Design and Synthesis of Small-molecule Inhibitors of the Hypoxia Inducible Factor-1 as Anticancer Therapeutics

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DESIGN AND SYNTHESIS OF SMALL-MOLECULE INHIBITORS OF THE HYPOXIA INDUCIBLE FACTOR-1 AS ANTICANCER THERAPEUTICS

by

ZEUS ALLEN ORENCIA DE LOS SANTOS

Under the Direction of Professor Binghe Wang

ABSTRACT

Throughout history, cancer has been severely plaguing mankind; the search for a cure to cancer had long been sought by scientists and still poses as one of the greatest challenges scientists have yet to overcome. Hypoxia in cells is a condition where there is little to no oxygen availability in its environment. In general, this event is detrimental since this can lead to cell necrosis or reoxygenation injuries. However, hypoxia, a prominent property of most solid tumors, activates the hypoxia-inducible factor (HIF-1) family of transcription factors that promotes angiogenesis. In this study, we describe the design and synthesis of small-molecule inhibitors of the HIF-1 pathway.

INDEX WORDS: Hypoxia, HIF-1, Cancer, Small-molecule inhibitors
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by

ZEUS ALLEN ORENCIA DE LOS SANTOS

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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Georgia State University

2014
DESIGN AND SYNTHESIS OF SMALL-MOLECULE INHIBITORS OF THE HYPOXIA INDUCIBLE FACTOR-1 AS ANTI-CANCER THERAPEUTICS

by

ZEUS ALLEN ORENCIA DE LOS SANTOS

Committee Chair: Binghe Wang
Committee: Kathryn B. Grant
Maged M. Henary

Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
August 2014
DEDICATION

To my family, especially my mum, Vicky, who never doubted me from the very beginning and has supported me through the myriad of academic decisions I have made, may it be good or otherwise, I dedicate this work to you.

Ad Maiorem Dei Gloriam
ACKNOWLEDGEMENTS

The road to the completion of this work had been both extremely tumultuous and enriching and I would not be able to make it through without the help of a lot of people. I would first like to thank Dr. Binghe Wang for his continuous support despite multiple failures that I have encountered as a student. He never doubted my skills and pushed me to always seek perfection whilst maintaining humility. I am forever indebted with the knowledge that he imparted with me for the last three years that I have been working in his lab both as an undergraduate and a graduate student. I would also want to thank my committee members, Dr. Maged Henary and Dr. Kathryn Grant. They have been such an essential part of my academic career. Dr. Henary has always pushed me to strive for academic excellence; rest assured that whenever I feel tired as I pursue future studies, his voice telling me to work harder will resonate and hopefully will be enough for me to keep going. Dr. Grant has inspired me to learn a different field of chemistry and how I can integrate it with my own research, for this I will be forever grateful. I also would like to thank Dr. Jyotsna Thota who ultimately pushed me to continue on as a chemist because she thought my knowledge would be wasted if I carried on with my plans on studying pharmacy. To this day, I still thank her because she was the first person who taught me how wonderful chemistry is.

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This thesis is for all of you! I hope I made all of you proud. Hoya Saxa.
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<th>Description</th>
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<tr>
<td>HIF-1</td>
<td>Hypoxia Inducible Factor 1</td>
</tr>
<tr>
<td>ARNT</td>
<td>Aryl hydrocarbon receptor nuclear translocator</td>
</tr>
<tr>
<td>PHD</td>
<td>Prolyl hydroxylase dioxygenase</td>
</tr>
<tr>
<td>FIH-1</td>
<td>Factor inhibiting HIF-1</td>
</tr>
<tr>
<td>ODD</td>
<td>Oxygen degradation domain</td>
</tr>
<tr>
<td>HRE</td>
<td>Hypoxia response element</td>
</tr>
<tr>
<td>pVHL</td>
<td>Von Hippel Lindau tumor suppressor protein</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Glucose transporter 1</td>
</tr>
<tr>
<td>LDHA</td>
<td>Lactose dehydrogenase A</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>CBP</td>
<td>CREB-binding protein</td>
</tr>
<tr>
<td>AP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>Luc</td>
<td>Luciferase</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure activity relationship</td>
</tr>
<tr>
<td>r.t.</td>
<td>Room temperature</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4]undec-7-ene</td>
</tr>
<tr>
<td>NaCNBH₄</td>
<td>Sodium cyanoborohydride</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>InCl₃</td>
<td>Indium (III) chloride</td>
</tr>
<tr>
<td>K₂CO₃</td>
<td>Potassium carbonate</td>
</tr>
<tr>
<td>KI</td>
<td>Potassium iodide</td>
</tr>
<tr>
<td>Cul</td>
<td>Copper (I) iodide</td>
</tr>
<tr>
<td>Symbol</td>
<td>Chemical Name</td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1 Cancer and Hypoxia

Among the persistent deadly diseases mankind still face, cancer is well known as one of the most varied and tenacious. Years of research have been spent towards the search for an effective therapy that minimizes the side effects current treatments come with. Although a lot has been achieved in this field, cancer is still the second leading cause of death in the U.S.A., second only to heart attack with 186 deaths occurring per 100,000 populations.\(^1\) Despite the tremendous amount of funding poured into research in this field, the sheer number of treatment pathways to explore is a daunting task.

Hypoxia, a pathological condition in which cells are deprived of an adequate oxygen supply, is generally detrimental to cells and tissues since the condition will eventually lead to necrosis. However, cancer cells thrive under such conditions.\(^2\) Hypoxia is commonly seen in progressing cancer cells; the inadequate oxygen supply in cells causes tissue hypoxia, which leads to the activation of several pathways, and further development and metastasis of the tumor.\(^2\) Cancer cells under such conditions turn on gene expressions that can alter metabolism and promote angiogenesis through multiple pathways, thereby allowing cells to fuel their progression. One example is the hypoxia-inducible factor 1 pathway (HIF-1). In tumor cells, the vasculature is different from normal cells. In addition to the normal vessels that the cells already have even before the tumor invades, most tumor cells develop microvessels that are brought about by neovascularization caused by the increased expression of genes that promote angiogenesis.\(^3\)
1.2 Hypoxia-Inducible Factor 1 Pathway (HIF-1)

There are three members of the hypoxia-inducible factor family whose activities rely upon the concentration of oxygen in the cell (Table 1).

<table>
<thead>
<tr>
<th>HIF</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>Alpha subunit, initiator of HIF function sequence</td>
</tr>
<tr>
<td>HIF-1β</td>
<td>Aryl hydrocarbon receptor nuclear translocator</td>
</tr>
<tr>
<td>HIF-2α</td>
<td>PAS domain protein</td>
</tr>
<tr>
<td>HIF-2β</td>
<td>Aryl hydrocarbon receptor nuclear translocator 2</td>
</tr>
<tr>
<td>HIF-3α</td>
<td>Alpha subunit (without transactivation domain)</td>
</tr>
<tr>
<td>HIF-3β</td>
<td>Aryl hydrocarbon receptor nuclear translocator 2</td>
</tr>
</tbody>
</table>

Although each subunit shares in the response to oxygen levels in the cell, the HIF-1 unit is the pathway that was focused on this study. The HIF-1 complex is a basic loop-helix-loop motif heterodimer that is composed of two major subunits called the HIF-1α and the HIF-1β. Levels of HIF-1α in the cells are regulated by the presence or absence of oxygen while HIF-1β is constitutively expressed as aryl hydrocarbon receptor nuclear translocator (ARNT). Under normal oxygen conditions, HIF-1α gets hydroxylated by prolyl hydroxylase dioxygenase (PHD) at proline 564 and 402 and by factor inhibiting HIF-1 (FIH-1) at asparagine 803 at its oxygen degradation domain (ODD) with an iron (Fe²⁺) and 2-oxoglutarate as co-substrates. Furthermore, HIF-1α gets acetylated by ADP-ribosylation factor domain protein 1 (ARD1) at lysine 532. This hydroxylation allows the HIF-1α to recruit the von Hippel-Lindau tumor suppressor protein (pVHL) that acts as an E3-ubiquitin ligase complex and tags the whole complex for ubiquitination and subsequent proteasomal degradation. This scenario is shown in Figure 1 as follows.
As depicted in Figure 2, prolyl hydroxylase loses its function to hydroxylate HIF-1α under hypoxic conditions. The HIF-1α subunit then gets translocated into the nucleus where it binds to another HIF subunit, HIF-1β.

Upon binding of HIF-1α with HIF-1β to form the active transcription factor of HIF-1, the complex then interact with its co-activator p300/CBP and binds to hypoxic
response elements (HREs) to act as a transcription factor for numerous genes.\(^9\) Because of the importance of oxygen in cells, it is not surprising that the HIF-1 complex is a transcription factor that controls more than 2\% of all human genes in arterial endothelial cells, directly or indirectly.\(^8\) Currently, it is well understood that the HIF-1 complex affects over 100 genes, most of which are vital to a cell’s survival. Among these genes are the vascular endothelial growth factor (VEGF), nitric oxide synthase (NOS), glucose transporter 1 (GLUT1), and lactose dehydrogenase A (LDHA).\(^10\) All of these genes that were mentioned play a massive role in the proliferation of a tumor cell since they fuel its growth through the formation of new vasculature and the creation of delivery pathways to sustain its propagation.

<table>
<thead>
<tr>
<th>Genes Activated by HIF-1</th>
<th>Effects on Cancer Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANGPT2, C-MET, ID2, NOS, PGF, PDGF, SCF, SDF-1, VEGF</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td>DEC1, MDR1</td>
<td>Genetic Instability</td>
</tr>
<tr>
<td>GLUT1, GP1, HK1, HK2, LDHA, PDK1, PKM2</td>
<td>Glucose Metabolism</td>
</tr>
<tr>
<td>TERT</td>
<td>Immortalization</td>
</tr>
<tr>
<td>NTSE</td>
<td>Immune Evasion</td>
</tr>
<tr>
<td>C-MET, EDN-1, FN-1, MMP-2, MMP-14, PLAU, SDF1</td>
<td>Invasion</td>
</tr>
<tr>
<td>ANGPT4, C-MET, CXCR4, LOX, MDR1, TWIST1, ZEB1</td>
<td>Metastasis</td>
</tr>
<tr>
<td>CA-IX, CA-XII</td>
<td>pH Regulation</td>
</tr>
<tr>
<td>C-MYC, ID2, IGF-2, NOS, PDGFB</td>
<td>Proliferation</td>
</tr>
<tr>
<td>ABCG2, JARID1B, OCT4</td>
<td>Stem Cell Maintenance</td>
</tr>
</tbody>
</table>

Many of the genes that the HIF-complex activates contribute to the defense mechanism of a cancer cell, as seen in Table 2. One of the most prominent features of the HIF-1 complex is that it activates genes that promote angiogenesis and glucose metabolism. Oftentimes, the cell’s environment gets further aggravated because of temporary occlusions and blocked vasculatures, a condition which
ultimately results into prompting the cell’s mechanism to begin the formation of new vasculature. With this acquired ability, the tumor cell can invade its surroundings to begin the growth of a secondary tumor and continue its malignancy. Once angiogenesis has commenced in tumor cells, nutrients can be supplied to these cells and their waste products removed. Cancer cells can also use these blood vessels to metastasize and pass through the blood vessels to distant sites where they can implant to start the growth of a new tumor. With this concurring metastasis, secondary tumor cells will need to develop their own ways of proliferation. In low oxygen conditions, tumor cells utilize anaerobic glycolysis to produce energy. Because of the low concentration of ATP that is produced using this cycle, tumor cells compensate by increasing glucose uptake through the increase of the production of glucose transporters such as GLUT1.\(^8\)

1.3 Chemo and Radiotherapy Resistance of Tumor Cells

Hypoxia in tumor cells has been shown to be a contributor to chemo and radioresistance. A factor that contributes to chemoresistance of tumor cells is the apparent pH changes that occur within the environment of the cells. It was previously thought that hypoxia and acidosis in tumor cells concomitantly occur because of the lactate produced when oxidative phosphorylation gets switched to anaerobic glycolysis; but this is not the case.\(^{11}\) It was shown that when tumor cells in hypoxic conditions are subjected to both buffered and non-buffered media, the cells survived in the buffered medium and the cells induced apoptosis in the non-buffered medium. One can infer from this study, therefore, that in hypoxic cells that are not experiencing acidosis, tumor cells can survive freely and have advantage over
healthy cells since programmed cell death would not commence. Most anti-cancer drugs that are on the market require molecular oxygen to function. Drugs such as melphalan, an antitumor alkylating agent, have a higher chance of permeating the tumor cells and interacting with their DNA under normoxic conditions rather than a hypoxic tumor cell. This phenomenon was seen when tumor cells were introduced into hypoxic conditions with drugs such as doxorubicin, another widely used anticancer drug. When doxorubicin was introduced to both normoxic and hypoxic tumor cells, the survival rate of the latter was increased. Furthermore, when cancer cells experience acidosis, their microenvironment becomes an area where cellular uptake of drugs is decreased. The acidic extracellular environment of cancer cells contributes to chemoresistance due to some anticancer drugs having a weakly basic character (i.e. doxorubicin, mitoxantrone, vincristine and vinblastine). As mentioned earlier, cancer cells have abnormal vasculatures and as such, there is limited blood flow to most areas in the cell that experiences hypoxia. Because of this, nutrients and drugs find it hard to reach these sites and thus contribute to chemoresistance. Another common way to treat cancer is through the use of radiotherapy. Radiotherapy does damage to tumor cells through induction of oxidative stress. When a malignant cancer cell is irradiated, either the DNA molecule itself or its environment that contains water molecule produce reactive oxygen species (ROS) that induce oxidative stress in the cell that can lead to DNA damage. However, upon radiation and production of ROS, a study found that radiation itself induced HIF-1 activity in the tumor cells that led to the expression of VEGF and basic fibroblast growth factor (bFGF) that enhances endothelial cell radioresistance. Because of these reasons, the HIF-1 complex is a very attractive target for cancer therapy.
1.4 HIF-1 Inhibitors

Throughout the years since its discovery and evident route for anticancer therapy, many have pursued HIF-1 complex inhibitors. Most of the advances in this endeavor have been focused on the synthesis of small molecules that inhibit the HIF-1 pathway. Many of the therapeutic candidates that have been synthesized and are currently in preclinical studies have the target of altering the concentration of the HIF-1α subunit by interfering with its translation, degradation or its transcriptional activity. One of the major subclasses of HIF-1 inhibitors consists mainly of heterocycle-based inhibitors that are derived from natural products.

To start this project, the laboratory of Dr. Erwin Van Mein in Emory University started the search for a small-molecule that shows potential HIF-1 complex inhibition using combinatorial chemistry. Some 10,000 compounds were screened from a 2,2-dimethylbenzopyran library. The chosen compounds were tested against human glioma cells that were transfected with an HRE-alkaline phosphatase reporter gene to measure their IC$_{50}$ values. From this library, a potential inhibitor, KCN-1 (Figure 3), was found, and was proven to be efficacious in inhibiting cancer growth.

![Figure 3 Molecular structure of KCN-1](image)

Although most HIF-inhibitors on the market affect the HIF-1α subunit by way of the HIF-1α’s concentration in the cell, KCN-1 was seen to affect the complex differently.
Through mechanistic studies, it was seen that KCN-1 interrupts the association of the HIF-1α/HIF-1β heterodimer from its interaction with its co-activators p300/CBP. This insight could lead to the synthesis of HIF-inhibitors that affect the HIF-1 complex through a different pathway.

2. DESIGN AND STRUCTURE OF INHIBITORS

2.1 KCN-1 Analogues

The main goal of the project was to synthesize more potent KCN-1 analogues with low to no toxicity. As seen in Figure 3, KCN-1 was divided into four regions for analogue synthesis: the benzopyran moiety (red), the nitrogen linker (blue), the sulfonamide moiety (green) and finally, the dimethoxy moiety (purple). Most of the changes done in this study were mostly focused on altering the nitrogen linker and the dimethoxy moiety in the hopes of synthesizing a more potent compound. Within this class, the most potent HIF-1 inhibitor previously synthesized by our group had an IC₅₀ of 0.25 µM (SRIV-64b, Figure 4) and was tested against KCN-1, showing a threefold increase in potency. With this lead, the synthesis of new HIF-1 sulfonamide analogues commenced.
2.1.1 Chemistry of Sulfonamide Analogues

The synthesis of such compounds started with the benzopyran moiety. With a hydroxyaryl aldehyde and a tertiary alkyl halide as a starting material, the two compounds were condensed in the presence of a non-nucleophilic base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in an SN1 fashion (Equation 1).

\[
\begin{align*}
\text{HO} & \quad \text{CHO} \quad \text{Cl} \quad \text{DBU, ACN} \\
0^\circ \text{C to r.t.} & \quad 6\text{h} \\
\end{align*}
\]

After this step, the cyclization of the ring occurred by subjecting the condensed product to basic conditions at 170 °C. (Equation 2).

\[
\begin{align*}
\text{的选择} & \quad \text{CHO} \\
\text{N,N-dimethylaniline} & \quad 170^\circ \text{C, 12h} \\
\end{align*}
\]

This ring closure is afforded through a Claisen rearrangement whose mechanism is shown in Figure 5. Once the allene moiety is formed, the oxyanion attacked the sp\(^2\) carbon to form the benzopyran moiety. However, the oxyanion could have attacked the sp carbon of the allene to form a benzofuran ring. The preference of the reaction to form the benzopyran ring can be rationalized by the angle of attack by the nucleophile.
If one were to analyze this mechanism using Baldwin’s rules of ring closure, it can be seen that both products shown in Figure 6 could form. However, if the orientation between the oxyanion nucleophile and the electrophilic allene carbon were to be considered, the main reason as to why the formation of the benzopyran path (green) is favored over its counterpart is probably due to the angle from which it attacked the electrophile and how the dimethyl group faced the anionic specie. The dimethyl group was orthogonal to the sp-carbon in this case, which exposed the terminal carbon (sp²) of the allene more to a nucleophilic attack.
After the synthesis of the left hand core, the nitrogen linker was introduced by reacting either an aliphatic or aromatic primary amine with the aldehyde to produce a secondary amine through reductive amination (Equation 3). The chosen nitrogen moieties for this scaffold in this study were aniline and cyclobutylamine (scaffolds for KCN-1 (X = C) and SR-IV-64b (X = N) respectively). Once the secondary amine had been formed, the next step was the introduction of the sulfonyl moiety by a sulfonylation reaction (Equation 4).

Different sulfonylation reactions were done to produce a variety of sulfonamide analogues (Scheme 1). The main group that was focused on in this scaffold was the
right hand core (sulfonyl moiety) to diversify the SAR studies done of previous analogues.

2.2 Structure Activity Relationships (SAR) of Previously Synthesized Analogues

As seen in Figure 7, the analogues synthesized follow a general scaffold in this study. The previous SAR profile demonstrated that on the left hand core, benzopyrans and pyrano [3,2b] pyridines showed an increase in inhibition activity. Although other fused rings are tolerated, it did not show an increase in potency as compared to KCN-1. The geminal dimethyl moiety on both the benzopyran and pyridinyl rings also showed significance since their loss in the scaffold led to a decrease in activity. For the nitrogen linker, the activity was at its best when the substituents on the nitrogen were an aromatic ring and cycloalkyl rings of less than 6-carbons in size; furthermore, it was seen that short alkyl chains were tolerated as well. It was also generally seen that the sulfonamide group itself (N-SO$_2$) was crucial for biological inhibition. Lastly, for the right hand core, it was seen that the activity was more pronounced if an aromatic group was present that had substituents on the para position as opposed to meta substituted aromatic rings.$^6$ In choosing the variation that were to be made with the compounds, the Lipinski’s Rule of Five was

---

$^a$ All of the SAR studies were obtained from Dr. Suazette Mooring’s studies.$^6$
selected to be the criteria to be followed. This allowed our group to synthesize compounds that followed general rules when considering what functional groups that were chosen to be introduced into the new analogues.

### 2.3 Manassantin B Scaffold

During the course of this project, about 200 compounds have been synthesized by our group. This library consists purely of sulfonamide analogues and only about 20% of the compounds in the library were active. In the hopes of finding a much more potent candidate of HIF-1 inhibitors, new scaffolds were being investigated as a potential HIF-1 inhibitors.

Manassantin B, a natural product that is extracted from the plant *Saururus cernuus* (common name: lizard’s tail) along with another extract, 4-O-demethylmanassantin have been discovered to affect the HIF-1 complex and inhibit its activity with IC$_{50}$ values in the range of 3 and 30 nM respectively.$^{19}$

![Figure 8 Molecular structure of Manassantin B](image)

Manassantin B and 4-O-demethylmanassantin only differ by one methoxy group on the right hand side of the molecule; however, studies show that this slight change gives Manassantin B a tenfold increase in its potency in inhibiting the HIF-1 activity.$^{19}$
With this knowledge in hand, the synthesis of analogues that followed this scaffold commenced. The structure of Manassantin B is very complex; it would have been very tedious if synthesis of a very similar analogue were to be done solely for trial purposes. If the structure were taken into consideration, there is a relative similarity between the left side and the right side of the molecule that is only separated by a furan ring. Therefore, it was decided to direct the synthesis of analogues towards creating compounds that looked similar to one side of the natural product.

![Simplified structure of scaffold based on Manassantin B]

2.3.1 Chemistry of Manassantin B-like analogues

Looking at the general structure shown in Figure 9, there are three structural groups that are important in this scaffold: the left core, the right core, and the hydroxymethine moiety. Different substitutions can be made with both left and right cores to see if they affect the HIF-1 complex as effectively as the natural product. The chemistry of these analogues usually starts with the preparation of both aryl groups (left and right core) separately before combining them (Equation 5). One of the aryl groups is usually prepared as a substituted aryl bromide and the other aryl group is usually prepared as a substituted aryl aldehyde. These two groups are condensed using n-butyllithium under an inert atmosphere at -78°C as seen in Equation 5.
Equation 5 Condensation of the two cores for Manassantin-B derived analogues. R₁ and R₂ groups are listed in Figure 10

2.4 Biological testing of analogues

After the synthesis of the HIF-1 inhibitors, our collaborators at the Van Meir laboratory performed the biological testing of the analogues to investigate their HIF-1-inhibiting activity. Using the plasmid construct with the six copies of the HRE of the VEGF gene that are upstream of a luciferase reporter gene, the IC₅₀ of each analogue was reported using a concentration curve at 0, 1, 2, 3 and 5 µM with SR-IV-64b (Fig. 4) as a standard to compare the IC₅₀ values to. In the results section of this study, any analogue that showed activity beyond 5 µM were not further tested and was designated to have activities >5 µM. These compounds’ activities were not further evaluated and thus their exact inhibiting activity, or lack thereof, was unknown. Furthermore, the IC₅₀ value of SR-IV-64b was not constant and had a range of 0.28 ± 0.12 µM in various experiments.

3. RESULTS AND DISCUSSION

3.1 Sulfonamide Analogues

Using the SAR profile that was generated from previous studies, a couple of new analogues were synthesized. The main variation that was done on these analogues
targeted the nitrogen linker and the right hand core. The left hand core kept the 2,2-dimethylbenzopyran group and the sulfonamide group was also retained. Below is the table of the sulfonamide analogues synthesized with their corresponding IC$_{50}$ and calculated LogP values which is the partition coefficient between hydrophobic and hydrophilic media.$^{5,20}$

Table 3 List of sulfonamide analogues synthesized with the 2,2-dimethylbenzopyran moiety kept intact

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Mol. Wt. (g/mol)</th>
<th>cLogP</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td><img src="image" alt="Structure 3" /></td>
<td>489.12</td>
<td>3.74</td>
<td>&gt;5</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Structure 4" /></td>
<td>467.14</td>
<td>3.80</td>
<td>&gt;5</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure 5" /></td>
<td>402.51</td>
<td>2.51</td>
<td>&gt;5</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Structure 6" /></td>
<td>441.12</td>
<td>2.92</td>
<td>&gt;5</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Structure 7" /></td>
<td>419.14</td>
<td>3.24</td>
<td>&gt;5</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Structure 8" /></td>
<td>430.14</td>
<td>2.48</td>
<td>&gt;5</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Structure 9" /></td>
<td>427.18</td>
<td>3.22</td>
<td>4.37</td>
</tr>
</tbody>
</table>

$^{5}$ All LogP values were calculated using a virtual computational chemistry program. (VCCLAB, Virtual Computational Chemistry Laboratory, [http://www.vcclab.org](http://www.vcclab.org), 2005.)
With the series of compounds synthesized and comparing it to KCN-1 and SRIV-64b, it can be seen that there was a decrease in biological activity once the right hand core was changed. Thus, it can be generalized that the dimethoxy group on both the para and meta positions of the aromatic ring on the sulfonamide analogues are necessary for biological activity. Of all the analogues that were synthesized in this class, only compound 9 was seen to remotely inhibit the activity of the HIF-1 complex. This also proves that the dimethoxy group on the right hand is arguably a required group for biological inhibition since compound 9 is the only compound in the group that remotely resembles the active analogue (Figure 4). Furthermore, it can also be noted that although almost all analogues synthesized were inactive, all of them had cLogP values that were less than five, which is considered to be the upper limit of desired lipophilicity based on Lipinski’s Rule of Five that was used throughout this study.

3.1.1 Morpholine group introduction

Another approach that was taken during this study was the introduction of other ring structures into the sulfonamide group. For this particular study, we were hoping that the introduction of the morpholine group into our compounds would improve the analogues’ aqueous solubility while maintaining potency without introducing any further cellular toxicity. The chemistry of the analogues synthesized with this group followed the same procedures that were mentioned previously. This procedure used 4-morpholinylbenzaldehyde as a starting material rather than its chromene counterpart (Scheme 1). The morpholine moiety was both introduced into the left hand core of the scaffold as well as the nitrogen linkers. The analogues were
subjected to same biological testing, and their IC$_{50}$ values were measured and LogP calculated.

Table 4 List of sulfonamide analogues synthesized with morpholine groups

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Mol. Wt.</th>
<th>cLogP</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>![Structure 10]</td>
<td>448.20</td>
<td>3.51</td>
<td>1.23</td>
</tr>
<tr>
<td>11</td>
<td>![Structure 11]</td>
<td>418.19</td>
<td>3.41</td>
<td>&gt;5</td>
</tr>
<tr>
<td>12</td>
<td>![Structure 12]</td>
<td>413.18</td>
<td>2.97</td>
<td>&gt;5</td>
</tr>
<tr>
<td>13</td>
<td>![Structure 13]</td>
<td>446.19</td>
<td>3.33</td>
<td>2.62</td>
</tr>
<tr>
<td>14</td>
<td>![Structure 14]</td>
<td>502.21</td>
<td>3.13</td>
<td>&gt;5</td>
</tr>
<tr>
<td>15</td>
<td>![Structure 15]</td>
<td>516.23</td>
<td>3.50</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

Looking at Table 4, the first change that was made was to introduce the morpholine ring into the left hand core of the scaffold. This was done in conjunction with the nitrogen linker having either a cyclobutyl or n-butyl substituent on the nitrogen atom (compounds 10-13). From this set, it can be seen that although the calculated LogP is comparably the same with the first set of sulfonamides synthesized, this set of analogues were relatively more active. This biological activity was perhaps caused by the combination of the morpholine group in tandem with an aliphatic nitrogen.
linker. The next change that was made in the scaffold was to introduce the morpholine group into the nitrogen linker and keep the 2,2-dimethylbenzopyran and dimethoxybenzyl group with the right hand and left hand core. As seen in Table 4, by introducing the morpholine group, there was not much difference that was seen in solubility; however, it is notable that compounds 14 and 15 lost their activities when the morpholine group was introduced. As it was previously mentioned that the dimethoxy group was necessary for biological activity; therefore, the morpholine group was not introduced into the right hand core.

3.2 Manassantin-B derived Analogues

For the synthesis of Manassantin B-derived analogues, the focus of variation was first directed towards the right hand side of the molecule as can be seen in the two compounds that were synthesized in Table 5. These compounds contain a thioether ring that replaced the normal benzopyran group. This was done to diversify the compounds that were already synthesized. The synthesis of such compounds was done using the procedure described below.

Scheme 2 Reaction procedure to synthesize the aryl-sulfane and thiophene moieties
Looking at compounds 18 and 19, one important observation can be made. The structural difference between Manassantin B and 4-O-demethylmanassantin was only one methoxy group at the meta position of Manassantin B. This difference gave a tenfold increase in potency to the said molecule. Reviewing the compounds in Table 5, it can be seen that even if the structure did not mirror the natural product, it is still very obvious that the compound lost its biological activity once the methoxy group on the meta position of the aryl was eliminated. This experiment gives a good starting point for the SAR profile on the right core since it proves how necessary the dimethoxy moiety is.

### 3.2.1 Importance of Hydroxymethine in the Scaffold

Variation was also done on this new scaffold by changing the hydroxymethine group of the molecule. The efforts to change the group on the left hand side of the molecule have been substantial and our group has developed analogues that have very promising IC\textsubscript{50} values. Therefore, for this particular study, it was decided to shift the focus to the hydroxymethine group and to observe its activity once it was changed based off of analogues that were previously synthesized. Five analogues whose variation was mainly focused on the left hand ring were chosen to see if their activity would change once the hydroxymethine group was varied. With this scaffold,
only the hydroxymethine group was changed and the right core of the analogues contained the dimethoxy groups in the para and meta positions of the aromatic ring. These five analogues were chosen as base compounds since they were either active as sulfonamide or as Manassantin B-like compounds. By oxidizing the hydroxy group into ketones using pyridinium chlorochromate, their activities were observed to see if any changes in the hydroxymethine group were significant enough in the gain or loss of biological inhibition.

The oxidation of the hydroxymethine group into its ketone counterpart was done using PCC oxidation and is described in Equation 8.

Moreover, the hydroxymethine compounds that were used to synthesize the ketone products are listed below.
Figure 11 List of hydroxymethine compounds used to make the ketone products.

In Figure 11, the figures for the five analogues whose left core was varied are drawn. The summary of their activities and their respective cLogP values are listed in Table 6.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Mol. Wt.</th>
<th>cLogP</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td><img src="image" alt="Structure 29" /></td>
<td>324.14</td>
<td>4.36</td>
<td>&gt;5</td>
</tr>
<tr>
<td>30</td>
<td><img src="image" alt="Structure 30" /></td>
<td>325.36</td>
<td>3.49</td>
<td>&gt;5</td>
</tr>
<tr>
<td>31</td>
<td><img src="image" alt="Structure 31" /></td>
<td>455.23</td>
<td>4.05</td>
<td>4.50</td>
</tr>
<tr>
<td>32</td>
<td><img src="image" alt="Structure 32" /></td>
<td>455.23</td>
<td>4.02</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>
As seen in Table 6 and the comparison that was made in Table 7 below, it is obvious that the hydroxyl group on the hydroxymethine carbon proved to be highly important since its elimination led to a complete depletion of HIF-1 activity, as clearly seen in the case of the most potent compound in the list, compound 32. This result gives a clear view of what future directions to take and focus can be directed on the left core of the molecule.

Table 7 Comparison of the data obtained for the hydroxymethine and ketone analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>cLogP</th>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>cLogP</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 24" /></td>
<td>&gt;5</td>
<td>4.23</td>
<td><img src="image" alt="Structure 29" /></td>
<td>&gt;5</td>
<td>4.36</td>
</tr>
<tr>
<td><img src="image" alt="Structure 25" /></td>
<td>2.25</td>
<td>3.47</td>
<td><img src="image" alt="Structure 30" /></td>
<td>&gt;5</td>
<td>3.49</td>
</tr>
<tr>
<td><img src="image" alt="Structure 26" /></td>
<td>0.89</td>
<td>3.55</td>
<td><img src="image" alt="Structure 31" /></td>
<td>4.50</td>
<td>4.05</td>
</tr>
<tr>
<td><img src="image" alt="Structure 27" /></td>
<td>0.20</td>
<td>3.52</td>
<td><img src="image" alt="Structure 32" /></td>
<td>&gt;5</td>
<td>4.02</td>
</tr>
<tr>
<td><img src="image" alt="Structure 28" /></td>
<td>&gt;5</td>
<td>2.63</td>
<td><img src="image" alt="Structure 33" /></td>
<td>&gt;5</td>
<td>3.06</td>
</tr>
</tbody>
</table>
3.2.2 Miscellaneous Compounds

In the hopes of further diversifying the library of the new scaffold, two compounds were made to test synthetic procedures that would mimic the reactions that were planned for the ketone moieties synthesized. The carbonyl group of the ketones synthesized was reacted with hydroxylamine and hydrazine to form the oxime and hydrazone groups respectively. These compounds, albeit slightly different from the original scaffold, were submitted for biological testing to observe their activity. The compounds are listed in Table 7 and their respective reaction procedures can be seen below.

Scheme 3 Reaction procedures for the synthesis of the miscellaneous compounds
Table 8 Miscellaneous compounds synthesized for synthetic purposes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Mol. Wt.</th>
<th>cLogP</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td><img src="image1" alt="Structure" /></td>
<td>257.11</td>
<td>3.19</td>
<td>&gt;5</td>
</tr>
<tr>
<td>35</td>
<td><img src="image2" alt="Structure" /></td>
<td>377.14</td>
<td>5.31</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

The two compounds that are seen in Table 7 were found to be inactive towards HIF-1 inhibition. However, the synthetic procedures that were used to make such compounds were successful and can be used to create new analogues that contain the oxime and hydrazone moieties.

4. Conclusion and Future Direction

Cancer has been plaguing humanity since the earliest records of medicine were made. Although medical advances have contributed immensely to the search for a good therapy to cure cancer, it is still a challenge for scientists to improve treatments by decreasing the therapies’ side effects while increase potency. The HIF-1 complex is a very attractive target for cancer therapy because of its role in tumor biology. Its role as an angiogenic promoter, among other things, can be exploited to create small molecules that would inhibit a tumor’s function and thereby inhibiting progression of cancer cells. In this study, a total of 22 compounds were synthesized that fell on three different classes of compounds. Most of the compounds that were synthesized in this study included sulfonamide analogues that give further diversity.
with the previously created library and will further elucidate the SAR profile of the sulfonamide class of HIF-1 inhibitors we have studied thus far. Some compounds were also synthesized that were based off of Manassantin B and have given us a new scaffold to investigate for HIF-1 inhibition. The last class of compounds that was synthesized were analogues of a potent Manassantin B-derived compound previously synthesized by other members of our group. These compounds proved that the hydroxymethine group in this particular scaffold holds great importance since its elimination leads to loss of inhibition. In the future, the observations made through this study will help in the synthesis of new and more potent HIF-1 inhibitors.
REFERENCES


13. Sullivan, R.; Pare, G. C.; Frederiksen, L. J.; Semenza, G. L.; Graham, C. H., Hypoxia-induced resistance to anticancer drugs is associated with decreased


Experimental:

All starting materials were obtained from Sigma-Aldrich, Acros Organics or Oakwood Chemicals and were used without further purification. Microwave heating was performed in a single-mode microwave cavity of a Discovery Synthesis System (CEM corp.) and the microwave-irradiated reactions were conducted in a heavy-walled glass vials sealed with Teflon septa. $^1$H and $^{13}$C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker Avance 400 NMR spectrometer using CDCl$_3$ containing tetramethylsilane (TMS) as internal calibration standard. Mass spectra analyses were performed by the mass spectrometry facilities at Georgia State University.

Procedures:

I. Sulfonamide analogues

\[ \text{4-((2-Methylbut-3-yn-2-yl)oxy)benzaldehyde (1)} \]

In a 100 mL round bottom flask, 2 g (0.015 mol) of p-hydroxybenzaldehyde was dissolved in dry acetonitrile (10 mL) and was cooled to 0 °C under inert atmosphere with argon gas. To this solution, 9.0 mL (0.06 mol) of DBU was added and the solution was allowed to stir for 30 min. 6.8 mL (0.06 mol) of 3-chloro-3-methylbut-1-yne was then added into the mixture drop wise. The solution was allowed to warm up to room temperature and was stirred overnight. The solution was concentrated and extracted with ethyl acetate (100 mL). The organic solution was washed with 1M HCl
solution (20 mL), 1M NaOH solution (20 mL), saturated NaHCO₃ solution (20 mL), and brine (20 mL) and was dried over Na₂SO₄. The organic layer was concentrated in vacuo and was taken directly to the next step.

![Chemical Structure](image)

**2,2-Dimethyl-2H-chromene-6-carbaldehyde (2)**

Using the impure product from the procedure above, the crude mixture was dissolved in N,N-dimethylaniline (<1 mL) and heated at 170 °C for 6 h. After cooling to room temperature, the organic layer was extracted with ethyl acetate (20 mL), and washed with IM HCl (20 mL x 3), saturated NaHCO₃ solution (20 mL), and brine 20 mL, and was dried over Na₂SO₄. The organic layer was concentrated in vacuo. The product was purified using flash column chromatography. Ethyl acetate and hexanes were used as eluent (1:8). Yield: 2 g, 37%. ^1H NMR (400 MHz, CDCl₃): δ 9.61 (s, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.28 (s, 1H), 6.64 (d, J = 8.0 Hz, 1H), 6.14 (d, J = 8.0 Hz, 1H), 5.47 (d, J = 8.0 Hz, 1H), 1.24 (s, 6H) ppm. ^13C NMR: δ 190.2, 158.3, 131.5, 131.3, 129.9, 127.7, 121.2, 121.0, 116.7 and 28.24 ppm.

**General procedure for the reductive amination of 2,2-dimethyl-2H-chromene-6-carbaldehyde:**

To a solution of 100 mg of aldehyde (1 eq.) and 21 mg of InCl₃ (0.15 eq.) in dry MeOH (10 mL) under Argon gas, the corresponding amount of amine (1.5 eq.) was added. The mixture was allowed to stir until completion as monitored by TLC. Upon consumption of the aldehyde, 36 mg of NaCNBH₄ (1.5 eq.) was added and was allowed to stir for 1 h. The reaction mixture was quenched with saturated 10 mL of
NH₄Cl solution. The organic layer was extracted with ethyl acetate (20 mL), washed with brine (20 mL) and dried over Na₂SO₄. The product was taken to the next step without further purification.

**General procedure for sulfonylation reactions of the secondary amine:**

In a 50 mL round-bottom flask, 50 mg of the crude secondary amine (1 eq.) in DCM (10 mL) was added the corresponding amounts of K₂CO₃ (2 eq.), then appropriate amounts of the sulfonyl chloride moiety (2 eq.). The reaction mixture was stirred at room temperature for 24 h. The solution was then washed with deionized water (10 mL) and the organic layer was extracted with DCM (10 mL x 2), washed with brine (20 mL), dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified using flash column chromatography. Ethyl acetate and hexanes were used as eluent (1:8).

\( \text{N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-N-phenyl-4-} \)
\( \text{(trifluoromethoxy)benzenesulfonamide (3)} \) – Yield: 18 mg, 18%. \(^1\)H NMR: \( \delta 8.13-8.11 \) (d, \( J = 8.8 \) Hz, 1H), 7.69 (d, \( J = 8.0 \) Hz, 2H), 7.44 (d, \( J = 8.8 \) Hz, 1H), 7.30 (d, \( J = 8.0 \) Hz, 2H), 7.23-7.22 (m, 2H), 6.96 (d, \( J = 3.2 \) Hz, 2H), 6.86 (d, \( J = 8.8 \) Hz, 1H), 6.59 (d, \( J = 8.0 \) Hz, 1H), 6.22 (d, \( J = 10.0 \) Hz, 1H), 5.57 (d, \( J = 10.0 \) Hz, 1H), 4.63 (s, 2H), 1.38 (s, 6H) ppm. \(^{13}\)C NMR: \( \delta 138.6, 137.3, 131.0, 129.8, 129.5, 129.4, 129.1, 129.0, 128.1, 127.7, 126.7, 122.0, 121.7, 121.2, 121.1, 120.6, 116.1, 54.6, 28.0 \) ppm. HRMS (ESI) \( m/z \) calculated for \( \text{C}_{25}\text{H}_{22}\text{F}_{3}\text{NO}_{4}\text{S} + \text{Na}^+ \): 512.1119, found 512.1106.
\[ \text{N-Cyclobutyl-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-4-} \\
\text{(trifluoromethoxy)benzenesulfonamide (4) – Yield: 66 mg, 48%.} \]

{\text{\textsuperscript{1}H NMR: } \delta 7.82 (d, J = 8.4 \text{ Hz, 2H}), 7.32 (d, J = 8.4 \text{ Hz, 2H}), 7.03 (d, J = 8.4 \text{ Hz, 1H}), 6.96 (s, 1H), 6.72 (d, J = 8.0 \text{ Hz, 1H}), 6.30 (d, J = 9.6 \text{ Hz, 1H}), 5.63 (d, J = 9.6 \text{ Hz, 1H}), 4.33 (s, 2H), 4.26-4.18 (m, 1H), 2.03-1.99 (m, 4H), 1.58 (m, 2H), 1.44 (s, 6H) ppm.} \text{ \textsuperscript{13}C NMR: } \delta 152.3, 151.9, 139.0, 131.1, 129.9, 129.1, 127.9, 125.3, 122.2, 121.3, 120.8, 116.2, 52.9, 48.2, 29.3, 28.0, 15.0 ppm. \text{HRMS (ESI) m/z calculated for } C_{23}H_{24}NO_{4}F_{3}S + \text{Na}^+: 490.1276, \text{found 490.1278.}}

\[ \text{N-Cyclobutyl-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-3,5-dimethylisoxazole-} \\
\text{4-sulfonamide (5) - Yield: 47 mg, 43%.} \]

{\text{\textsuperscript{1}H NMR: } \delta 7.02 (d, J = 8.4 \text{ Hz, 1H}), 6.91 (s, 1H), 6.72 (d, J = 8.4 \text{ Hz, 1H}), 6.31-6.28 (d, J = 10.0 \text{ Hz, 1H}), 5.65-5.62 (d, J = 10.0 \text{ Hz, 1H}), 4.41 (s, 2H), 4.28-4.10 (m, 1H), 2.58 (s, 3H), 2.39 (s, 3H) 2.09-2.04 (m, 4H), 1.62-1.52 (m, 2H) 1.43 (s, 6H) ppm.} \text{ \textsuperscript{13}C NMR: } \delta 172.7, 157.3, 152.4, 131.3, 129.5, 127.8, 125.2, 122.1, 121.4, 117.5, 116.3, 52.2, 47.8, 29.3, 27.9, 15.0, 12.8, 11.1 ppm. \text{HRMS (ESI) m/z calculated for } C_{21}H_{26}N_{2}O_{4}S + \text{Na}^+: 425.1511, \text{found 425.1529.}
**N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-3,4-difluoro-N-phenylbenzenesulfonamide (6)** - Yield: 30 mg, 37%. $^1$H NMR: $\delta$ 7.50 (t, $J = 8.8$ Hz, 1H), 7.43 (d, $J = 8.4$ Hz, 1H), 7.32-7.26 (m, 4H), 6.98-6.96 (m, 2H), 6.89 (d, $J = 10.8$ Hz, 2H), 6.62 (d, $J = 10.0$ Hz, 1H), 6.24 (d, $J = 10.0$ Hz, 1H), 5.59 (d, $J = 10.0$ Hz, 1H), 4.65 (s, 2H), 1.41 (s, 6H) ppm. $^{13}$C NMR: $\delta$ 152.6, 138.4, 131.0, 129.4, 129.1, 129.0, 128.3, 127.6, 126.7, 122.0, 121.2, 118.0, 117.8, 116.2 ppm. HRMS (ESI) $m/z$ calculated for C$_{24}$H$_{21}$NO$_3$F$_2$S $+$ Na$^+$: 464.1108, found 464.1097.

**N-Cyclobutyl-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-3,4-difluorobenzenesulfonamide (7)** - Yield: 37 mg, 29%. $^1$H NMR: $\delta$ 7.61-54 (m, 2H), 7.29-7.28 (m, 1H), 7.03 (d, $J = 8.0$ Hz, 1H), 6.96 (s, 1H), 6.72 (d, $J = 8.0$ Hz, 1H), 6.30 (d, $J = 9.6$ Hz, 1H), 5.64 (d, $J = 9.6$ Hz, 1H), 4.32 (s, 2H), 4.23-4.19 (m, 1H), 2.06-1.99 (m, 4H), 1.55-1.52 (m, 2H), 1.44 (s, 6H) ppm. $^{13}$C NMR: $\delta$ 152.4, 148.9, 137.4, 131.1, 129.7, 128.0, 125.3, 122.1, 121.3, 118.1, 117.9, 117.0, 116.8, 116.3, 52.8, 48.2, 29.3, 28.0, 15.0 ppm. HRMS (ESI) $m/z$ calculated for C$_{22}$H$_{23}$NO$_3$F$_2$S $+$ Na$^+$: 442.1264, found 442.1255.
4-Cyano-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-N-phenylbenzenesulfonamide (8) - Yield: 15 mg, 30%. $^1$H NMR: δ 7.77 (q, $J = 8.4$ Hz, 4H), 7.27 (t, $J = 5.2$, 3H), 6.95-6.93 (m, 2H), 6.89-6.85 (m, 2H), 6.62 (d, $J = 8.0$ Hz, 1H), 6.23 (d, $J = 10.0$ Hz, 1H), 5.60 (d, $J = 10.0$ Hz, 1H), 4.66 (s, 2H), 1.41 (s, 6H) ppm. $^{13}$C NMR: δ 152.7, 143.2, 138.2, 132.7, 131.1, 129.5, 129.2, 129.1, 128.4, 128.2, 127.3, 126.7, 122.0, 121.2, 116.3, 116.2, 54.9, 28.0 ppm. HRMS (ESI) $m/z$ calculated for C$_{25}$H$_{22}$N$_2$O$_3$S + Na$^+$: 453.1249, found 453.1260.

$N$-Cyclobutyl-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-4-ethoxybenzenesulfonamide (9) - Yield: 16 mg, 36%. $^1$H NMR: δ 7.69 (d, $J = 8.0$ Hz, 2H), 7.04 (d, $J = 8.4$ Hz, 1H), 6.98 (s, 1H), 6.93 (d, $J = 8.8$ Hz, 2H), 6.70 (d, $J = 8.0$ Hz, 1H), 6.29 (d, $J = 10.0$ Hz, 1H), 5.60 (d, $J = 10.0$ Hz, 1H), 4.26 (s, 2H), 4.18-4.10 (m, 1H), 4.10-4.05 (m, 2H), 2.00-1.90 (m, 4H), 1.52-1.44 (m, 5H), 1.42 (s, 6H) ppm. $^{13}$C NMR: δ 162.1, 152.1, 131.8, 130.9, 130.7, 129.1, 127.9, 125.3, 122.4, 121.2, 116.1, 114.5, 63.9, 52.9, 48.2, 29.2, 28.0, 15.1, 14.6 ppm. HRMS (ESI) $m/z$ calculated for C$_{24}$H$_{29}$O$_4$NS + Na$^+$: 450.1710, found 450.1709.

$N$-Butyl-3,4-dimethoxy-N-(4-morpholinobenzyl)benzenesulfonamide (10) - Yield: 10 mg, 23%. $^1$H NMR: δ 7.47 (d, $J = 8.0$ Hz, 1H), 7.28 (d, $J = 5.2$ Hz, 2H), 7.18 (d, $J = 8.0$ Hz, 2H), 6.95 (d, $J = 8.4$ Hz, 1H), 6.86 (d, $J = 8.4$ Hz, 2H), 4.26 (s, 2H), 3.97 (s, 3H), 3.92 (s, 3H), 3.87 (t, $J = 4.4$ Hz, 4H), 3.16 (t, $J = 9.2$ Hz, 4H), 3.09 (t, $J = 7.6$ Hz, 2H), 1.39-1.34 (m, 2H), 1.32-26 (m, 2H), 1.18 (t, $J = 7.2$ Hz, 3H) ppm. $^{13}$C
NMR: δ 152.3, 149.0, 132.2, 129.4, 121.0, 115.5, 110.6, 110.0, 66.9, 56.2, 51.0, 49.3, 47.3, 29.9, 19.9, 13.6 ppm. HRMS (ESI) m/z calculated for C_{23}H_{33}N_2O_5S [(M + H)^+]: 449.2110, found 449.2094.

**N-Butyl-4-methoxy-N-(4-morpholinobenzyl)benzenesulfonamide (11)** – Yield: 48 mg, 36%. ¹H NMR: δ 7.76 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 4.23 (s, 2H), 3.87-3.85 (m, 4H), 3.84 (s, 3H), 3.15-3.13 (m, 4H), 3.04 (t, J = 7.6 Hz, 2H), 1.35-1.27 (m, 2H), 1.17-1.09 (m, 2H), 0.76-0.72 (t, J = 7.2 Hz, 3H) ppm. ¹³C NMR: δ 162.6, 132.1, 129.4, 129.2, 115.6, 114.2, 66.9, 55.6, 51.1, 49.3, 47.3, 30.0, 19.9, 13.6 ppm. HRMS (ESI) m/z calculated for C_{22}H_{31}O_{4}N_2S [(M + H)^+]: 419.1999, found 419.1980.

**N-Butyl-4-cyano-N-(4-morpholinobenzyl)benzenesulfonamide (12)** – Yield: 21 mg, 33%. ¹H NMR: δ 7.92 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.4, 2H), 7.12 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 4.31 (s, 2H), 3.89-3.86 (m, 4H), 3.17-3.11 (m, 6H), 1.38-1.34 (m, 2H), 1.19-1.13 (m, 2H), 0.80 (t, J = 7.2 Hz, 3H) ppm. ¹³C NMR: δ 151.1, 144.9, 132.8, 139.4, 137.6, 126.5, 117.5, 115.9, 115.4, 66.8, 50.9, 49.1, 47.4, 29.9, 19.7, 13.6 ppm. HRMS (ESI) m/z calculated for C_{22}H_{28}O_{3}N_3S [(M + H)^+]: 414.1846, found 414.1857.
**N-Cyclobutyl-3,4-dimethoxy-N-(4-morpholinobenzyl)benzenesulfonamide (13)** –
Yield: 81 mg, 70%. \(^1\)H NMR: \(\delta\) 7.39 (d, \(J = 8.4\) Hz, 1H), 7.28-7.20 (m, 3H), 6.91 (d, \(J = 8.4\) Hz, 1H), 6.85 (d, \(J = 8.4\), 2H), 4.32 (s, 2H), 4.24-4.18 (m, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.86 (m, 4H), 3.15 (m, 4H), 2.02-1.89 (m, 4H), 2.05-1.90 (m, 4H), 1.55-1.25 (m, 2H) ppm. \(^1\)C NMR: \(\delta\) 152.3, 150.5, 149.0, 132.2, 129.8, 128.2, 120.9, 115.6, 110.6, 109.7, 66.9, 56.1, 56.2, 52.9, 49.4, 48.0, 29.7, 29.3, 15.1 ppm. HRMS (ESI) m/z calculated for C\(_{23}\)H\(_{31}\)O\(_5\)N\(_2\)S [(M + H)\(^+\)]: 446.1948, found 447.1949.

**N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-3,4-dimethoxy-N-(2-morphinoethyl)benzenesulfonamide (14)** – Yield: 31 mg, 49%. \(^1\)H NMR: \(\delta\) 7.45 (d, \(J = 8.4\) Hz, 1H), 6.93 (t, \(J = 9.6\) Hz, 2H), 6.85 (s, 1H), 6.67 (d, \(J = 8.4\) Hz, 1H), 6.22 (d, \(J = 10.0\) Hz, 1H), 5.58 (d, \(J = 10.0\) Hz, 1H), 4.23 (s, 2H), 3.93 (s, 3H), 3.89 (s, 3H), 3.59 (s, 4H), 3.19 (t, \(J = 7.2\) Hz, 2H), 2.32 (t, \(J = 7.2\) Hz, 2H), 2.26 (s, 4H), 1.96 (s, 6H) ppm. \(^1\)C NMR: \(\delta\) 152.7, 152.5, 149.1, 131.8, 131.3, 129.1, 128.3, 126.5, 122.0, 121.4, 121.9, 116.3, 110.6, 109.8, 66.8, 57.3, 56.3, 56.2, 53.6, 52.2, 44.4, 27.9 ppm. HRMS (ESI) m/z calculated for C\(_{26}\)H\(_{35}\)O\(_6\)N\(_2\)S [(M + H)\(^+\)]: 503.2210, found 503.2204.
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N-((2,2\text{-dimethyl}-2H\text{-chromen}-6\text{-yl})\text{methyl})-3,4\text{-dimethoxy}-N-(3\text{-morpholinopropyl})\text{benzenesulfonamide (15)} \] – Yield: 16 mg, 14%.

\( ^1\text{H NMR:} \ \delta \) 7.43 (d, \( J = 8.0 \text{ Hz}, \ 1\text{H} \)), 7.26 (s, \( 1\text{H} \)), 6.98-6.93 (m, \( 2\text{H} \)), 6.87 (s, \( 1\text{H} \)), 6.68 (d, \( J = 8.4 \text{ Hz}, \ 1\text{H} \)), 6.23 (d, \( J = 10.0 \text{ Hz}, \ 1\text{H} \)), 5.60 (d, \( J = 10 \text{ Hz}, \ 1\text{H} \)), 4.19 (s, \( 2\text{H} \)), 3.94 (s, \( 3\text{H} \)), 3.91 (s, \( 3\text{H} \)), 3.60 (s, \( 4\text{H} \)), 3.12 (t, \( J = 7.6 \text{ Hz}, \ 2\text{H} \)), 2.22 (s, \( 4\text{H} \)), 2.18 (t, \( J = 8.0 \text{ Hz}, \ 2\text{H} \)), 1.59-1.52 (m, \( 2\text{H} \)), 1.40 (s, \( 6\text{H} \)) ppm. \( ^{13}\text{C NMR:} \ \delta \) 152.8, 152.4, 149.1, 131.6, 131.3, 129.3, 128.4, 126.5, 121.9, 121.3, 121.0, 116.3, 110.6, 109.8, 66.9, 56.3, 55.9, 53.4, 52.0, 46.2, 28.0, 25.4 ppm. HRMS (ESI) \( \text{m/z calculated for C}_{27}\text{H}_{37}\text{O}_{6}\text{N}_{2}\text{S} \) [(M + H)\( ^{+} \)]: 517.2367, found 517.2366.

II. Hydroxymethine analogues

\( (4\text{-bromophenyl})(2\text{-chboroallyl})\text{sulfane (16)} \)

In a 50 mL three-neck round bottom flask, 100 mg (1 eq.) of 4-bromothiophenol, 146 mg of \( \text{K}_2\text{CO}_3 \) (2 eq.), 150 mg (1.7 eq) of KI and 5 mg (0.05 eq) of Cul were mixed in acetone (10 mL) and was put under argon gas. The solution was then heated up to 55\(^\circ\text{C} \) for 15 min and 106 mg (1.8 eq) of 2,3-dichloroprop-1-ene was added. The reaction mixture stirred for 6 h and was allowed to cool down to room temperature. The mixture was treated with DI \( \text{H}_2\text{O} \) (20 mL) and diethyl ether (20 mL). The ethereal layer was washed with brine (20 mL), dried over \( \text{Na}_2\text{SO}_4 \), and concentrated in vacuo.
The product was purified using flash column chromatography. Hexanes were used as eluent. Yield: 46 mg, 33%. $^1$H NMR: $\delta$ 7.44 (d, $J = 8.4$ Hz, 2H), 7.27 (d, $J = 8.4$ Hz, 2H), 5.27 (s, 2H), 3.70 (s, 2H). $^{13}$C NMR: $\delta$ 137.5, 133.8, 132.6, 132.1, 121.3, 115.1, 42.8 ppm.

**5-bromo-2-methylbenzo[b]thiophene (17)**

In a 50 mL round bottom flask, the sulfane product was treated with <1 mL of $N,N$-dimethylaniline and was stirred at 170$^\circ$C for 12 h. The solution was taken up in ethyl acetate (20 mL), washed with IM HCl (20 mL x 3), saturated NaHCO$_3$ solution (20 mL), brine 20 mL and was dried over Na$_2$SO$_4$. The organic layer was concentrated in vacuo. The product was purified using flash column chromatography. Hexanes were used as eluent (1:8). Yield: 19 mg, 48%. $^1$H NMR: $\delta$ 7.77 (d, $J = 1.2$ Hz, 1H), 7.59 (d, $J = 8.8$ Hz, 1H), 7.34 (dd, $J = 8.8$ Hz, 1.6 Hz, 1H), 6.90 (s, 1H), 2.59 (s, 3H) ppm. $^{13}$C NMR: $\delta$ 132.2, 129.4, 126.3, 125.2, 123.3, 120.9, 16.2 ppm.

2-Bromo-5-((2-methylbut-3-yn-2-yl)oxy)pyridine (20a)

In a 100 mL round bottom flask, 2 g (0.012 mol) of 6-bromopyridin-3-ol was dissolved in dry acetonitrile (10 mL) and was cooled to 0$^\circ$C under Argon gas. To this solution, 9.0 mL (0.05 mol) of DBU was added and the solution was allowed to stir for 30 min. 6.8 mL (0.05 mol) of 3-chloro-3-methylbut-1-yne was then added into the mixture drop wise. The solution was allowed to warm up to room temperature and was stirred overnight. The solution was concentrated and was taken up with ethyl acetate (100 mL). The organic solution was washed with 1M HCl solution (20 mL),
1M NaOH solution (20 mL), saturated NaHCO₃ solution (20 mL), brine (20 mL) and was dried over Na₂SO₄. The organic layer was concentrated in vacuo and was taken directly to the next step.

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\text{Br} \\
\text{N} \\
\text{O} \\
\text{Br}
\]

**6-Bromo-2,2-dimethyl-2H-pyrano[3,2-b]pyridine (20)**

Using the impure product from the procedure above, the crude mixture was dissolved in N,N-dimethylaniline (<1 mL) at 170°C for 6 h. The solution was taken up in ethyl acetate (20 mL), washed with IM HCl (20 mL x 3), saturated NaHCO₃ solution (20 mL), brine 20 mL and was dried over Na₂SO₄. The organic layer was concentrated in vacuo. The product was purified using flash column chromatography. Hexanes were used as eluent. Yield: 239.4 mg, 34%. ¹H NMR:  δ 7.10 (d, J = 8.4 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 6.40 (d, J = 10.0 Hz, 1H), 5.83 (d, J = 10.0 Hz, 1H), 1.41 (s, 6H) ppm. ¹³C NMR: δ 149.1, 142.1, 136.0, 131.3, 127.4, 125.9, 123.0, 28.2 ppm.

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\text{Boc} \\
\text{N} \\
\text{O} \\
\text{Br}
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**Tert-butyl 4-((4-bromobenzyl)oxy)piperidine-1-carboxylate (21)**

In a 100 mL round bottom flask, 2 g (0.012 mol) of tert-butyl 4-hydroxypiperidine-1-carboxylate and 295 mg (0.8 mmol) of TBAI was dissolved in dry THF (10 mL) and was cooled to 0°C under inert atmosphere with Argon gas. To this solution, 480 mg (0.02 mol) of NaH was added and the solution was allowed to stir for 30 min. 2.4 g (0.008 mol) of 4-bromobenzyl bromide was then added into the mixture. The solution was allowed to warm up to room temperature and was stirred overnight. The solution
was quenched with saturated NH$_4$Cl solution (20 mL) and the organic layer was extracted up with ethyl acetate (50 mL), washed with brine (20 mL) and was dried over Na$_2$SO$_4$. The organic layer was concentrated in vacuo and was purified using flash column chromatography. Hexanes were used as eluent. Yield: 2 g, 64%. $^1$H NMR: $\delta$ 7.44 (d, $J = 8.4$ Hz, 2H), 7.20 (d, $J = 8.4$ Hz, 2H), 4.48 (s, 2H), 3.77-3.74 (m, 2H), 3.55-3.51 (m, 1H), 3.12-3.06 (m, 2H), 1.82 (bs, 2H), 1.60-1.57 (m, 2H), 1.44 (s, 9H) ppm. $^{13}$C NMR: $\delta$ 154.8, 137.9, 131.5, 129.1, 121.3, 79.5, 74.2, 69.1, 60.4, 41.0, 34.7, 31.6, 31.0, 28.4, 25.3, 22.7, 21.0, 14.2, 14.1 ppm.

**Tert-butyl 4-((3-bromobenzyl)oxy)piperidine-1-carboxylate (22)**

In a 50 mL round bottom flask, 120 mg (0.60 mmol) of tert-butyl 4-hydroxypiperidine-1-carboxylate and 15 mg (0.04 mmol) of TBAI was dissolved in dry THF (10 mL) and was cooled to 0°C under inert atmosphere with Argon gas. To this solution, 24 mg (1.0 mmol) of NaH was added and the solution was allowed to stir for 30 min. 100 mg (0.40 mmol) of 4-bromobenzyl bromide was then added into the mixture. The solution was allowed to warm up to room temperature and was stirred overnight. The solution was quenched with saturated NH$_4$Cl solution (20 mL) and the organic layer was extracted up with ethyl acetate (20 mL), washed with brine (20 mL) and was dried over Na$_2$SO$_4$. The organic layer was concentrated in vacuo and was purified using flash column chromatography. Hexanes were used as eluent. Yield: 167 mg, 75%. $^1$H NMR: $\delta$ 7.51(s, 1H), 7.42 (d, $J = 6.4$ Hz, 1H), 7.28-7.20 (m, 2H), 4.53-4.52 (s, 2H), 3.79-3.78 (m, 2H), 3.57-3.55 (m, 1H), 3.14-3.10 (m, 2H), 1.86 (bs, 2H), 1.61-
1.59 (m, 2H), 1.28 (s, 9H) ppm. $^{13}$C NMR: δ 154.8, 141.1, 130.6, 130.3, 130.0, 125.8
122.6, 79.5, 74.4, 69.0, 31.0, 28.4 ppm.

**General procedure for the synthesis of hydroxymethine products:**

A flask of the aryl bromide (1 eq.) was degassed and anhydrous THF (10 mL) was
added under nitrogen gas. The solution was cooled to -78°C and stirred for 1 h. n-
BuLi (0.9 eq) was then added to the solution drop wise. To this solution, the
aldehyde (1.1 eq) was added dropwise and the reaction mixture was stirred for 1 h
and was allowed to warm up to room temperature. The mixture was then quenched
with saturated ammonium chloride solution (20 mL) and the organic layer was
extracted with ethyl acetate (20 mL x 2), washed with brine (25 mL x 1), dried over
Na$_2$SO$_4$ and concentrated in vacuo. The product was purified using flash column
chromatography. Ethyl acetate and hexanes were used as eluent (1:3).

![Structural formula of the product](image)

**3,4-Dimethoxyphenyl)(2-methylbenzo[b]thiophen-5-yl)methanol (18)** – Yield: 3
mg, 12%. $^1$H NMR: δ 7.70 (s, 1H), 7.67 (d, $J = 4.0$ Hz, 1H), 7.23 (d, $J = 8.4$ Hz, 1H),
6.94 (s, 1H), 6.91 (d, $J = 8.4$ Hz, 1H), 6.82 (d, $J = 8.4$ Hz, 1H), 5.90 (s, 1H), 3.86 (s,
3H), 3.84 (s, 3H), 2.58 (s, 3H) ppm, 2.20 (d, $J = 2$ Hz, 1H). HRMS (ESI) m/z
calculated for C$_{18}$H$_{18}$O$_3$S + Na$^+$: 337.0874, found 337.0883.
(2,4-Dimethoxyphenyl)(2-methylbenzo[b]thiophen-5-yl)methanol (19) – Yield: 3 mg, 14%. $^1$H NMR: δ 7.67 (d, J = 4.8 Hz, 2H), 7.24 (s, 1H), 7.07 (d, J = 4.0 Hz, 1H), 6.94 (s, 1H), 6.47 (s, 1H), 6.44 (d, J = 8.4 Hz, 1H), 6.10 (d, J = 4.8 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 2.92 (d, J = 5.2 Hz, 1H), 2.57 (s, 3H) ppm. HRMS (ESI) m/z calculated for C$_{18}$H$_{18}$O$_3$S + Na$: 337.0874$, found 337.0863.

(3,4-Dimethoxyphenyl)(2,2-dimethyl-2H-chromen-6-yl)methanol (24) - Yield: 104 mg, 63%. $^1$H NMR: δ 7.09 (d, J = 8.0 Hz, 1H), 6.98 (s, 1H), 6.94 (s, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.29 (d, J = 9.6 Hz, 1H), 5.72 (s, 1H), 5.61 (d, J = 9.6 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 2.20 (s, 1H), 1.43 (s, 6H) ppm. $^{13}$C NMR: δ 152.4, 149.0, 148.4, 136.7, 136.3, 130.9, 127.4, 124.6, 122.3, 121.1, 118.8, 116.2, 110.9, 109.7, 76.3, 75.5, 55.9, 55.9, 28.0, 28.0 ppm. HRMS (ESI) m/z calculated for C$_{20}$H$_{22}$O$_4$ + Na$: 349.1416$, found 349.1419.

(3,4-Dimethoxyphenyl)(2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methanol (25) – Yield: 69 mg, 53%. $^1$H NMR: δ 6.95 (d, J = 8.0 Hz, 1H), 6.87 (d, J = 5.2 Hz, 2H), 6.80 (d, J = 4.4 Hz, 2H), 6.79 (dd, J = 8.4, 4 Hz, 2H), 6.50 (d, J = 10.0 Hz, 1H) 5.85 (d, J = 10.0 Hz, 1H), 5.58 (s, 1H), 5.14 (bs, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 1.43 (s, 6H) ppm. $^{13}$C NMR: 152.8, 149.1, 148.7, 148.6, 139.2, 136.1, 135.3, 124.0, 123.6, 121.1, 119.5, 119.3, 111.0, 110.4, 74.4, 55.9, 55.8, 28.3 ppm.
**Tert-butyl 4-((4-((3,4-dimethoxyphenyl)(hydroxy)methyl)benzyl)oxy)piperidine-1-carboxylate (26)** - Yield: 877 mg, 54%. $^1$H NMR: $\delta$ 7.35 (d, $J = 8.0$ Hz, 2H), 7.30 (d, $J = 8.4$ Hz, 2H), 6.91 (d, $J = 1.6$ Hz, 1H), 6.88-6.86 (m, 1H), 6.80 (d, $J = 8.4$ Hz, 1H), 5.78 (s, 1H), 4.52 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.77-3.73 (m, 2H), 3.57-3.51 (m, 1H), 3.11-3.05 (m, 2H), 2.39 (s, 1H), 1.84-1.82 (m, 2H), 1.58-1.55 (m, 2H), 1.44 (s, 9H) ppm. $^{13}$C NMR: $\delta$ 149.1, 143.3, 137.9, 136.6, 127.6, 126.5, 118.9, 110.9, 109.7, 79.5, 75.8, 74.0, 69.6, 60.4, 55.9, 55.8, 28.4, 21.0, 14.2 ppm.

**Tert-butyl 4-((3-((3,4-dimethoxyphenyl)(hydroxy)methyl)benzyl)oxy)piperidine-1-carboxylate (27)** – Yield: 41 mg, 33%. $^1$H NMR: $\delta$ 7.74 (s, 1H), 7.66 (d, $J = 7.6$ Hz, 1H), 7.57 (d, $J = 7.2$ Hz, 1H), 7.50-7.34 (m, 2H), 7.38 (d, $J = 8.4$ Hz, 1H), 6.89 (d, $J = 8.4$ Hz, 1H), 5.80 (s, 1H), 4.62 (s, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 3.76 (bs, 2H), 3.60 (m, 1H), 3.14-3.08 (m, 2H), 2.46 (s, 1H), 1.85 (bs, 2H), 1.62 (bs, 2H), 1.48 (s, 9H) ppm. $^{13}$C NMR: $\delta$ 154.8, 149.0 148.4, 144.3, 138.8, 136.7, 128.5, 126.5, 125.7, 125.5, 119.0, 110.9, 109.9, 79.5, 75.7, 74.1, 69.8, 55.9, 55.8, 41.2, 34.1, 31.0, 28.4 ppm.
(3,4-Dimethoxyphenyl)(4-morpholinophenyl)methanol (28) - Yield: 167 mg, 55%.

$^1$H NMR: $\delta$ 7.25 (d, $J = 8.0$ Hz, 2H), 6.92 (s, 1H), 6.87-6.80 (m, 4H), 5.70 (s, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.82 (m, 4H), 3.12-3.10 (m, 4H), 2.80 (bs, 1H) ppm. $^{13}$C NMR: $\delta$ 150.6, 148.9, 148.2, 136.9, 135.7, 127.5, 118.8, 115.6, 110.8, 109.7, 75.4, 66.8, 66.5, 55.9, 55.87, 49.3 ppm. HRMS (ESI) $m/z$ calculated for C$_{19}$H$_{24}$NO$_4$ [(M + H)$^+$] 330.1705, found 330.1710.

III. Ketone derivatives from hydroxymethine products

General procedure for the ketone products:

To a solution of 50 mg of hydroxymethine product (1 eq.) in DCM (10 mL) was added Celite (33 mg Celite/ hydroxymethine product) as solid support. The reaction mixture was then charged with the appropriate amounts of PCC (2 eq) and was allowed to stir at room temperature for 24 h. The solution mixture was filtered and washed with DCM (20 mL x 3) and concentrated in vacuo. The crude product was purified using flash column chromatography. Ethyl acetate and hexanes were used as eluent (1:10).

(3,4-Dimethoxyphenyl)(2,2-dimethyl-2H-chromen-6-yl)methanone (29) – Yield: 77 mg, 74%. $^1$H NMR: $\delta$ 7.49 (d, $J = 8.4$ Hz, 1H), 7.41 (s, 1H), 7.34 (s, 1H), 7.28 (d, $J = 8.4$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 1H), 6.73 (d, $J = 8.4$ Hz, 1H), 6.26 (d, $J = 10.0$ Hz, 1H), 5.58 (d, $J = 10.0$ Hz, 1H), 3.87(s, 3H), 3.85 (s, 3H), 1.39 (s, 6H) ppm. $^{13}$C NMR:
δ 194.3, 171.1, 156.7, 152.5, 149.0, 131.9, 131.2, 130.8, 130.7, 128.6, 124.7, 121.7, 120.6, 115.8, 112.2, 109.7, 60.3, 56.1, 56.0, 28.4, 21.0, 14.2 ppm. HRMS (ESI) m/z calculated for C_{20}H_{21}O_4 [(M + H)^+]: 325.1440, found 325.1433.

**(3,4-Dimethoxyphenyl)(2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methanone (30)** – Yield: 44 mg, 64%. \(^{1}H\) NMR: δ 7.87-7.83 (m, 2H), 7.79 (s, 1H), 7.15 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 5.976 (d, J = 10.0 Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 1.53 (s, 6H) ppm. \(^{13}C\) NMR: δ 190.9, 153.0, 152.0, 148.6, 147.7, 139.6, 135.7, 130.0, 126.6, 126.1, 123.8, 123.1, 113.3, 109.8, 78.2, 56.1, 56.0, 28.7 ppm. HRMS (ESI) m/z calculated for C_{19}H_{20}O_4N [(M + H)^+]: 326.1387, found 326.1392.

**Tert-butyl 4-((4-(3,4-dimethoxybenzoyl)benzyl)oxy)piperidine-1-carboxylate (31)** – Yield: 594 mg, 68% \(^{1}H\) NMR: δ 7.69 (d, J = 8.4 Hz, 2H), 7.42-7.39 (m, 3H), 7.31 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 4.58 (s, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.75-3.72, (m, 2H), 3.56-3.54 (m, 1H), 3.10-3.04 (m, 2H), 1.96 (s, 1H), 1.82 (bs, 2H), 1.59-1.53 (m, 2H), 1.40 (s, 9H) ppm. \(^{13}C\) NMR: δ 195.1, 171.0, 154.8, 153.0, 148.9, 143.0, 137.4, 130.2, 130.0, 126.9, 125.4, 112.1, 109.8, 79.4, 74.5, 69.3, 60.3, 56.0, 55.9, 41.1, 31.0, 28.4, 21.0, 14.2 ppm. HRMS (ESI) m/z calculated for C_{26}H_{33}O_6N + Na\(^+\): 478.2200, found 478.2199.
**Tert-butyl 4-((3-(3,4-dimethoxybenzoyl)benzyl)oxy)piperidine-1-carboxylate (32)**

- Yield: 16 mg, 54%. ¹H NMR: δ 7.73 (s, 1H), 7.75 (d, J = 7.2 Hz, 1H), 7.56 (d, J = 7.2 Hz, 1H), 7.49 (s, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.37 (dd, J = 8.4, 1.6 Hz, 1H), 6.88 (d, J = 8.4, 1H), 4.61 (s, 2H), 3.96 (s, 3H), 3.94 (s, 3H), 3.76 (m, 2H), 3.61-3.57 (m, 1H), 3.13-3.07 (m, 3H), 1.86 (bs, 2H), 1.66-1.58 (m, 2H), 1.45 (s, 9H) ppm. ¹³C NMR: δ 195.5, 154.5, 153.1, 149.0, 139.1, 138.4, 130.9, 130.2, 129.0, 128.6, 128.2, 125.5, 112.1, 109.8, 79.5, 74.5, 69.5, 56.1, 56.0, 31.0, 28.4 ppm. HRMS (ESI) m/z calculated for C₂₆H₃₃O₆N⁺: 478.2200, found 478.2197.

**IV. Miscellaneous compounds:**

**Procedure for the synthesis of oxime product (34)**
To a solution of 133 mg of bis(4-methoxyphenyl)methanone (1 eq.) in MeOH (10 mL) was added 0.22 mL of pyridine (5 eq.) The solution was then charged with 153 mg of hydroxylamine hydrochloride (4 eq.) and was allowed to stir at room temperature for 36 h. The mixture was concentrated and the solid obtained was dissolved in DCM (20 mL). The solution was washed with 1M HCl solution (20 mL), saturated NaHCO$_3$ solution (20 mL), brine (20 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The product was purified using flash column chromatography. Ethyl acetate and hexanes were used as eluent (1:6). Yield: 79 mg, 56%. $^1$H NMR: δ 7.44-7.40 (m, 4H), 6.99 (d, $J$ = 8.4 Hz, 2H), 6.86 (d, $J$ = 8.4 Hz, 2H), 3.86 (s, 3H), 3.82 (s, 3H) ppm. $^{13}$C NMR: δ 160.7, 160.0, 157.0, 131.2, 129.5, 129.3, 125.0, 113.8, 113.6, 55.4, 55.3 ppm. HRMS (ESI) m/z calculated for C$_{15}$H$_{16}$NO$_3$ [(M + H)$^+$]: 258.1130, found 258.1123.

![Structure](image.png)

**Procedure for the synthesis of the hydrazone product (35):**

In a heavy-walled glass vial sealed with a Teflon septum, 40 mg of bis(4-methoxyphenyl)methanone (1 eq) was dissolved in MeOH (5 mL) was mixed with 160 mg of (4-nitrophenyl)hydrazine (4 eq.) and 0.66 mL of pyridine (5 eq.). The solution was irradiated with microwave for 5 min at 150 °C at 35W. The mixture was concentrated and the solid obtained was dissolved in DCM (20 mL). The solution was washed with 1M HCl solution (20 mL), saturated NaHCO$_3$ solution (20 mL), brine (20 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The product was
purified using flash column chromatography. Ethyl acetate and hexanes were used as eluent (1:8). Yield: 63 mg, 79%. $^1$H NMR: $\delta$ 8.15 (d, $J = 9.2$ Hz, 2H), 7.89 (s, 1H), 7.56 (d, $J = 8.8$ Hz, 2H), 7.27 (d, $J = 8.4$ Hz, 2H), 7.13 (d, $J = 8.4$ Hz, 2H), 7.08 (d, $J = 8.8$ Hz, 2H), 6.90 (d, $J = 8.8$ Hz, 2H), 3.91 (s, 3H), 3.85 (s, 3H) ppm. $^{13}$C NMR: $\delta$ 160.6, 160.5, 149.6, 148.8, 139.9, 132.2, 130.5, 130.3, 128.6, 126.1, 123.8, 115.2, 115.2, 113.7, 113.5, 111.8, 55.4, 55.3 ppm. HRMS (ESI) $m/z$ calculated for C$_{21}$H$_{20}$O$_4$N$_3$ [(M + H)$^+$]: 378.1448, found 378.1444.
APPENDIX

Section 1:

$^1$H NMR of compound 2 53
$^{13}$C NMR of compound 2 54
$^1$H NMR of compound 3 55
$^{13}$C NMR of compound 3 56
$^1$H NMR of compound 4 57
$^{13}$C NMR of compound 4 58
$^1$H NMR of compound 5 59
$^{13}$C NMR of compound 5 60
$^1$H NMR of compound 6 61
$^{13}$C NMR of compound 6 62
$^1$H NMR of compound 7 63
$^{13}$C NMR of compound 7 64
$^1$H NMR of compound 8 65
$^{13}$C NMR of compound 8 66
$^1$H NMR of compound 9 67
$^{13}$C NMR of compound 9 68
$^1$H NMR of compound 10 69
$^{13}$C NMR of compound 10 70
$^1$H NMR of compound 11 71
$^{13}$C NMR of compound 11 72
$^1$H NMR of compound 12 73
$^{13}$C NMR of compound 12 74
\( ^1\text{H NMR of compound 13} \)

\( ^13\text{C NMR of compound 13} \)

\( ^1\text{H NMR of compound 14} \)

\( ^13\text{C NMR of compound 14} \)

\( ^1\text{H NMR of compound 15} \)

\( ^13\text{C NMR of compound 15} \)

\( ^1\text{H NMR of compound 16} \)

\( ^13\text{C NMR of compound 16} \)

\( ^1\text{H NMR of compound 17} \)

\( ^13\text{C NMR of compound 17} \)

\( ^1\text{H NMR of compound 18} \)

\( ^13\text{C NMR of compound 18} \)

\( ^1\text{H NMR of compound 19} \)

\( ^13\text{C NMR of compound 19} \)

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\( ^13\text{C NMR of compound 21} \)

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\( ^1\text{H NMR of compound 25} \)

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Section II:

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HRMS spectrum of compound 8 123
HRMS spectrum of compound 9 125
HRMS spectrum of compound 10 127
HRMS spectrum of compound 11 129
HRMS spectrum of compound 12 131
HRMS spectrum of compound 13 133
HRMS spectrum of compound 14 135
HRMS spectrum of compound 15 137
HRMS spectrum of compound 18 139
HRMS spectrum of compound 19 141
HRMS spectrum of compound 29 143
HRMS spectrum of compound 30 145
HRMS spectrum of compound 31 147
HRMS spectrum of compound 32 149
HRMS spectrum of compound 33 151
HRMS spectrum of compound 34 153
HRMS spectrum of compound 35 156
The image contains a spectrum graph with chemical shifts and peaks labeled. The spectrum is labeled with various parameters and chemical structures. The chemical structure depicted in the image includes atoms labeled with 'Ome' and 'Boc'.
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
29508 formula(e) evaluated with 118 results within limits (all results (up to 1000) for each mass)
Elements Used:

Minimum: -1.5
Maximum:  
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100% MeOH + 0.1% HCOOH 
SARAH_ZD-33_BWANG-ACCU_08-31-2012_ESI-POS01 119 (1.256) AM (Cen,2, 80.00, Ar,5000.0,556.28 
173.0941 
1.04e4
Elemental Composition Report

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
24118 formula(e) evaluated with 80 results within limits (all results (up to 1000) for each mass)
Elements Used:

Minimum:

Maximum:

Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula
--- | --- | --- | --- | --- | --- | ---
490.1278 | 490.1276 | 0.2 | 0.4 | 10.5 | 1.0 | C23 H24 N O4 F3 S Na
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
4305 formula(e) evaluated with 20 results within limits (all results (up to 1000) for each mass)
Elements Used:

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Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
18747 formula(e) evaluated with 66 results within limits (all results (up to 1000) for each mass)
Elements Used:

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Elemental Composition Report

Single Mass Analysis
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Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
14806 formula(e) evaluated with 47 results within limits (all results (up to 1000) for each mass)
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Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM  /  DBE: min = -1.5, max = 200.0
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Monoisotopic Mass, Even Electron Ions
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![Image of molecular structure]
**SPECTRUM - simulation:**

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Elemental Composition Report

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
959 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)

Elements Used:

Minimum:
800.0  5.0  -1.5

Maximum:
449.2094  449.2104  -1.0  -2.2  -0.5  1253.7  C15 H37 N4 O7 S2
<table>
<thead>
<tr>
<th></th>
<th>1.1</th>
<th>2.4</th>
<th>9.5</th>
<th>595.9</th>
<th>C19 H29 N8 O3 S</th>
</tr>
</thead>
<tbody>
<tr>
<td>449.2083</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.6</td>
<td>-3.6</td>
<td>8.5</td>
<td>832.1</td>
<td>C23 H33 N2 O5 S</td>
</tr>
<tr>
<td>449.2110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>3.8</td>
<td>0.5</td>
<td>1086.3</td>
<td>C11 H33 N10 O5 S2</td>
</tr>
<tr>
<td>449.2077</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Chemical Structure](image)
SPECTRUM - simulation:

<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>419.1980</td>
<td>419.1999</td>
<td>-4.52</td>
<td>8.5</td>
<td>C22 H31 O4 N2 S</td>
</tr>
</tbody>
</table>
SPECTRUM - simulation:

<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>414.1857</td>
<td>414.1846</td>
<td>2.71</td>
<td>10.5</td>
<td>C22 H28 O3 N3 S</td>
</tr>
</tbody>
</table>
SPECTRUM - simulation:

<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>447.1949</td>
<td>447.1948</td>
<td>0.18</td>
<td>9.5</td>
<td>C23 H31 O5 N2 S</td>
</tr>
</tbody>
</table>

![Chemical Structure](image-url)
### SPECTRUM - simulation:

```
<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>503.2204</td>
<td>503.2210</td>
<td>-1.24</td>
<td>10.5</td>
<td>C26 H35 O6 N2 S</td>
</tr>
</tbody>
</table>
```
<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>517.2366</td>
<td>517.2367</td>
<td>-0.16</td>
<td>10.5</td>
<td>C27 H37 O6 N2 S</td>
</tr>
</tbody>
</table>
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
138 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:
Minimum:
Maximum:
Mass    Calc. Mass    mDa    PPM    DBE    i-FIT    Formula

238.0791  338.0972  339.0929  450.1881  556.2771  651.1966
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
226 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:

Minimum:  800.0  5.0  -1.5
Maximum:  200.0
Mass       Calc. Mass   mDa    PPM    DBE   i-FIT   Formula
<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>297.0939</td>
<td>297.0949</td>
<td>-1.0</td>
<td>-3.4</td>
<td>10.5</td>
<td>48.0</td>
<td>C18 H17 O2 32S</td>
</tr>
<tr>
<td></td>
<td>297.0950</td>
<td>-1.1</td>
<td>-3.7</td>
<td>3.5</td>
<td>12.4</td>
<td>C12 H18 O7 23Na</td>
</tr>
<tr>
<td></td>
<td>297.0925</td>
<td>1.4</td>
<td>4.7</td>
<td>7.5</td>
<td>32.6</td>
<td>C16 H18 O2 23Na 32S</td>
</tr>
</tbody>
</table>

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
180 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:

Minimum:
-1.5

Maximum:
800.0   5.0   200.0

Mass   Calc. Mass   mDa  PPM  DBE   i-FIT  Formula     |
297.0939 | 297.0949   | -1.0 | -3.4 | 10.5 | 48.0  | C18 H17 O2 32S |
297.0950   | 297.0950   | -1.1 | -3.7 | 3.5  | 12.4  | C12 H18 O7 23Na |
297.0925   | 297.0925   | 1.4  | 4.7  | 7.5  | 32.6  | C16 H18 O2 23Na 32S |
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-80  H: 1-80  O: 1-80

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>325.1433</td>
<td>325.1440</td>
<td>-0.7</td>
<td>-2.2</td>
<td>10.5</td>
<td>362.7</td>
<td>C20 H21 O4</td>
</tr>
</tbody>
</table>
in 50% MeOH + 0.1% HCOOH

ZEUS_ZDII95B_ESI_POS_BWANG_02272014 371 (6.905) Cm (365:401)

QtofMicro

27-Feb-2014 17:11:58

TOF MS ES+

4.60e3

m/z

100

%
**Elemental composition search on mass 326.1392**

<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>326.1392</td>
<td>326.1387</td>
<td>1.58</td>
<td>10.5</td>
<td>C_{19}H_{20}O_{4}N</td>
</tr>
<tr>
<td>326.1400</td>
<td>326.1400</td>
<td>-2.52</td>
<td>15.5</td>
<td>C_{20}H_{16}N_{5}</td>
</tr>
</tbody>
</table>

![Chemical structure image]
Elemental composition search on mass 456.2379

m/z = 451.2379-461.2379

<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>456.2379</td>
<td>456.2381</td>
<td>-0.36</td>
<td>10.5</td>
<td>C_{26} H_{34} O_{6} N</td>
</tr>
<tr>
<td>456.2367</td>
<td>456.2367</td>
<td>2.58</td>
<td>11.0</td>
<td>C_{24} H_{32} O_{5} N_{4}</td>
</tr>
<tr>
<td>456.2394</td>
<td>456.2394</td>
<td>-3.29</td>
<td>15.5</td>
<td>C_{27} H_{30} O_{2} N_{5}</td>
</tr>
</tbody>
</table>

Diagram: Boc-N-CO-CH-CH-CO-CH=O-CH=O
<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Delta (ppm)</th>
<th>RDB equiv.</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>328.1538</td>
<td>328.1543</td>
<td>-1.75</td>
<td>9.5</td>
<td>C19 H22 O4 N</td>
</tr>
</tbody>
</table>
Leu Enk ITSD 556.2771
ZEUS_ZDII105A_HRESI_POS_03052014_2 458 (8.514) AM (Cen,4, 80.00, Ar,6000.0,556.28,0.80); Cm (448.493) 4.48e4

Elemental
Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
267 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>258.1123</td>
<td>258.1130</td>
<td>-0.7</td>
<td>-2.7</td>
<td>8.5</td>
<td>62.4</td>
<td>C15 H16 N O3</td>
</tr>
</tbody>
</table>
Elemental composition search on mass 378.1444

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{m/z} & 378.1444-383.1444 \\
\hline
\text{m/z} & \text{Theo.} & \text{Delta (ppm)} & \text{RDB equiv.} & \text{Composition} \\
378.1444 & 378.1448 & -1.14 & 13.5 & \text{C}_{21} \text{H}_{20} \text{O}_{4} \text{N}_3 \\
378.1462 & 378.1462 & -4.69 & 13.0 & \text{C}_{23} \text{H}_{22} \text{O}_5 \\
\hline
\end{array}
\]