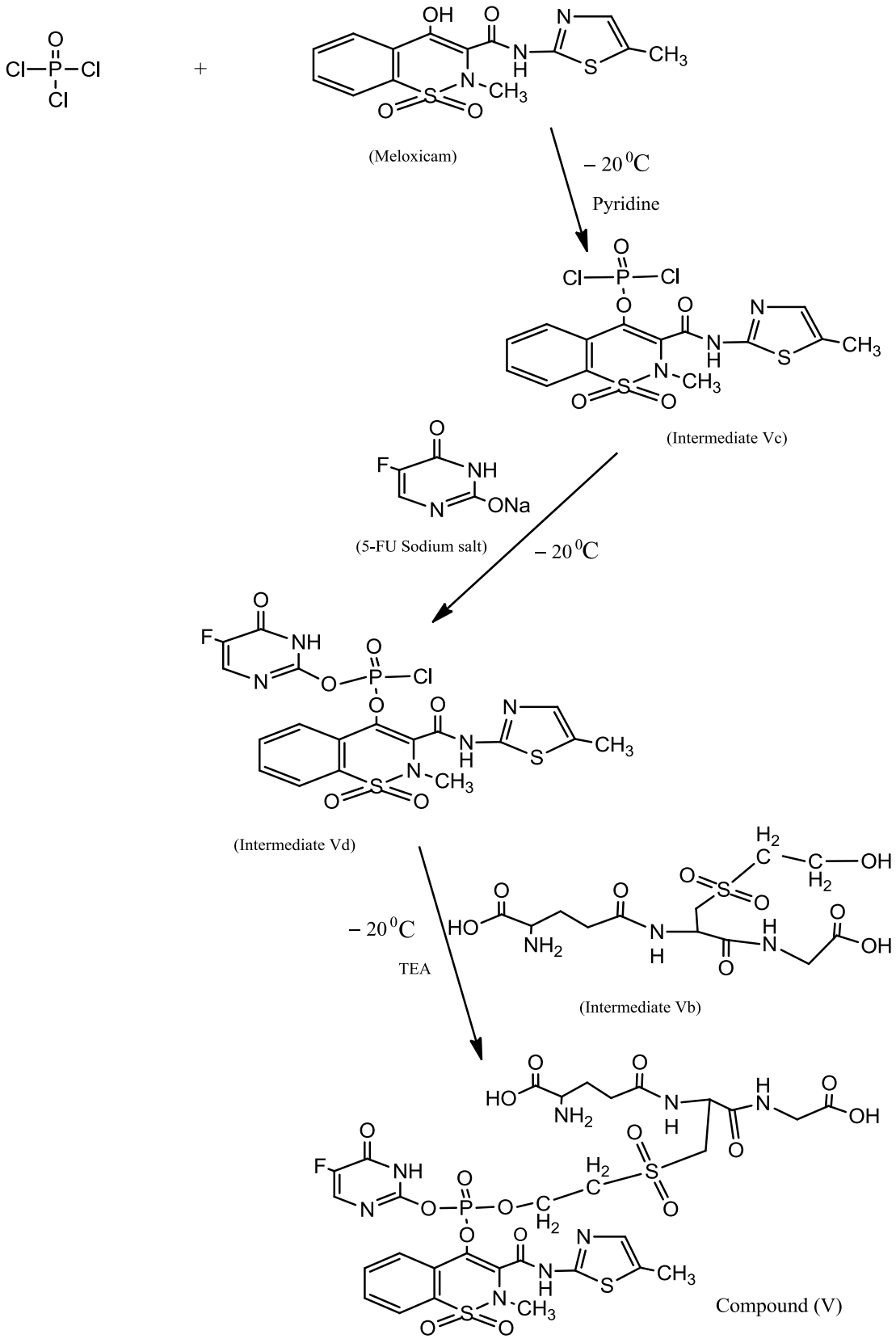


(Intermediate Va)

H_2O_2



(Intermediate Vb)



Scheme (3): Chemical synthesis of compound V.

Table 1 : The IR characteristic bands of the final compounds.

Compounds	Bands(cm^{-1})and Their Interpretation
I	2931, 2831 (C−H) for CH_2 stretching, 1660 (C=O of 2^o amide), 1620 (C=C of benzene), 1346 &1163 (asymmetrical & symmetrical stretching vibration of sulfone), 1267 (P=O stretching), 1064 (single C−F stretching), 941 (P−O stretching vibration of P−O−C aryl).
II	2955 & 2870 (C−H) of CH_3 stretching, 2925 & 2870 (C−H) of CH_2 stretching, 1651 (C=O stretching of 2^o amide), 1631, 1492 (C=C of benzene), 1384&1365 (gem dimethyl bending), 1249 (P=O stretching), 1064 (single C−F stretching), 881 (P−O stretching vibration of P−O−C aryl).
III	2893 (gem dimethyl stretching vibration), 1681 (C=O stretching of 2^o amide), 1581&1492 (C=C of benzene), 1581&1340 (asymmetric and symmetric stretching of nitro group), 1400, 1380 (gem dimethyl bending), 1300 (P=O stretching), 1114 (single C−F stretching vibration).
IV	1678 (C=O of 2^o amide), 1595 (C=C of benzene), 1554 (Amide II band), 1529&1330 (asymmetrical & symmetrical stretching vibration of nitro group), 1330&1170 (asymmetrical & symmetrical stretching of sulfone group), 1296 (P=O stretching), 1066 (single C−F stretching), 941 (P−O stretching vibration of P−O−C aryl).
V	3431 (NH stretching vibration of 2^o amide), 3000-2500 (OH stretching vibration of COOH), 2983, 2922 & 2850 (C−H) of CH_3 &CH_2 stretching vibration, 1629 (C=O stretching of 2^o amide), 1600, 1498 (C=C of benzene), 1554 (amide II band), 1440 (OH bending of acid), 1352 & 1182 (asymmetrical & symmetrical stretching of sulfone), 1246 (P=O stretching), 1240 (C−O stretching of acid),1066 (single C−F stretching), 923 (P−O stretching vibration of P−O−C aryl).

Table 2 : The elemental microanalysis of the final compounds.

Compound	Elemental microanalysis%		
	Element	**Calculated**	**found**
I	C	48.29	48.689
	H	3.74	3.901
	N	13.00	13.349
	O	17.32	17.798
	S	9.92	10.569
II	C	59.88	60.491
	H	5.83	5.903
	N	8.38	8.799
	O	15.95	16.496
	S	–	–
III	C	53.18	53.678
	H	4.46	4.590
	N	8.09	8.419
	O	24.64	25.090
	S	–	–
IV	C	43.38	43.791
	H	2.73	2.811
	N	12.65	13.010
	O	24.08	24.599
	S	9.65	10.271
V	C	39.65	39.900
	H	3.77	3.890
	N	12.33	12.790
	O	28.17	28.667
	S	10.58	11.010

The proposed mechanism to account for substitution processes occurring at the phosphoryl phosphorus atom for synthesis of compounds I, II, III and IV and their intermediates via pentavalent intermediates.The reactions were preceded through nucleophilic substitution mechanism which involves the formation of a true penta-coordinate intermediate generally thought to have trigonal bipyramidal geometry, and constructed by apical approach of the attacking nucleophile with departure of the displaced group at the opposite apex. (17) The first step in the synthesis of compound V (synthesis of intermediate Va) include the reaction of potassium salt of glutathione with 2-chloroethanol to form thioether (sulfide) through nucleophilic substitution reaction. (18) The second step in the synthesis of compound V (synthesis of intermediate Vb) includes oxidation of thioether with hydrogen peroxide to afford sulfone compound. (18) Finally, the synthesis of intermediates Vc, Vd, and compound V follow the same mechanism that shown for synthesis of compound I, II, III, and IV.

HPLC analysis

Qualitative identifications were made by using HPLC technique; this was done by comparison of retention times obtained at identical chromatographic conditions of compound III containing sodium dithionite (the sample) and p-aminophenol (the standard). The informations obtained from HPLC method of analysis reveal that retention time for p-aminophenol was 2.1 minute for its peak to appear in the chart of HPLC analysis. The nitro group in compound III was reduced to amino group by using sodium dithionite as reducing agent (in vitro) which lead to release of p-aminophenol from compound III. This was proved by using HPLC analysis in which the same retention time was appeared in the chart (2.1 minute) of compound III which indicate that the p-aminophenol was released from compound III as shown in figure 1.

Expected enzymatic hydrolysis of the compounds:

The expected enzymatic hydrolysis of compounds I and II in the body include addition of hydroxyl group to the para position of the phenyl moiety due to the effect of cytochrome P450 enzymes, [19] then tyrosinase enzyme [20] catalyze the addition of another hydroxyl group and the oxidation of these phenolic substrates into the corresponding o-quinone, which rearrange into o-diol followed by hydrolysis, thereby releasing the active drug. Enzymatic hydrolysis of compounds III and IV include reduction of the nitro group that was performed using sodium dithionite as previously described [16] then rearrangement reaction, followed by hydrolysis, there by releasing the active drug. Finally, the expected enzymatic hydrolysis of compound V include activation by tyrosine -7 from the GST enzyme that abstracts a proton from compound V, resulting in a β-elimination reaction, subsequently generating the active drug.[21]

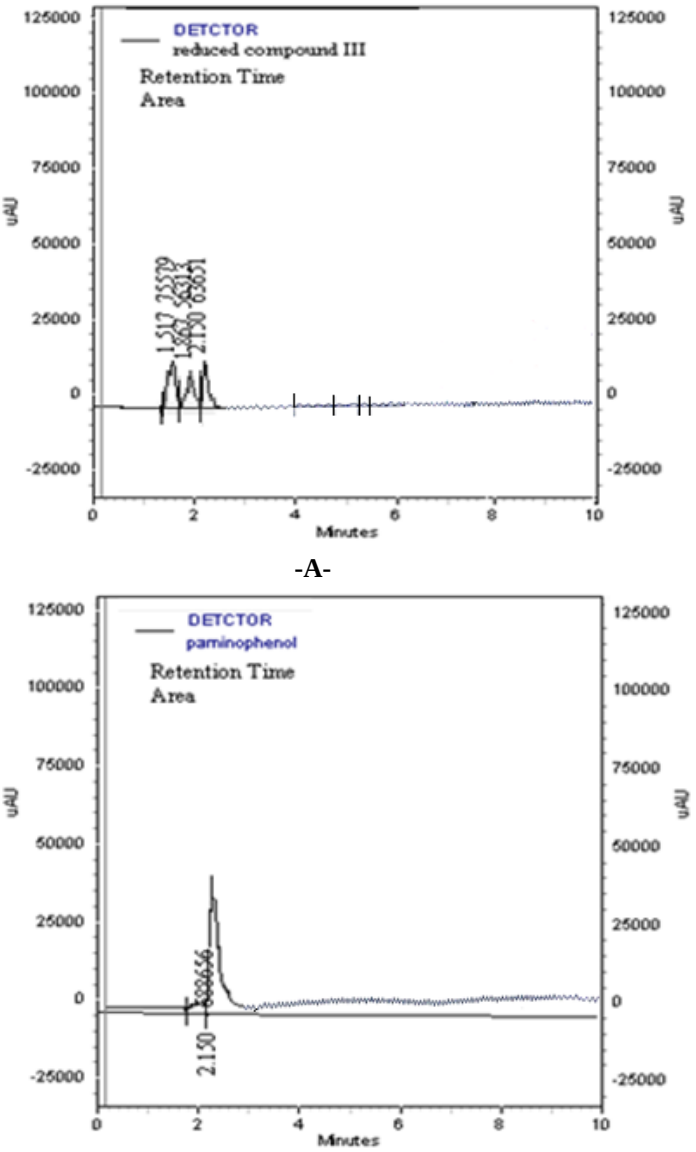


Figure 1: HPLC analysis of reduced compound III (A) and p-aminophenol standard (B).

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