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Abstract

Enzyme cyclooxygenase (COX) inhibition with non-steroidal anti-inflammatory drugs (NSAIDs) has long been utilized to treat inflammation and relieve pain. Several studies have shown that NSAIDs have also cancer preventative and tumor regressive effects. Prostaglandin E2 which acts as an inflammatory mediator influences many mechanisms that plays a significant role in tumorigenesis such as cell proliferation, angiogenesis, and metastasis. COX overexpression is a characteristic feature of most malignant tumors and contributes to poor outcomes in multiple malignancies. It has been reported that cancer incidence can be reduced by 25-40% in patients regularly taking low dose COX inhibitor aspirin on a daily basis, with the most compelling evidence acquired for colorectal cancer. We envisioned that NSAID conjugates derived from 2-alkoxycarbonyl allyl esters would have cytotoxicity enhancing prodrug properties with dual anti-inflammatory and intracellular alkylation. In the current work, 2-alkoxycarbonyl allyl ester conjugates of several common NSAIDs have been synthesized and tested for their cell proliferation inhibition properties in breast (MDA-MB-231, 4T1), pancreatic (MIA PaCa-2), and colorectal adenocarcinoma (WiDr) cell lines. Several of the synthesized derivatives exhibit good potency against all four cancer cell lines. The synthesized compounds have also been tested for their COX inhibition properties.
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<th>Abbreviation</th>
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<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti Inflammatory Drug</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>CdCl₂</td>
<td>Cadmium chloride</td>
</tr>
<tr>
<td>MEF</td>
<td>Mefenamic acid</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>CDI</td>
<td>1'-Carbonyldiimidazole</td>
</tr>
<tr>
<td>RGD</td>
<td></td>
</tr>
<tr>
<td>EPB</td>
<td>Epirubicin</td>
</tr>
<tr>
<td>HBTU</td>
<td>(2-(1H-benzotriazol-1-y1)-1,1,3,3-tetramethyluronium hexafluorophosphate)</td>
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<td>DIPEA</td>
<td>Diisopropylethylamine</td>
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<td>PIA</td>
<td>phospho-ibuprofen amide</td>
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<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>ADT</td>
<td>anethole dithiolethione</td>
</tr>
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<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
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<tr>
<td>PyBOP</td>
<td>benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate</td>
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<tr>
<td>POCl₃</td>
<td>Phosphoryl chloride</td>
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<tr>
<td>TEA</td>
<td>Triethylamine</td>
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<tr>
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<td>Tetrohydrofuran</td>
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<tr>
<td>CH₃OH</td>
<td>Methanol</td>
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<td>NaOH</td>
<td>Sodium hydroxide</td>
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<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
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<td>Pd/C</td>
<td>Palladium on carbon</td>
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<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>BH</td>
<td>Baylis-Hillman</td>
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<td>DABCO</td>
<td>1,4-diazabicyclo[2.2.2]octane</td>
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<tr>
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<td>K₂CO₃</td>
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<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
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<tr>
<td>NMO</td>
<td>N-methylmorpholine-N-oxide</td>
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CHAPTER 1: INTRODUCTION

NSAIDs have recently been exploited for having a second purpose in the body besides anti-inflammatory properties. There are many cancers that are related to chronic inflammatory conditions such as the link between inflammatory bowel disease and colon cancer. There is strong evidence to suggest that taking an NSAID regularly can help decrease your risk of developing colon cancer; and in this regard NSAIDs have been exploited for their potential as anticancer agents.

In 2012, Hanahan and Weinberg identified several distinct hallmarks that lead to tumor growth (Figure 1a). Inflammation has been recognized as one of the important markers of cancer development and growth and consequently, developing anti-inflammatory agents that are specific towards cancer cells will be highly useful towards discovering novel anticancer agents.

![Figure 1a: Hallmarks of cancer](image)

When experiencing inflammation, an enzyme called cyclooxygenase (COX) is involved. There are two types of COX enzymes, COX-1 and COX-2. COX-1 is present throughout normal and cancer cells. It is responsible for many biological pathways such as helping to develop stomach lining and making platelets for the removal of clots. The second enzyme, COX-2, is
only present when there is an inflammation. It is no surprise that tumors being inflammatory in nature also overexpress COX-2 enzyme. Many NSAIDs are not able to distinguish between inhibiting COX-1 or COX-2, which is why most NSAIDs have gastrointestinal side effects.

COX-2 is commonly found in premalignant lesions, carcinoma \textit{in situ}, invasive cancer, and metastatic disease. COX-2 is responsible for forming prostaglandin (PGE2), and this upregulation can be found responsible for many different pathways in tumorigenesis including immunosuppression, mutagenesis, anti-apoptosis, metastasis, mitogenesis, and angiogenesis (\textbf{Figure 1b}).

\textbf{Figure 1b:} Role of COX-2 in cancer development

Some of the commonly used NSAIDs for various pain related ailments are shown in \textbf{Figure 1c}. 
Owing to the importance of cyclooxygenase in cancer progression, there have been several synthetic and biological studies for the development of novel NSAID based cyclooxygenase inhibitors as anticancer agents. The following are few representative synthetic methods that have been reported for the preparation of NSAID based anticancer agents.
Metallo derivatives of NSAIDs

Tabrizi et al. synthesized mefenamic acid potassium salt 2 from NSAID mefenamic acid 1. The salt was added to a methanolic solution of CdCl₂ to provide the complex [Cd(mef)₂(CH₃OH)]ₙ 3 (Scheme 1.1). It exhibited an IC₅₀ value of 0.12±0.56 µM against MCF-7 breast cancer cell line and 0.86±0.21 µM against T-24 bladder cancer cell line.⁶

![Scheme 1.1: Synthesis of mefenamic acid cadmium complexes⁶](image)

Coyle et al. reported the reaction of NSAID salicylic acid 5 with silver oxide to form dimeric Ag(I) complexes (Scheme 1.3). These derivatives exhibited both antifungal and anticancer properties. The complex 6 was tested on squamous carcinoma of tongue Cal-27,
hepatocellular carcinoma Hep-G2 and kidney adenocarcinoma A-496 cell lines using an MTT assay. The compound exhibited cell proliferation inhibition of 51, 9, and 32 μM respectively.\textsuperscript{8}

Scheme 1.3: Synthesis of dimeric Ag(I) complexes of salicylic acid\textsuperscript{8}

Rodrigo \textit{et al.} synthesized a diruthenium(I,II)-ketoprofen complex using ketoprofen 7 and [Ru\(_2\)Cl(O\(_2\)CCH\(_3\))\(_4\)] \textsuperscript{8} in the presence of lithium chloride (Scheme 1.4). This complex 9 was tested against COX-2 overexpressing HT-29 cell line and Caco-2 cell line with low COX-2 levels. Cytotoxicity profile of this complex in these two cell lines did exhibit low cell proliferation inhibition, signifying that COX-2 inhibition by these drugs did not affect the cytotoxicity.\textsuperscript{9}

Scheme 1.4: Synthesis of diruthenium(II,III)–ketoprofen complex from ketoprofen\textsuperscript{9}
Gust et al. synthesized several [cyclopentadienyl]metalcarbonyl complexes of acetylsalicylic acid. Cyclopentadienyl alcohols 10 were prepared by the reaction of cyclopentadiene with sodium hydride followed by the addition of alcohol. The alcohol was coupled with acetylsalicylic acid chloride in the presence of pyridine to form (cyclopentadienyl)alkyl-2-acetoxybenzoate 11. This derivative was further treated with thallium ethoxide to obtain η^5-[(cyclopentadienyl)alkyl-2-acetoxybenzoate]thallium 12. This complex was further functionalized into various metal complexes 13-17 (Scheme 1.5). These complexes were evaluated for COX-2 enzyme inhibition, and prop-Cp-ASS-metal complexes 13-17 were found to have more inhibitory effects compared to the parent aspirin. The complexes 13-17 were also tested for their cytotoxicity in MCF-7 and MDA-MB-231 breast cancer cells and HT-29 colon cancer cells. 12 exhibited high cytotoxicity in the range of 1.4 to >50 uM in MCF-7, 1.9 to >50 μM in MDA-MB-231 and 4.6 to >50 µM in HT-39 cells.10
**Scheme 1.5:** Synthesis of [cyclopentadienyl]metalcarbonyl complexes of acetylsalicylic acid\textsuperscript{10}
Non-metallo derivatives of NSAIDS

Inulin 18 was coupled with ibuprofen 19 in the presence of CDI to synthesize nanoparticalized derivative for targeted delivery of epirubicin, an anticancer drug. RGD conjugated EPB loaded nanoparticles were then used to encapsulate the inulin-ibuprofen polymer 20 (Scheme 1.6). These nanoparticles were tested against BGC832 cell line and were found to have relatively non-cytotoxic compared to the parent drug epirubicin. They were also evaluated for their anticancer efficacy in H22 xenograft model in male ICR mice and were found to have increased tumor growth inhibition compared to free epirubicin.\(^{11}\)

![Scheme 1.6: Synthesis of inulin-ibuprofen polymer\(^{11}\)](image)

Babu et al. synthesized mefenamic acid based novel indole analogues starting from indole derivatives. Mefenamic acid in 1 was reduced with lithium aluminum hydride followed by alkylation with propargyl bromide to give the corresponding alkylated product 21. Treatment of 21 with o-iodoaniline in the presence of copper catalyst and palladium-carbon provided the indolyl mefenamic acid derivative 22 (Scheme 1.7). These compounds were then tested for their
anticancer properties on breast cancer cell line MCF-7 to provide an IC$_{50}$ ranging from 0.75-10 μM.$^{12}$

![Synthesis of mefenamic acid indole derivatives](image)

**Scheme 1.7:** Synthesis of mefenamic acid indole derivatives$^{12}$

Rigas *et al.* synthesized phospho-ibuprofen amides to increase the stability of phospho-ibuprofen derivatives. Ibuprofen 19 was coupled to 4-aminobutanol in the presence of HBTU and diisopropylethylamine (DIPEA) to obtain N-(4-hydroxybutyl)-2-(4-isobutylphenyl)propenamide 23. This alcohol 23 was converted into phospho-ibuprofen amide (PIA) 24 by reacting the alcohol with diethylchlorophosphate in the presence of DIPEA and DMAP (Scheme 1.8). This derivative PIA was further nanoparticalized by encapsulating in liposomes. This liposome encapsulated PIA was tested against several non-small cell lung carcinoma cell lines and it was found to be 10 times more potent than ibuprofen. Pharmacokinetic studies of this PIA showed that this form exhibits metabolic stability against
hydrolysis with carboxylesterases. Liposome encapsulated PIA also exhibited significant tumor growth inhibition compared to ibuprofen in A549 xenograft model.\textsuperscript{13}

\begin{center}
\begin{tikzpicture}
\node[anchor=east] at (0,0)\textsuperscript{13}{\textit{Scheme 1.8}: Synthesis of phospho-ibuprofen amides\textsuperscript{13}};
\end{tikzpicture}
\end{center}

Zawidlak-\Węgrzyńska, \textit{et al.} synthesized novel conjugates of oligo(3-hydroxybutyrate) and ibuprofen. The ibuprofen was made into a sodium salt \textbf{25} and then reacted with the oligo(3-hydroxybutyrate) \textbf{26} to give the oligomers of ibuprofen \textbf{27}. This was further tested for anticancer properties on two colon cancer cell lines, HT-29 and HCT 116, using an MTT assay. These IC\textsubscript{50} values ranged from 37-77 µM in the HT-29 line and 31-53µM in the HCT 116 cell line.\textsuperscript{14}

\begin{center}
\begin{tikzpicture}
\node[anchor=east] at (0,0)\textsuperscript{14}{\textit{Scheme 1.9}: Synthesis of Ibuprofen Oligo(3-hydroxybutyrate) Derivatives\textsuperscript{14}};
\end{tikzpicture}
\end{center}

Wittine \textit{et al.} conjugated NSAIDs ibuprofen \textbf{19} and ketoprofen \textbf{7} with 3-hydroxypropylamides. Ibuprofen and ketoprofen benzotriazolides \textbf{28} and \textbf{29} were reacted with 3-
hydroxypropylamine in presence of trimethylamine to provide corresponding amide products 30 and 31 (Scheme 1.10). The compounds were then tested on nine different cell lines (L1200, Molt 4/C8, CEM, HeLa, MIA PaCa-2, SW 620, MCF-7, H 460, and WI 380). These compounds did not exhibit any significant cytotoxic properties and the IC50 values ranged from 55-273 µM in cell lines L1200, Molt 4/C8, and CEM.\textsuperscript{15}

Scheme 1.10: Synthesis of ketoprofen and ibuprofen 3-hydroxypropylamide derivatives\textsuperscript{15}

Vannini et al. synthesized NOSH-aspirin, a molecule capable of releasing hydrogen
sulfide and nitric oxide intracellularly. 2-hydroxybenzaldehyde 32 was coupled with 4-bromobutanoic acid in the presence of DCC and catalytic DMAP to obtain bromo derivative 33. This bromide undergoes S$_{N}2$ type reaction with silver nitrate to get the O-nitrated compound 34. The aldehyde 34 was oxidized to carboxylic acid using Pinnick oxidation protocol. The carboxylic acid 35 was further coupled with ADT-OH to give the desired NOSH-NSAID product 36 (Scheme 1.11). The compound was shown to have an IC$_{50}$ of 48 µM on colon cancer line HT-29 and 57 µM on HCT 15.\textsuperscript{16}

![Scheme 1.11: Synthesis of NOSH-aspirin derivative\textsuperscript{16}](image)

Jacob and Tazawa synthesized an aspirin-glucose derivative by coupling aspirin 37 with dimethyl acetal protected glucose 38 using DCC as a coupling agent. The dimethyl acetal
protecting group was deprotected under acidic conditions to give the glucose aspirin conjugate 39 (Scheme 1.12). This derivative was then tested on three cancer cell lines breast, pancreatic, prostate (SKBR3, PC3, PANC-1). The IC\textsubscript{50} for this compound ranged from 200-800 µM.\textsuperscript{17}

![Scheme 1.12: Synthesis of glucose-aspirin conjugate\textsuperscript{17}](image)

Zhang et al. synthesized cytotoxic podophyllotoxin-NSAID conjugates of aspirin and ibuprofen. The carboxylic acid group aspirin 37 or ibuprofen 19 were coupled with hydroxyl group in podophyllotoxin 40 with EDC in the presence of DMAP as a catalyst resulting in the products 41 and 42 (Scheme 1.13). These derivatives were tested for an IC\textsubscript{50} in three cell lines, Bel-7402, Bel-7402/5-FU and L-O2. The aspirin derivative had IC\textsubscript{50} values of 0.09, 0.065 and
0.19 µM respectively; the ibuprofen podophyllotoxin derivative had IC$_{50}$ values in the 18.88, 10.27, and 7.38 µM range.$^{18}$

Scheme 1.13: Synthesis of podophyllotoxin-NSAID derivatives with aspirin and ibuprofen$^{18}$

Chennamaneni et al. synthesized several derivatives of sulindac for the treatment of cancer. 2-(5-fluoro-2-methyl-1H-inden- 3-yl)acetic acid 43 was coupled with an amine in the presence of PyBOP and base. The resulting amide 44 was further reacted with an aldehyde in the presence of a base to give the target sulindac amides 45 and 46 (Scheme 1.14). The cytotoxic effects were tested on HT29 colon cancer cells. The most promising derivatives were the 4-
methylsulfinyl derivative 45 and the 4-chloro derivative 46 with IC$_{50}$ values of 11.3 and 12.4 μM, respectively.$^{19}$

\[
\begin{align*}
\text{F} & \quad \text{OH} & \quad \text{R}_2\text{NH}_2 & \quad \text{PyBOP} & \quad \text{TEA} & \quad \text{DMF} & \quad \rightarrow & \quad \text{F} & \quad \text{NHR} & \quad \text{R}_2\text{CHO} & \quad \text{NaOH} & \quad \text{CH}_3\text{OH} & \quad \rightarrow & \quad \text{F} & \quad \text{NHR}_1 \\
43 & \quad & & & & & & & & & & & & 44 & & & & & & & & & & & & 45 & \quad & 11.3\pm3.5\mu\text{M} & & & & & & & & & & & & 46 & \quad & 12.4\pm6.4\mu\text{M} \\
\end{align*}
\]

**Scheme 1.14:** Synthesis of sulindac derivatives$^{19}$

Fogli *et al.* synthesized sulindac hydroxamic acid derivatives to see if substitution of the carboxylic acid group for hydroxamic acid could increase the toxicity of the derivative. (O-(tert-butyl-dimethylsilyl)hydroxylamine) was added to sulindac 47 in the presence of EDC to obtain O-silylated hydroxamates 48. Trifluoroacetic acid deprotected the silyl group to give the product hydroxamic acid 49. The IC$_{50}$ values of the derivatives ranged from 32-64 μM against MIA PaCa-2 cells, and provided IC$_{50}$ values ranged from 6-62.5 μM in COLO320 cells.$^{20}$
**Scheme 1.15:** Synthesis of sulindac hydroxamic acid derivatives$^{20}$
Gobec et al. synthesized NSAID analogs for AKR1C3 inhibition. AKR1C3 is a human enzyme that belongs to aldo-keto reductase superfamily. This enzyme is responsible for the conversion of androstenedione and estrone into testosterone and estrogen, respectively, in presence of NADPH cofactor. This enzyme plays a key role in the progression of hormone dependent breast and prostate cancers. Various NSAID analogs such as N-acylanthranilic acids, 2-benzoylbenzoic acids, benzophenones, and phenoxybenzoic acids 50-52 were synthesized and evaluated for their activity against human recombinant AKR1C3 (Figure 1d). These analogs exhibited IC₅₀ values ranging from 0.68 to 180 µM.²¹

![Chemical structures of NSAID analogs](image)

**Figure 1d:** NSAID analogs for AKR1C3 inhibition²¹
Vela´zquez et al. synthesized novel NONO-NSAID derivatives using aspirin and ibuprofen. The acid chloride 53 and O\(^2\)-acetoxymethyl 1-[N-(2-hydroxyethyl)-N-methylamino]diazen-1-ium-1,2-diolate 54 were coupled together in triethylamine forming the desired product 55. Ibuprofen 19 and O\(^2\)-acetoxymethyl 1-[N-(2-hydroxyethyl)-N-methylamino]diazen-1-ium-1,2-diolate 54 were coupled together in the presence of DCC and trimethylamine to give the product 56. These compounds were tested for their inhibitory activity on COX-1 and COX-2 enzymes but did not show any noticeable effects.\(^{22}\)

**Scheme 1.16**: Synthesis of NONO-aspirin and NONO-ibuprofen\(^{22}\) Lu et al. synthesized celecoxib based coumarin sulfonamides. Various substituted chalcones were synthesized using dimethyl oxalate. These compounds were further coupled with 4-hydrazinylbenzenesulfonamide and hydrolyzed to obtain carboxylic acid 57, which was treated with hydrazine hydrate to form the amides 58. This was further functionalized to coumarin derivative 59 using POCl\(_3\) (Scheme 1.17). All derivatives were tested against four cancer cell lines (HeLa, HepG2, F10, A549) and two non-cancer cell lines (293T, L02). The IC\(_{50}\) values of compounds against HeLa cell lines were between 0.36 and 16.19µM and HepG2 cell lines were range from 0.85 to 21.19 µM. All compounds also exhibited less toxicity on the non-cancerous cell line than celecoxib.\(^{23}\)
Scheme 1.17: Synthesis of celecoxib based coumarin sulfonamides$^{23}$
CHAPTER 2: RESULTS AND DISCUSSION

Baylis-Hillman (BH) reaction is an important C-C bond forming reaction reported in organic chemistry. This reaction was originally discovered in the 1970s and it received wide attention in the last three decades as evidenced by thousands of scientific publications and numerous review articles.\textsuperscript{24-28} This reaction can be easily carried out by simply mixing aldehydes/aldimines/activated ketones with $\alpha$, $\beta$ unsaturated esters, ketones, or nitriles, in the presence of a nucleophilic base such as DABCO (\textbf{Scheme 2.1}).\textsuperscript{24-28} This reaction typically does not require usage of any solvents, heating, or any special reaction conditions. BH reaction provides good reaction yields, with complete atom economy. BH reaction also provides densely functionalized allyl alcohols and imines in one step. The product alcohols can be further functionalized by converting the alcohol into a leaving group such as an acetate or converting the alcohol into allyl bromide, followed by treatment with wide variety of nucleophiles containing C, O, S, N atoms etc (\textbf{Scheme 2.1}).\textsuperscript{24-28}
The allyl bromides 1 and 2 derived from BH alcohols are highly useful synthetic intermediates and our group has utilized these bromides for the synthesis of a wide variety of medicinally useful natural and synthetic products.\(^{24-28}\) The allyl bromides 1 and 2 offer an interesting choice of reactive sites for the incoming nucleophiles, involving a direct S\(_{N2}\) attack, S\(_{N2'}\) attack with allylic rearrangement, or a simple 1,4-addition to provide functionalized synthetic intermediates (Scheme 2.2).
Our long-standing interest in developing small molecule therapeutics using the BH reaction has prompted us to utilize BH bromide 1 and 2 as a starting material. The bromides 1 and 2 are highly reactive and based on their structures, these bromides could act as DNA alkylating cytotoxic agents. However, these bromides suffer from serious drawbacks such as low chemical stability at room temperature, low metabolic stability, and high reactivity with the potential for serious side effects. We envisioned that α-carboxycarbonyl allyl esters 3-12 derived from carboxylic acids and BH bromides would retain the $S_N2$, or $S_N2'$, or 1,4 addition properties with decreased reactivity and improved chemical stability to be developed as potential anticancer agents (Scheme 2.3). In this regard, recently, we carried out a study involving the synthesis of α-carboxycarbonyl allyl esters derived from various carboxylic acids.²⁹
We have evaluated the cell proliferation inhibition studies of the synthesized compounds on several cancer cell lines. Our structure-activity relationship (SAR) study indicated that aromatic carboxylic compounds in general provided higher cell proliferation inhibition properties compared to aliphatic carboxylic acids. Introduction of electron donating or electron withdrawing group did not have much effect on the cell proliferation inhibition. Methoxy carbonyl group was found to be the optimal structural entity and removal of the double bond completely eliminated the biological activity, emphasizing the importance of S<sub>N</sub>2/S<sub>N</sub>2’ mechanism. The IC<sub>50</sub> values of the α-carboxycarbonyl allyl esters 3-12 were found to be in the range from 3 - 100 µM against breast and pancreatic cancer cell lines, MDA-MB-231, 4T1, and MiaPaCa-2 (Figure 2.1).
**Figure 2.1:** IC$_{50}$ values of various α-carboxycarbonyl allyl esters
The lead candidate compound 12 upon daily intraperitoneal administration was well tolerated in healthy CD-1 mice as evidenced by normal body weight gains compared to vehicle treated mice (Figure 2.2A). The lead candidate 12 also exhibited significant tumor growth inhibition in a triple negative breast cancer xenograft model MDA-MB-231 (Figure 2.2). 29

![Graph A](image-url)

![Graph B](image-url)

**Figure 2.2:** A) Systemic toxicity study of 12 in CD-1 mice; B) Anticancer efficacy study of 12 in MDA-MB-231 tumor xenograft model

As described in the introduction, inflammation has been recognized as one of the important tumor markers that can be targeted for anticancer efficacy in many solid tumors. Although several NSAIDs have been used as anticancer agents, and/or chemopreventative agents, the general lack of cytotoxicity precludes them to be used as primary chemotherapeutic
agents. In the present work, we hypothesize that conjugating the BH bromide with carboxy containing NSAIDs would lead to α-carboxycarbonyl allyl esters of NSAIDs with enhanced cytotoxic properties. These esters would also release the parent NSAIDs upon interacting with intracellular nucleophilic components either in S\textsubscript{N}2 or S\textsubscript{N}2' fashion (Figure 2.3). We envisaged that these dual mechanistic properties should provide higher anticancer efficacy than using NSAIDs alone.

![Diagram](image)

**Figure 2.3:** Hypothesis: Conjugates of NSAID carboxylic acids with BH bromides

With the above hypothesis in mind, initially we chose salicylic acid 13 for conjugating with BH bromide 1 derived from formaldehyde. The reaction of salicylic acid 13 with BH bromide 1 in the presence of potassium carbonate in DMSO provided methoxycarbonylallyl ester 15 (Scheme 2.4). The pure 15 could be readily obtained in 89% yield by silica gel column chromatography using 2% ethyl acetate in hexanes as eluent. Using the similar protocol, we also synthesized carboxycarbonyl allyl ester 16 from acetylsalicylic acid 14 (aspirin) in 90% yield.
Scheme 2.4: Synthesis of carboxycarbonyl allyl esters from salicylic acid and aspirin

The carboxy esters 15 and 16 were evaluated against human triple negative breast cancer cell line MDA-MB-231 and highly metastatic murine breast cancer cell line 4T1, pancreatic cancer cell line MIAPaCa-2 and colorectal adenocarcinoma cell line WiDr. The cell proliferation inhibition studies were carried out using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. MTT is reduced to formazan through mitochondrial reductase with an absorbance at 590nm. Cell cultures in 96-well plates were incubated with the compounds for 72 h, and MTT values expressed as percent of vehicle-only (control) wells. The IC$_{50}$ value was calculated for each compound as the dose required to suppress the MTT signal to 50% of control values. These assays were carried out a minimum of three trials and the IC$_{50}$ values were calculated using GraphPad Prism 6 Software. Gratifyingly, the IC$_{50}$ values for 15 and 16 were found to be in the range from 4 - 17 µM for the above-mentioned cell lines (Figure 2.4). It is important to note here that the parent NSAIDs salicylic acid 13 and acetylsalicylic acid 14 did not show any cell proliferation inhibition against any of the cell lines that we used even at 100 µM concentration.
Encouraged by significant cell proliferation inhibition at low micromolar concentrations, we planned to synthesize and explore the biological properties of several carboxy containing NSAIDs. In this regard, we chose six other commercially available NSAIDs, namely ketoprofen 17, ibuprofen 18, fenamic acid 19, mefenamic acid 20, meclofenamic acid 21 and niflumic acid 22 (Figure 2.5). Ibuprofen is an over-the-counter NSAID used for pain, ketoprofen is typically used for toothaches. Fenamic acid itself is not an NSAID but it is the parent molecule for the three NSAIDs mefenamic acid, meclofenamic acid, and niflumic acid. All three of these are commonly used for joint or muscle pain as well as pains related to menstruation such as cramps or headaches. All these NSAIDs provide the therapeutic benefit by inhibition of COX-1 and COX-2.
Synthesis of carboxycarbonyl allyl esters 23 to 28 involved the similar procedure employed in scheme 2.4, by stirring the NSAID carboxylic acids with BH bromide 1 in the presence of potassium carbonate in DMSO at room temperature (Scheme 2.5). All the crude products were purified by silica gel column chromatography with a mixture of hexanes and ethyl acetate as eluents. All the pure products were characterized by $^1$H NMR, $^{13}$C NMR, and mass spectroscopy.
The cell proliferation inhibition studies were carried out using MTT assay as described above. The BH esters derived from aliphatic carboxylic acid NSAIDs ketoprofen and ibuprofen 23, 24 showed modest cytotoxic properties against all four cancer cell lines. Carboxycarbonyl allyl ester of ketoprofen 23 provided IC\textsubscript{50} values in the range from 17 µM to >100 µM. Carboxycarbonyl allyl ester of ibuprofen 24 exhibited IC\textsubscript{50} values in the range from 80 µM to >100 µM. These modest cell proliferation inhibition properties are consistent with our earlier published work that aliphatic carboxylic acids in general provide less cytotoxic properties than aromatic carboxylic acids\textsuperscript{29}. The other aromatic carboxylic acid containing NSAID
carboxycarbonyl allyl esters 25-28 showed significant cell proliferation inhibition against all four cell lines. Carboxycarbonyl allyl esters of fenamic acid 25, mefenamic acid 26, meclofenamic acid 27 and niflumic acid 28 exhibited IC₅₀ values ranged from 16-37 µM, 5-21 µM, 5-21 µM, and 15-25 µM, respectively.

Encouraged by significant increase in cytotoxic values of carboxycarbonyl allyl esters 23-28 derived from formaldehyde BH bromide 1, we also synthesized carboxycarbonyl allyl esters 29-32 using benzaldehyde BH bromide 2 and NSAIDs 13, 14, 17 and 18. The corresponding BH bromide 2 was synthesized in two steps starting from benzaldehyde which upon condensation with methyl acrylate in the presence of DABCO provided the BH alcohol, which was then brominated using HBr and H₂SO₄. The bromide 2 upon reaction with NSAIDs 13, 14, 17 and 18 in the presence of potassium carbonate in DMSO provided the crude esters 29-32 (Scheme 2.6). The pure products were readily obtained using silica gel chromatography using a mixture of hexane and ethyl acetate as eluents. All the synthesized products were characterized by ¹H NMR, ¹³C, and mass spectrometry.
Scheme 2.6: Synthesis of benzaldehyde BH ester 2 and the corresponding NSAID esters 29-32

MTT based cell proliferation inhibition properties against two breast cancer cell lines, MDA-MB-231 and 4T1 of carboxycarbonyl esters 29-32 exhibited slightly decreased IC$_{50}$ values compared to carboxycarbonyl allyl esters 23-28 derived from formaldehyde BH bromide 1. Again, the aromatic carboxycarbonyl allyl esters of NSAIDs salicylic acid 29 and acetylsalicylic acid 30 provided higher cell proliferation inhibition than aliphatic carboxycarbonyl allyl esters of ketoprofen 31 and ibuprofen 32. Owing to the decreased cell proliferation inhibition properties and lower water solubility, we did not synthesize carboxycarbonyl allyl esters from the NSAIDs 19-22. From this SAR study, it is quite evident that β-unsubstitution is more preferred than β-
substitution in the double bond region. These results also provide credence for our hypothesis that $S_N2'$ mechanism may be operative in providing the biological activity.

Since our proposed mechanism for enhanced cell proliferation property involves the double bond for $S_N2$, $S_N2'$ and 1,4 addition, we sought to understand its critical role by saturating the double bond. For this purpose, we chose dihydroxylation of double bond in carboxycarbonylallyl esters 15, 16, 23-28. These allyl esters were treated with a catalytic amount of osmium tetroxide in the presence of N-methylmorpholine N-oxide in acetone to afford the crude diols 33-40 (Scheme 2.7). The crude compounds were purified by silica gel column chromatography by using hexane and ethyl acetate (1:1) as eluents to obtain pure products in 65 - 78% yield.

![Scheme 2.7](image-url)
**Scheme 2.7:** General scheme for dihydroxylation of carboxycarbonyl allyl esters

Cell proliferation studies using MTT assay indicated that none of these diolic carboxycarbonyl esters 33-40 did not exhibit any significant inhibition even at 100 µM concentration against all four cancer cell lines. These results are not surprising as we have observed similar loss of activity in the case of simple carboxylic acids in our earlier work.29

As a part of SAR study, we synthesized a simple allyl ester 41 from salicylic acid 13 which has limited capability of S_N2 or S_N2’. Reaction of salicylic acid with allyl bromide under basic conditions provided the corresponding allyl ester 41 in 71% yield (Scheme 2.8). As expected, the biological evaluation of 41 against two breast cancer cell lines, MDA-MB-231 and 4T1 indicated no cell proliferation inhibition properties even at 100 µM concentration. The loss of cytotoxic activity also reaffirms the critical role of the double bond with α-carboxycarbonyl group in providing the biological activity via S_N2, S_N2’, and 1,4 addition pathways.

![Scheme 2.8: Synthesis of salicylic acid allyl ester 41](image)

**Scheme 2.8:** Synthesis of salicylic acid allyl ester 41
Conclusion and Future Directions

In conclusion, we have synthesized, purified, and characterized several new compounds based on various commercially available NSAIDs. All the synthesized compounds were evaluated for cell proliferation inhibition using MTT assay against four cancer cell lines, MDA-MB-231, 4T1, MiaPaCa-2, and WiDr. All the 2-alkoxycarbonyl allyl esters conjugates of NSAIDs exhibited significantly higher cell proliferation inhibition than the parent NSAIDs. In general, β-unsubstitution in the double bond provided higher biological activity than β-substituted derivatives. Aromatic carboxylic acid containing NSAID esters of BH bromides exhibited higher cell proliferation inhibition than aliphatic carboxylic acid containing NSAIDs. Structure activity relationship studies also indicated that double bond in 2-alkoxycarbonyl allyl ester was a critical component for cell proliferation inhibition, and saturation of the double bond resulted in complete loss of activity, confirming that $S_N2/S_N2'$ mechanism plays a key role in providing the biological activity. Based on the higher cell proliferation inhibition properties, four carboxycarbonylallyl esters derived from salicylic acid, acetyl salicylic acid, mefenamic acid and meclofenamic acid were designated as lead candidate compounds for further studies.

The future directions of this project will involve evaluation of COX inhibition of all the lead compounds and identification of primary lead candidate compound that exhibits higher cell proliferation inhibition, and COX inhibition. The primary lead will also be evaluated for systemic toxicity study in healthy CD-1 mice and anticancer efficacy studies in mice tumor models using WiDr colorectal adenocarcinoma cell line and pancreatic cancer cell line MiaPaCa-2.
CHAPTER 3: Experimental Procedures and Spectral Characterization

General procedure for the synthesis of 2-(bromomethyl)acrylate-NSAIDs

A mixture of NSAID (1.0 eq, 42.7 mmol) and methyl 2-(bromomethyl)acrylate (1.1 eq, 46.9 mmol) in 30ml DMSO were first stirred at room temperature for 30 minutes, followed by the addition of potassium carbonate (3.0 eq, 128 mmol). The reaction mixture was stirred for 1 hour at rt. After it was confirmed that the reaction was completed using a TLC (10% EtOAc/hexane), water was added to the reaction mixture in order to dissolve the excess potassium carbonate, and extracted with three times diethyl ether. The crude product was purified by silica gel column chromatography using hexanes and ethyl acetate as eluents.
2-(methoxycarbonyl)allyl 2-hydroxybenzoate

$^1$H NMR (500 MHz, CDCl$_3$):
δ 10.61 (s, 1H), 7.83 (dd, $J = 2.0, 4.5$ Hz, 1H), 7.43 (m, 1H), 6.94 (dd, $J = 1, 8.5$ Hz, 1H), 6.84 (m, 1H), 6.41 (s, 1H), 5.93 (s, 1H), 5.05 (s, 2H), 3.78 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):
δ 169.40, 165.38, 161.73, 135.89, 134.77, 129.83, 127.98, 119.20, 117.61, 112.12, 62.99, 52.09

HRMS (ESI) m/z: calc’d for C$_{12}$H$_{12}$O$_5$ [M+Na]$^+$ : 259.0577, found 259.0777
2-(methoxycarbonyl)allyl 2-acetoxybenzoate

$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 8.04 (dd, $J$ = 1.5, 8 Hz, 1H), 7.57 (m, 1H), 7.32 (m, 1H), 7.11 (dd, $J$ = 1.5, 8.5 Hz, 1H), 6.42 (s, 1H), 5.93 (s, 1H), 5.02 (s, 2H), 3.80 (s, 3H), 2.32 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):
$\delta$ 169.64, 165.59, 163.81, 150.78, 135.03, 134.08, 131.81, 127.9, 126.06, 123.89, 122.96, 63.00, 52.09, 20.98

HRMS (ESI) m/z: calc’d for C$_{14}$H$_{14}$O$_5$[M+Na]$^+$: 301.0683, found 301.0906
3-methoxy-2-oxobut-3-en-1-yl 2-(4-isobutylphenyl)propanoate

\[ \text{H NMR (500 MHz, CDCl}_3\text{):} \]
\[ \delta 7.21 (d, J = 8.0 \text{ Hz}, 1\text{H}), 7.09 (d, J = 8.0 \text{ Hz}, 1\text{H}), 6.22 (s, 1\text{H}), 5.54 (s, 1\text{H}), 4.81 (dd, J = 14.0, 50 \text{ Hz}, 2\text{H}), 3.76 (q, J = 7.0 \text{ Hz}, 2\text{H}), 3.71 (s, 3\text{H}), 2.45 (d, J = 7.0 \text{ Hz}, 2\text{H}), 1.87-1.82 (m, 1\text{H}), 1.51 (d, J = 7.0 \text{ Hz}, 3\text{H}), 0.89 (d, J = 6.5 \text{ Hz}, 6\text{H}) \]

\[ \text{C NMR (125 MHz, CDCl}_3\text{):} \]
\[ \delta 173.95, 165.54, 140.65, 137.46, 135.18, 129.33, 127.21, 126.76, 62.31, 51.88, 45.07, 45.00, 30.18, 22.33, 18.24 \]

\[ \text{HRMS (ESI) m/z: calc'd for C}_{18}\text{H}_{24}\text{O}_4\text{[M+Na]}^+: 327.1567, \text{found 327.1789} \]
methyl 2-(((2-(3-benzoylphenyl)propanoyl)oxy)methyl)acrylate

$^1$H NMR (500 MHz, CDCl$_3$):
\[ \delta 7.79-7.4 (m, 8H), 6.26 (s, 1H), 5.64 (s, 1H), 4.80 (dd, J = 14.5, 23 Hz, 2H), 3.81 (q, J = 7.0 Hz, 1H), 3.70 (s, 3H), 1.55 (d, J = 7.0 Hz, 3H) \]

$^{13}$C NMR (125 MHz, CDCl$_3$):
\[ \delta 196.36, 173.32, 165.46, 140.55, 137.95, 137.44, 135.02, 132.51, 131.49, 130.03, 129.20, 129.07, 128.55, 128.31, 127.34, 62.73, 51.97, 45.31, 18.29 \]

HRMS (ESI) m/z: calc’d for C$_{21}$H$_{12}$O$_5$ [M+Na]$^+$ : 375.1203, found 375.1433
2-(methoxycarbonyl)allyl 2-(phenylamino)benzoate

$^1$H NMR (500 MHz, CDCl$_3$):

$\delta$ 9.42 (s, 1H), 8.00 (d, $J = 8.0$ Hz, 1H), 7.36-7.24 (m, 7H), 7.10 (t, $J = 7.5$ Hz, 1H), 6.74 (t, $J = 7.0$ Hz, 1H), 6.43 (s, 1H), 5.97 (s, 1H), 5.06 (s, 2H), 3.82 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):

$\delta$ 167.72, 165.70, 148.22, 140.62, 135.71, 134.37, 131.60, 129.38, 127.43, 123.70, 122.64, 117.21, 114.05, 111.48, 62.45, 52.11

HRMS (ESI) m/z: calc’d for C$_{18}$H$_{17}$NO$_4$[M+Na]$^+$: 334.105, found 334.127
2-(methoxycarbonyl)allyl 2-((2,3-dimethylphenyl)amino)benzoate

$^{1}H$ NMR (500 MHz, CDCl$_3$):

$\delta$ 9.21 (s, 1H), 8.00 (d, $J = 10.0$ Hz, 1H), 7.26-6.67 (m, 7H), 6.44 (s, 1H), 5.99 (s, 1H), 5.08 (s, 2H), 3.83 (s, 3H), 2.34 (s, 3H), 2.18 (s, 3H)

$^{13}C$ NMR (125 MHz, CDCl$_3$):

$\delta$ 167.89, 165.73, 149.71, 138.59, 138.23, 135.44, 134.41, 132.54, 131.42, 127.27, 126.88, 125.95, 123.19, 116.08, 113.73, 110.37, 62.31, 52.09, 20.62, 14.00

HRMS (ESI) m/z: calc’d for C$_{20}$H$_{21}$NO$_2$ [M+Na]$^+$ : 362.1363, found 362.1603
2-(methoxycarbonyl)allyl 2-((2,6-dichloro-3-methylphenyl)amino)benzoate

**$^1$H NMR (500 MHz, CDCl$_3$):**

$\delta$ 9.28 (s, 1H), 8.03 (d, $J = 8.0$ Hz, 1H), 7.32-7.26 (m, 2H), 7.12 (d, $J = 8.5$ Hz, 1H), 6.77 (t, $J = 8.0$ Hz, 1H), 6.45 (s, 1H), 6.33 (d, $J = 8.5$ Hz, 1H), 6.00 (s, 1H), 5.09 (s, 2H), 3.83 (s, 3H), 2.42 (s, 3H)

**$^{13}$C NMR (125 MHz, CDCl$_3$):**

$\delta$ 167.75, 165.73, 147.73, 136.49, 135.33, 135.08, 134.34, 134.22, 131.31, 131.28, 128.59, 127.73, 127.51, 117.37, 113.79, 111.26, 62.51, 52.09, 20.63

**HRMS (ESI) m/z:** calc’d for C$_{19}$H$_{17}$Cl$_2$NO$_4$ [M+Na]$^+$ : 416.0427, found 416.0687
2-(methoxycarbonyl)allyl 2-((3-(trifluoromethyl)phenyl)amino)nicotinate

$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 10.28 (s, 1H), 8.42 (d, $J = 7.0$ Hz, 1H), 8.29 (d, $J = 10.0$ Hz, 1H), 8.09 (s, 1H), 7.88 (d, $J = 8.5$ Hz, 1H), 7.43 (t, $J = 16.0$ Hz, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 6.80 (m, 1H), 6.46 (s, 1H), 5.97 (s, 1H), 5.09 (s, 2H), 3.83 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):
$\delta$ 166.79, 165.52, 155.78, 153.32, 140.26, 134.89, 131.12(qurtet), 129.18, 128.31, 125.26, 123.55, 123.09, 119.10, 117.16, 114.10, 107.13, 63.23, 52.17

HRMS (ESI) m/z: calc’d for C$_{18}$H$_{15}$F$_3$N$_2$O$_4$ [M+Na]$^+$: 403.0876 found 403.1146
Synthesis of Benzaldehyde Baylis Hillman Compounds

(E)-2-(methoxycarbonyl)-3-phenylallyl 2-hydroxybenzoate

$^1$H NMR (500 MHz, CDCl$_3$):

$\delta$ 10.71 (s, 1H), 8.10 (s, 1H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.37 (m, 6H), 7.00 (d, $J = 8.5$ Hz, 1H), 6.87 (t, $J = 8.0$ Hz, 1H), 5.23 (s, 2H), 3.87 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):

$\delta$ 169.76, 167.51, 161.68, 146.40, 135.82, 134.06, 130.06, 129.81, 129.43, 128.98, 126.01, 119.21, 117.60, 112.33, 60.09, 52.43

HRMS (ESI) m/z: calc’d for C$_{18}$H$_{16}$O$_5$ [M+Na]$^+$ : 335.089, found 335.1116
\[(E)-2-\text{(methoxycarbonyl)-3-phenylallyl 2-acetoxybenzoate}\]

\(^1\text{H NMR (500 MHz, CDCl}_3):\]
\[\delta 8.07 \text{ (s, 1H), 8.03 (d, } J = 8.0 \text{ Hz, 1H), 7.57 (m, 1H), 7.42 (m, 5H), 7.32 (t, } J = 7.5 \text{ Hz, 1H), 7.11 (d, } J = 8.5 \text{ Hz, 1H), 5.16 (s, 2H), 3.86 (s, 3H), 2.29 (s, 3H)}\]

\(^13\text{C NMR (125 MHz, CDCl}_3):\]
\[\delta 169.61, 167.28, 164.22, 150.75, 146.13, 134.06, 134.01, 132.04, 129.74, 129.52, 128.87, 126.40, 126.07, 123.90, 123.13, 60.07, 52.39, 20.92\]

\text{HRMS (ESI) m/z: calc’d for C}_{20}\text{H}_{18}\text{O}_{6}[\text{M+Na}]^+: 377.0996, \text{found 377.1254}\]

\begin{center}
\includegraphics[width=0.8\textwidth]{methyl\text{-(E)-2-\text{(((2-(3-benzoylphenyl)propanoyl)oxy)methyl)-3-phenylacrylate}}}
\end{center}

\(^1\text{H NMR (500 MHz, CDCl}_3):\]
\[\delta 7.96 \text{ (s, 1H), 7.76-7.20 (m, 14H), 5.00 (d, } J = 12.0 \text{ Hz, 1H), 4.85 (dd, } J = 12, 73 \text{ Hz, 1H), 3.87 (q, } J = 7 \text{ Hz, 1H), 3.78 (s, 3H), 1.56 (d, } J = 7.5 \text{ Hz, 3H)}\]
$\textsf{\textsuperscript{13}C NMR (125 MHz, CDCl}_3\text{)}$:

$\delta$ 196.39, 173.64, 167.81, 145.80, 140.72, 137.98, 137.46, 134.04, 132.49, 131.50, 130.03, 129.58, 129.33, 129.02, 128.65, 128.55, 128.30, 126.35, 59.83, 52.23, 45.34, 18.18

HRMS (ESI) m/z: calc’d for C$_{27}$H$_{24}$O$_5$ [M+Na]$^+$ : 451.1516, found 451.1825

![methyl (E)-2-(((2-(4-isobutylphenyl)propanoyl)oxy)methyl)-3-phenylacrylate](image)

$\textsf{\textsuperscript{1}H NMR (500 MHz, CDCl}_3\text{)}$:

$\delta$ 7.87 (s, 1H), 7.22-7.02 (m, 9H), 4.80 (dd, $J = 11.5, 94$ Hz, 1H), 3.67 (s, 3H), 2.37 (d, $J = 7.0$ Hz, 2H), 1.76 (m, 1H), 1.43 (d, $J = 7.0$ Hz, 3H), 0.81 (m, 6H)

$\textsf{\textsuperscript{13}C NMR (125 MHz, CDCl}_3\text{)}$:

$\delta$ 174.35, 167.31, 145.83, 140.56, 137.65, 134.10, 129.44, 129.39, 128.58, 127.55, 126.52, 59.63, 52.17, 45.07, 45.04, 40.93, 30.20, 22.39, 22.37, 18.27

HRMS (ESI) m/z: calc’d for C$_{24}$H$_{28}$O$_4$ [M+Na]$^+$ : 403.188, found 403.2148
allyl 2-hydroxybenzoate

$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 10.71 (s, 1H), 7.85 (s, 1H), 7.42 (s, 1H), 6.95 (s, 1H), 6.85 (s, 1H), 6.00 (m, 1H), 5.40 (d, $J =$ 17.0 Hz, 1H), 5.29 (d, $J =$ 5.5 Hz), 4.81 (s, 2H)

$^{13}$C NMR (125 MHz, CDCl$_3$):
$\delta$ 169.80, 161.72, 135.75, 131.58, 129.92, 119.15, 118.88, 117.59, 112.38, 65.79
Synthesis of Baylis Hillman NSAID Diols

To compound (2 mmol) in acetone (10 mL) was added N-methylmorpholine-N-oxide (NMO) (5 mmol) and catalytic osmium tetroxide (0.02 mmol). Reaction was stirred at room temperature for an hour. Upon completion (TLC), acetone was removed under reduced pressure and residue obtained was diluted with 50 mL of water and the crude product was extracted with ethyl acetate (3×25mL) and purified via column chromatography (1:5, EtOAc:Hexanes) to obtain the diol (70%).
2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl 2-acetoxybenzoate

\[ \text{H NMR (500 MHz, CDCl}_3\text{):} \]
\[ \delta 7.99 (d, J = 8.0 \text{ Hz}, 1\text{H}), 7.63 (t, J = 7.5 \text{ Hz}, 1\text{H}), 7.36 (t, J = 7.5 \text{ Hz}, 1\text{H}) 7.16 (d, J = 8.0 \text{ Hz}, 1\text{H}), 4.50 (s, 2\text{H}), 3.93-3.81 (m, 7\text{H}), 2.40 (s, 3\text{H}) \]

\[ \text{C NMR (125 MHz, CDCl}_3\text{):} \]
\[ \delta 173.02, 169.85, 163.71, 150.91, 134.29, 131.62, 126.11, 123.89, 122.41, 77.62, 66.13, 64.71, 53.45, 21.00 \]
2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl 2-hydroxybenzoate

\[ \text{H NMR (500 MHz, CDCl}_3\text{):} \]
\[ \delta 10.47 \text{ (s, 1H), 7.74 (d, } J = 10.0 \text{ Hz, 1H), 7.46 (t, } J = 8.0 \text{ Hz, 1H), 6.97 (d, } J = 8.5 \text{ Hz, 1H), 6.87 (t, } J = 15.0 \text{ Hz, 1H), 4.51 (dd, } J = 11.5, 21.0 \text{ Hz, 2H), 3.92-3.80 (m, 7H)} \]

\[ \text{C NMR (125 MHz, CDCl}_3\text{):} \]
\[ \delta 172.97, 169.40, 161.68, 136.18, 129.82, 119.40, 117.69, 111.77, 110.00, 66.27, 64.68, 53.64 \]

\[ \text{HRMS (ESI) m/z: calc'd for C}_{12}H_{14}O_{7} [\text{M+Na}]^+: 293.0632, \text{ found 293.0848} \]
methyl 2,3-dihydroxy-2-(((2-(4-isobutylphenyl)propanoyl)oxy)methyl)propanoate

[DIASTEREOMERS] (1:1)

\(^1\)H NMR (500 MHz, CDCl\(_3\)):
δ 7.18-7.08 (m, 8H), 4.26-4.16 (m, 4H), 3.77-3.64 (m, 14H), 2.55-2.43 (m, 6H), 1.88-1.80 (m, 2H), 1.47 (t, \(J=7\) Hz, 6H), 0.89 (d, \(J = 7.5\) Hz, 12H)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)):
δ 174.19, 172.87, 172.75, 140.75, 140.69, 137.27, 137.11, 129.33, 127.20, 127.17, 66.04, 65.80, 64.62, 64.55, 45.00, 44.98, 44.83, 30.17, 22.33, 18.13, 18.08

HRMS (ESI) m/z: calc’d for C\(_{18}\)H\(_{26}\)O\(_6\) [M+Na]\(^+\) : 361.1622, found 361.1898
methyl 3-((2-(3-benzoylphenyl)propanoyl)oxy)-2-hydroxy-2-(hydroxymethyl)propanoate

[DIASTEREOMERS] (1:1)

$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 7.81 (m, 9H), 4.33-4.21 (m, 2H), 3.85-3.62 (m, 8H), 1.53 (m, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):
$\delta$ 196.52, 196.48, 173.42, 173.39, 172.78, 172.71, 140.42, 140.33, 138.04, 137.99, 137.36, 132.61, 132.59, 131.52, 131.46, 130.10, 130.09, 129.23, 129.17, 129.11, 128.51, 128.47, 128.33, 66.07, 65.91, 64.59, 64.57, 60.40, 53.32, 53.23, 45.22, 45.19, 21.03, 18.13, 18.11, 14.18

HRMS (ESI) $m/z$: calc’d for C$_{21}$H$_{22}$O$_7$ [M+Na]$^+$ : 409.1258, found 409.1557
2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl 2-(phenylamino)benzoate

\[ \text{H NMR (500 MHz, CDCl}_3\text{):} \]
\[ \delta 9.32 (s, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.36-7.11 (m, 9H), 6.73 (t, J = 7.0 Hz, 1H), 4.48 (dd, J = 10.5, 32 Hz, 2H), 3.94-3.82 (m, 7H) \]

\[ \text{C NMR (125 MHz, CDCl}_3\text{):} \]
\[ \delta 173.22, 167.72, 148.30, 140.47, 134.57, 131.54, 129.39, 123.85, 122.78, 117.20, 114.05, 110.96, 65.88, 64.74, 53.55 \]

\[ \text{HRMS (ESI) m/z: calc'd for C}_{18}\text{H}_{19}\text{NO}_6 [M+Na]^+: 368.1105, \text{found 368.1375} \]
2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl2-((2,3-dimethylphenyl)amino) benzoate

$^1$H NMR (500 MHz, CDCl$_3$):

$\delta$ 9.12 (s, 1H), 7.87 (d, $J = 8.5$ Hz, 1H), 7.27-7.04 (m, 4H), 6.73 (d, $J = 8.5$ Hz, 1H), 6.66 (t, $J = 8.0$ Hz, 1H), 4.49 (dd, $J = 9.5$, 30 Hz, 2H), 3.95-3.82 (m, 7H), 2.33 (s, 3H), 2.17 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$):

$\delta$ 173.26, 167.95, 149.81, 138.45, 138.27, 134.65, 132.64, 131.38, 127.03, 125.99, 123.34, 116.17, 113.71, 109.80, 77.72, 65.87, 64.75, 53.52, 20.61, 13.99

HRMS (ESI) m/z: calc’d for $C_{20}H_{23}NO_6$ [M+Na]$^+$ : 396.1418, found 396.1702
2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl2-((2,6-dichloro-3-methylphenyl)amino)benzoate

\[ \text{H NMR (500 MHz, CDCl}_3\text{)}: \]
\( \delta \) 9.18 (s, 1H), 7.90 (d, 1H), 7.32-7.26 (m, 2H), 7.12 (d, \( J=12.5 \text{Hz} \), 1H), 6.75 (t, \( J= 7.5 \text{ Hz} \), 1H), 6.31 (d, \( J= 8.5 \text{ Hz} \), 1H), 4.53 (dd, \( J=12 \text{ Hz} \), \( J=17 \text{ Hz} \), 1H), 3.86-3.72 (m, 5H), 2.40 (s, 3H)

\[ \text{C NMR (125 MHz, CDCl}_3\text{)}: \]
\( \delta \) 173.26, 167.77, 147.82, 136.52, 134.94, 134.48, 134.36, 131.31, 128.71, 127.75, 117.49, 113.78, 110.71, 77.64, 66.00, 64.75, 53.57, 53.55, 20.61

\[ \text{HRMS (ESI) m/z}: \text{calc’d for C}_{19}\text{H}_{19}\text{Cl}_2\text{NO}_6 \text{[M+Na]}^+: 450.0482, \text{found} 450.0799 \]
2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl2-((3-(trifluoromethyl)phenyl)amino)nicotinate

$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 10.18 (s, 1H), 8.50 (d, $J = 5.0$ Hz, 1H), 8.24 (d, $J = 8.0$ Hz, 1H), 8.14 (s, 1H), 7.92 (d, $J = 8.5$ Hz, 1H), 7.50 (t, $J = 8.0$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 1H), 6.87 (m, 1H), 4.59 (dd, $J = 11.0$, 39.5 Hz, 2H), 4.01-3.89 (m, 6H)

$^{13}$C NMR (125 MHz, CDCl$_3$):
$\delta$ 173.06, 166.74, 155.79, 153.58, 140.26, 140.07, 132.04 (quartet), 129.23, 123.73, 119.32, 119.29, 117.37, 114.21, 106.68, 66.30, 64.71, 53.69

HRMS (ESI) m/z: calc’d for C$_{18}$H$_{17}$F$_3$N$_2$O$_6$ [M+H]$^+$ : 415.1111, found 415.1395
Biological Evaluation

Table 1: IC$_{50}^{*}$ values of NSAID-Baylis Hillman derivatives in MiaPaCa-2, MDA-MB-231, 4T1, and WiDr cell lines using MTT assay

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<th>Compound</th>
<th>MiaPaCa-2</th>
<th>MDA-MB-231</th>
<th>4T1</th>
<th>WiDr</th>
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Diol
References
