

## DESIGN AND SYNTHESIS OF DICLOFENAC AND INDOMETHACIN – CONJUGATES WITH GEMCITABINE AS A POSSIBLE MUTUAL PRODRUGS

**Dhurgham Q. Shaheed, Ahmed Kareem Hussein Mubarak\*, Akeel Abdulkadium  
Jassim Aboalard, Hayder Kadhium Abbas, Mohammed Dakhil ALRekabi, and  
Ahmed Hashim Hussein**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Kufa,  
Najaf, Iraq.

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**\*Correspondence for  
Author**

**Dr. Ahmed Kareem  
Hussein Mubarak**

Department of  
Pharmaceutical  
Chemistry, Faculty of  
Pharmacy, University of  
Kufa, Najaf, Iraq.

### ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed classes of drugs and their long-term regimens have been greatly shortened due to their gastrointestinal side effects. COX-2 plays a pivotal role throughout oncogenesis and here we exploit the rationale to explore the use of NSAID (Diclofenac and indomethacin) in combination with current anticancer drug (Gemcitabine) for the prevention and/or treatment of cancer. The study describes design and synthesis of mutual pro-drug of NSAID and gemcitabine, which is designated to generate the complementary pharmacological action as a single chemical entity with improved drug targeting. The synthesis is conferred by FT-IR, CHNS and physiochemical properties. The newly designed mutual pro-drug is

expected to reduce the side effects of NSAIDs on the gastrointestinal (GI) tract with improvement of the oral bioavailability and targeting of gemcitabine.

**KEYWORDS:** Diclofenac, indomethacin, gemcitabine, drug targeting, mutual prodrug.

### INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed classes of drugs throughout the world. The overall worldwide production of about 50,000 tons a year reflects the importance of this substance even today.<sup>[1]</sup> NSAIDs are used extensively to alleviate inflammation, pain, rheumatoid arthritis, and osteoarthritis. Long-

term regimens of NSAIDs have been greatly shortened due to their gastrointestinal side effects.<sup>[2]</sup> The pharmacological activity of NSAIDs is related to their ability to inhibit the activity of the enzyme cyclooxygenases (COXs), involved in the biosynthesis of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>). It is well known that COX exists in two isoforms, namely COX-I and COX-II, which are regulated differently.<sup>[3]</sup> COX-I is constitutively expressed in stomach to provide cytoprotection in the GIT.<sup>[4]</sup> COX-II is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells.<sup>[5]</sup> Targeting of inflammatory states with non-steroidal anti-inflammatory drugs (NSAID'S) is an attractive proposition for cancer prevention. There is abundant epidemiological and experimental evidence that NSAID'S can inhibit tumor development in a number of organs and such drugs have given positive results in human intervention studies.<sup>[6]</sup>

In addition to the well-established pathophysiological role of COX-2 in inflammation, recent evidence implies that this isoform may also be involved in multiple biologic events throughout the tumorigenic process. Many epidemiological studies demonstrate that non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of a wide range of tumors.<sup>[7]</sup> Interestingly, substantial experimental clinical evidence indicates a role for NSAIDs in the prevention of various types of cancer, especially when combined with chemotherapy.<sup>[8, 9]</sup> NSAIDs may also be associated with reduced risk of cancers of bladder, breast, esophagus, lung, ovary, prostate, stomach, liver, pancreas, tongue and glioblastoma multiform.<sup>[10]</sup> NSAIDs may have attractive effects other than analgesic and anti-inflammatory effect, these include: the novel NSAID derivatives as potential pro-drugs for anticancer therapy or chemo preventive applications with less toxic side effects have been synthesized. It has been proved that phosphoramidate derivatives of fenoprofen, ketoprofen, ibuprofen, indomethacine and diclofenac possess significantly higher anti-proliferative activities.<sup>[11, 12]</sup>

The main disadvantage associated with cancer treatment is the development of drug resistance by the cancer cells. A cancer that is not totally eliminated by administration of a single drug will, sooner or later, become resistant to that drug. Resistance to chemotherapeutics and molecularly targeted drugs can result from one of two general causes: host factors and specific genetic or epigenetic alterations in the cancer cells which include poor absorption, rapid metabolism, excretion of a drug, resulting in low serum levels and poor tolerance to effects of a drug.<sup>[13]</sup> The design of cancer chemotherapy has become increasingly sophisticated, yet there is no cancer treatment that is 100% effective against

disseminated cancer. Entry of a mutual pro-drug into the cancerous tissues exposes both the sensitive and the resistant cells. As with any combination strategy, the two drugs simultaneously target the cells separately and thereby augment their anticancer activity.<sup>[14]</sup> Nowadays anticancer, cardiovascular, antiviral, antipsychotic antioxidant, anti-inflammatory and antibacterial drugs are best utilizing the concept of mutual pro-drug designing for their better effect.

## MATERIALS AND METHODS

### Materials

All reagents and anhydrous solvents were of analytical grade type and used as received from the commercial suppliers (Merck, Germany; Reidel De-Haen, Germany; Sigma-Aldrich, Germany and BDH, England). Diclofenac and indomethacin was supplied by the SDI Company, Iraq.

### Conversion of Diclofenac sodium to free Diclofenac

The procedure was performed as described earlier.<sup>[15]</sup> In short, 2 g of Diclofenac sodium (6.3 mmol) was dissolved in 20 ml absolute ethanol with stirring for 10 min at room temp. Then 3 ml of HCl (2N) was added wisely with stirring for 1 hr. Excess cold water was added to solution and the precipitate of free diclofenac was filtered off and dried in oven at 60 °C.

### Synthesis of Diclofenac–Gemcitabine and Indomethacin-Gemcitabine conjugate

500 mg of Diclofenac acid (Ia) (2 mmol) or 715 mg of Indomethacin (Ib) (2 mmol) was dissolved in 20 ml of chloroform followed by addition of 412 mg of *N,N'*-Dicyclohexylcarbodiimide DCC (2 mmol). The reaction mixture was stirred at room temperature for 1 hour (solution A). 600 mg of Gemcitabine Hcl (2mmol) and 20 mg of DMAP were dissolved in 30 ml DMF (solution B). Then solution A and B were mixed and stirred for 48 hour at room temp. The precipitate of *N,N'*-Dicyclohexylurea DCU was filtered off and solvent of filtrate was removed under reduced pressure. Cold water (90 ml) was added to the obtained residue and the ppt was collected and recrystallized from absolute ethanol.

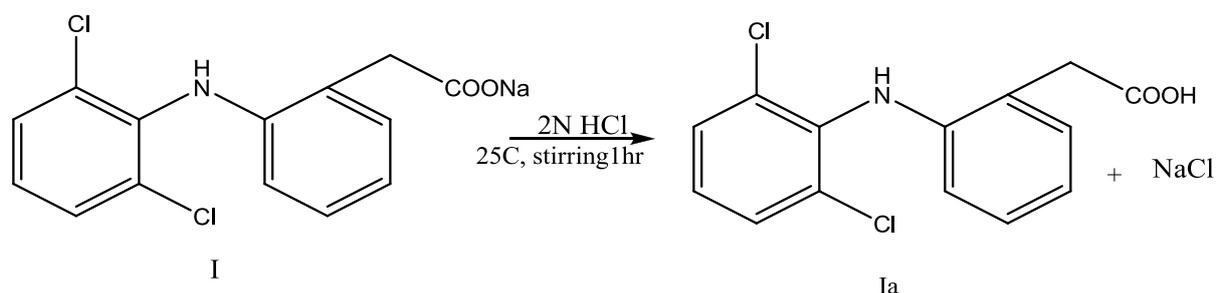
### Analysis of compound

Melting points were determined by capillary method on Electrical melting point apparatus SMP30 Stuart, England. To check the purity and progress of reactions, ascending thin layer chromatography (TLC) was run on DC-Kartan SI alumina (0.2 mm) plates. The identification

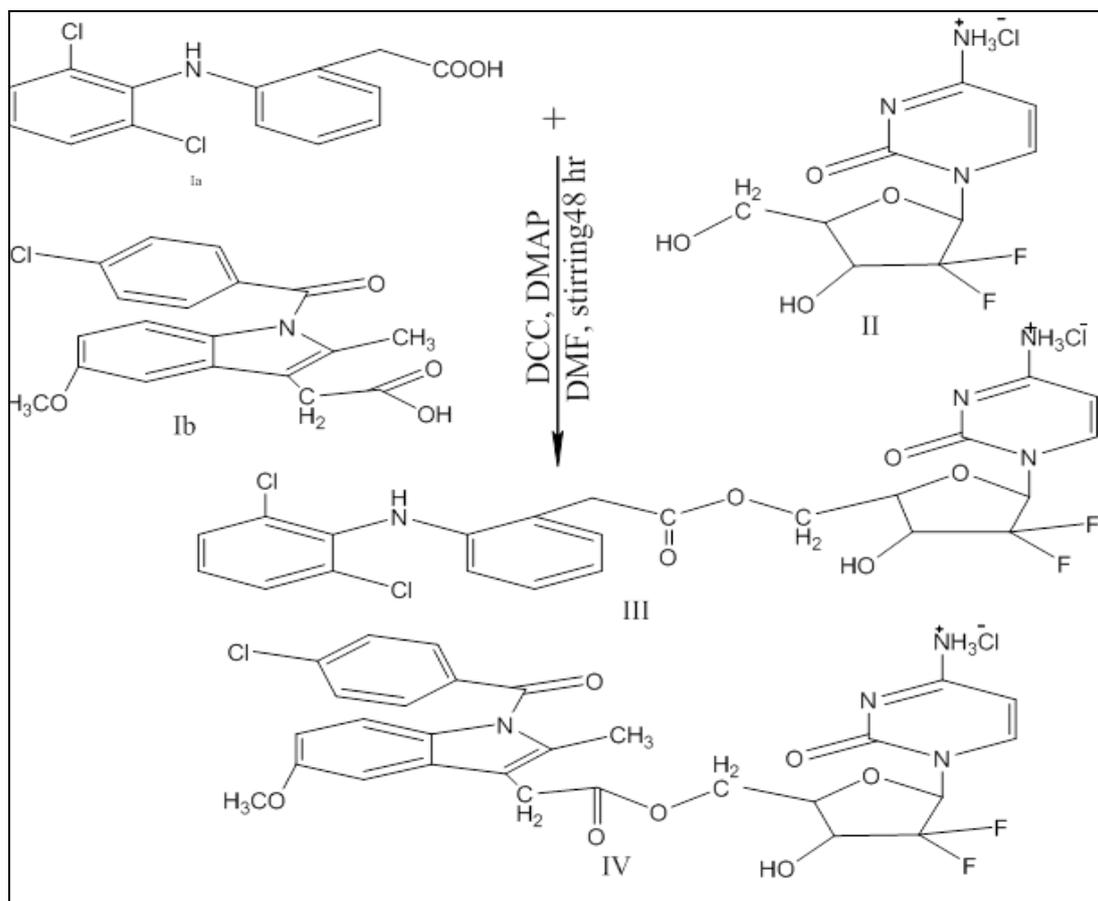
of compounds was done using a U.V. detector and the chromatograms were eluted with Chloroform:Methanol (8.5:1.5). IR spectra were recorded on a FTIR-spectrophotometer Shimadzu as KBr disks. CHNS microanalysis was done using a Euro EA 3000 elemental analyzer (Italy).

## RESULTS AND DISCUSSION

The Conversion of Diclofenac sodium to free Diclofenac is presented in figure 1. The synthesis scheme is presented in figure 2. The general routes outlined in the schemes were used to synthesize all compounds described here.



**Figure 1: Conversion of Diclofenac sodium (I) to free Diclofenac (Ia).**



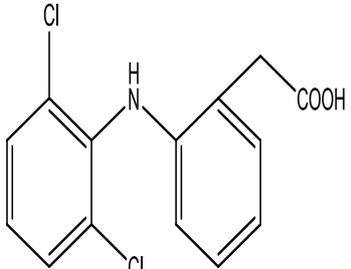
**Figure 2: The scheme for synthesis of Diclofenac-Gemcitabine & indomethacin - Gemcitabine conjugate (III & IV).**

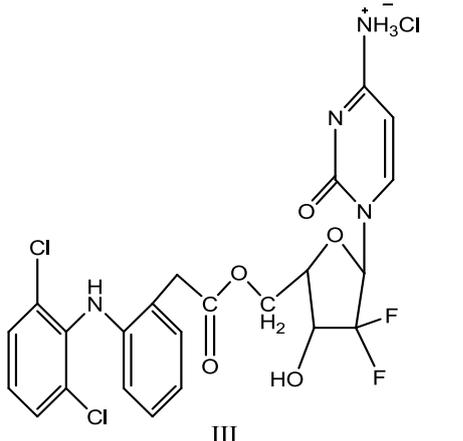
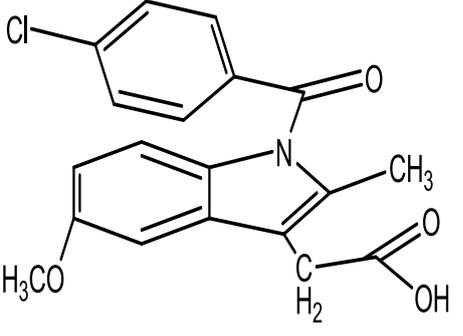
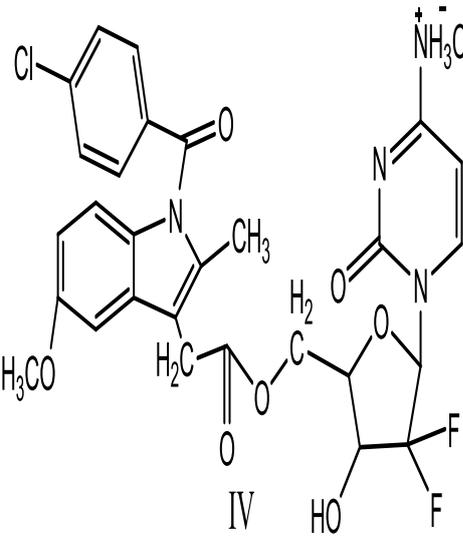
After synthesis the compounds were analyzed and the percent yield, physical appearance, melting point and TLC results were listed in table 1. Calculated Elemental Analysis for compound III were: C- 47.81; H- 3.66; N- 9.70 and observed Elemental Analysis were: C- 45.2; H- 3.1; N- 9.5. Calculated Elemental Analysis for compound IV were: C- 52.5; H- 4.10; N- 8.7 and observed Elemental Analysis were: C- 49.1; H-3.45; N- 6.73. The FT-IR spectra are shown in table 2.

**Table 1: The percent yield, physical appearance, melting point and  $R_f$  values of the target products.**

Compounds	Chemical formula	Molecular weight	Description	% yield	Melting point °C	$R_f$ value
<i>Ia free diclofenac</i>	$C_{14}H_{11}Cl_2NO_2$	296.15	White powder	85	170-173	0.62
<i>III(Diclo-G)</i>	$C_{23}H_{21}Cl_3F_2N_4O_5$	576.05	Faint yellow	60	semisolid	0.69
<i>IV (Indo-G)</i>	$C_{28}H_{26}Cl_2F_2N_4O_7$	638.11	yellow	52	123-128	0.78

**Table 2: IR characteristic bands of the synthesized compounds.**

Compounds	Bands ( $cm^{-1}$ )	Interpretation
 <p style="text-align: center;"><i>Ia</i></p>	2500-3310	O-H broad stretching vibration of carboxylic acid & N-H stretching vibration of secondary amine
	2989, 2889.	asy.&sym.(C-H) stretching vibration of $CH_3$
	3078.	(C-H) stretching of aromatic
	1710	(C=O) stretching vibration of acid
	1273.02	(C-O) stretching vibration of acid
	3323.35	(N-H) stretching vibration of 2ndary amine(N-H) bending of secondary amide
	1505.33	
	1305.81	(C-N) stretching vibration of 2ndary amine
	1577.77	
	1451.22	(C=C) stretching vibration of aromatic
	750.69,709.80,	Aromatic out of plane( C-H)
	765.74	Ortho-1,2,3-Subst.
1089.09	(Ar-Cl) stretching vibration	

 <p>III</p> <p>[1-((5S)-5-((2-(2-(2,6-dichlorophenylamino)phenyl)acetoxy)methyl)-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-aminium chloride]</p>	<p>3327.21 3070 2927.94,2850.79 1735 1705 1492.9, 1577, 1452.4 1627.92 ; 1089 748.38</p>	<p>(N-H) stretching vibration of ammonium C-H stretching vibration of aromatic C-H stretching vibration for CH<sub>2</sub>&amp;CH<sub>3</sub> C=O stretching vibration of ester C=O stretching vibration of ketone C=C stretching vibration of aromatic overlap with N-H bending vibration C=N stretching vibration Ar-Cl stretching vibration C-H bend vibrationing out of plane of aromatic</p>
 <p>Ib</p>	<p>2500-3200 Broad band centered in 3000, 3022 1720 1693 1068 &amp; 1085</p>	<p>O-H stretching vibration of carboxyl aromatic C-H stretching vibration. C=O stretching vibration of carboxyl. C=O stretching vibration of amide C-O-C stretching vibration of ether. chlorobenzene stretching vibration</p>
 <p>IV</p> <p>[1-(5-((2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-</p>	<p>3329.14 3050 absence of broad band of O-H stretching vibration of carboxyl of indomethacin 2927, 2850 1170 1735.6 1222</p>	<p>N-H stretching vibration of ammonium of gemcitabine C-H stretching vibration of aromatic due to esterification. C-H stretching vibration for CH<sub>2</sub>&amp;CH<sub>3</sub> C=O stretching vibration of ketone C=O stretching vibration of synthesized ester (C-O) stretching vibration of synthesized ester</p>

yl)acetoxy)methyl)-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-aminium chloride]	1 1082 1 1	<i>chlorobenzene stretching vibration</i>
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Pharmacological studies consistently demonstrate that COX-2 inhibitors hinder tumor growth and metastasis in dose-dependent manner in various animal models. Importantly, several investigators have shown that COX-2 inhibitors may act additively or synergistically with currently used cytotoxic and molecularly targeted agents. Here we present a broad overview of the growing evidence that COX-2 plays a pivotal role throughout oncogenesis and summarize the rationale to explore the use of COX-2 inhibitors for the prevention and/or treatment of cancer as a single agent or in combination with current anticancer modalities [7, 16]. Chemically gemcitabine is a nucleoside analog in which the hydrogen atoms on the 2' carbon of deoxycytidine are replaced by fluorine atoms. As with fluorouracil and other analogues of pyrimidines, the triphosphate analogue of gemcitabine replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. The process arrests tumor growth, as only one additional nucleoside can be attached to the "faulty" nucleoside, resulting in apoptosis. Another target of gemcitabine is the enzyme ribonucleotide reductase (RNR). The diphosphate analogue binds to RNR active site and inactivates the enzyme irreversibly. Once RNR is inhibited, the cell cannot produce the deoxyribonucleotides required for DNA replication and repair, and cell apoptosis is induced.<sup>[17]</sup>

The mutual prodrug is an efficient approach for drug optimization, the term 'mutual prodrug' refers to two or more therapeutic compounds bonded via a covalent chemical linkage. Regardless of being similar to pro-drug it differs in having inactive group replacement by active group, which are coupled directly or indirectly by a cleavable spacer.<sup>[18]</sup> The designated mutual pro-drug is oriented into three directions:

- 1- Derivatization of the carboxylate moiety in NSAIDs would eliminate their ability to inhibit COX-1 without significantly affecting their COX-2 inhibitory properties and the development of bio-reversible ester derivatives, by temporarily masking the acidic group of NSAIDs, as a promising mean of reducing or abolishing the GI toxicity.
- 2- Gemcitabine is administered by the intravenous route, since it is extensively metabolized by the gastrointestinal tract, so when coupled with NSAID as single chemical entity in an

ester mutual pro-drug expected to enhance oral bioavailability of the parent gemcitabine. The mutual pro-drug of indomethacin-5-FU improve oral bioavailability of 5-FU.<sup>[19]</sup>

- 3- In general, COX-2 is expressed in 40% to 80% of neoplastic cells in human cancers and the extent and intensity of expression is greater in cancerous than in non-cancer cells. Moreover, well and moderately differentiated cancers have significantly higher COX-2 expression than poorly differentiated cancers. In contrast, COX-2 is not detected in the vasculature of normal tissues, so, the mutual pro-drugs of NSAIDs, gemcitabine are oriented to target cancerous cells.<sup>[20]</sup> The designated mutual pro-drug is the application to the principle of combination of NSAIDs (Diclofenac and indomethacin with chemo therapeutic agent gemcitabine) as single chemical entity with combined complementary therapeutic action.

## CONCLUSION

In the present study, the combined derivatives of NSAIDs (Indomethacin and Diclofenac) with anticancer anti metabolite drug (Gemcitabine) have been designed and synthesized as possible mutual pro-drug. It is expected to enhance oral bioavailability of Gemcitabine and improve therapeutic action on specific targets.

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