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The Potential Efficacy of M13 Analogs Used as Therapeutic Treatment for IBD

by

Jennifer Vo

Under the Direction of Didier Merlin, Ph.D.

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

Master of Interdisciplinary Studies

in the Institute for Biomedical Sciences

Georgia State University

2023

ABSTRACT

Inflammatory bowel disease (IBD) is a chronic inflammatory condition which can affect all parts of the gastrointestinal tract and increase the risk of developing colitis-associated cancers. Researchers have attempted to create therapies that can alleviate their symptoms and potentially reduce the severity of the disease. Currently, anti-TNF-alpha is a biologics therapy proven to be effective within IBD-afflicted patients, yet its drawbacks include its loss of efficacy and high likelihood for side effects. Some researchers have investigated anti-inflammatory natural products as alternative drug candidates to treat IBD. Ginger is a great resource as it exhibits strong anti-inflammatory effects, mainly due to its active components including 6-shogaol. In addition, the *in vivo* metabolite of 6-shogaol, M13, has demonstrated higher anti-inflammatory properties but is more hydrophilic than 6-shogaol. In this study, we investigated the potential efficacy of M13 analogs to see if they exhibit more favorable properties than M13. After testing the drugs' *in vitro* efficiency, we found several more potent drug candidates (effective in reducing cancer cell growth). Further wound-healing tests of these analogs are still ongoing, but for now, we can conclude some of the M13 analogs show promising effects as treatment against IBD and colitis-associated cancer.

INDEX WORDS: IBD, Inflammatory Bowel Diseases, Ginger, M13 analogs

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2023

The Potential Efficacy of M13 Analogs Used as Therapeutic Treatment for IBD

by

Jennifer Vo

Committee Chair: Dr. Didier Merlin

Committee: Dr. Chunhua Yang

Electronic Version Approved:

Office of Academic Assistance – Graduate Programs

Institute for Biomedical Sciences

Georgia State University

January 2024

DEDICATION

I would like to dedicate this paper to my family for the continual support in my education and dreams. To my coworkers, thank you for your encouragement. To my friend who is now hundreds of miles away, this paper is also for you as without your constant support and encouragement, I would not have taken on this opportunity and be where I am today.

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation towards Dr. Didier Merlin, Dr. Chunhua Yang, and Dr. Dingpei Long. To Dr. Merlin, thank you for giving me the opportunity to take part in your research and make an impact. To Chunhua, thank you being a great mentor to me and teaching me all I need to know in the lab room. To Dingpei, thank you for lending your expertise and guiding me in my experiments. To all, I'm thankful for the warmth and kindness you have shown me throughout my time at GSU!

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1. INTRODUCTION

Inflammatory bowel disease (IBD) is a disease which causes chronic inflammation of the gastrointestinal (GI) tract (CDC, 2022). Crohn's Disease (CD) and ulcerative colitis (UC) are two major types of conditions which fall under IBD. One of the main differentiating characteristics between the two subgroups is the location of inflammation. In UC cases, inflammation typically occurs within the large intestines, starting from the rectum and gradually spreading up to the colon. For Crohn's disease, while it often favors the small intestine, inflammation can occur at any part of the GI tract, leading to patchy areas of affected and non-affected tissues (CDC, 2022).

Historically, IBD was thought to be a "White man's disease" as prevalence was higher among the United States and Europe. However, recent studies have combatted this notion and found that though IBD is more prevalent with Caucasians, individuals from all ethnicities, genders, and age groups can also be affected with any form of IBD (Shi et al., 2018). Within the United States alone, the number of diagnosed cases were estimated to include 6 million individuals in 2017, which is double the amount recorded back in 2015 (Xu et al., 2021). While the total number of confirmed diagnoses are still higher within Western countries, the rates for IBD have also steadily increased among other countries, such as Japan, Singapore, and Latin America, as the years passed (Loftus, 2004). It has been speculated that these incidences were starting to appear in other countries due to the increasing industrialization and adaptation of a Western diet (Shi et al., 2018).

Although symptoms can vary within each person and the type of IBD condition, most individuals tend to experience symptoms such as diarrhea, bloody stool, abdominal pain, weight loss, and fatigue. Those with IBD may encounter moments of remission (timeframe with no symptoms) followed by periods of relapse (reoccurrence of symptoms). The intervals between these two periods can range from weeks to years. Unfortunately, as the severity of the disease

worsens, the intervals between remission and relapse become shorter. Due to the spontaneity of the disease and its associated symptoms, IBD can potentially impact one's quality of life and affect their normal day-to-day routine. As a result, most IBD-affected individuals struggle with cases of anxiety and depression as their disease progressively worsens (Eugenicos & Ferreira, 2021).

1.1 Causes of IBD

The underlying cause for IBD is still unknown, but researchers have identified potential factors which could contribute to the onset of the disease. These factors include but are not limited to genetic susceptibility, being a smoker, use of antibiotics, and diet.

From a genetics perspective, nucleotide binding oligomerization domain containing 2, or NOD2, is one of the main genes linked to the possible development of IBD. NOD2 is typically expressed within Paneth cells, which are intestinal epithelial cells responsible for the secretion of antimicrobial properties. When NOD2 is mutated, Paneth cells are unable to properly recognize and destroy invasive microbes within the environment (Jarmakiewicz-Czaja et al., 2022). Because the entrance of these microbes is not regulated, an influx of unwanted bacteria can occur and cause dysbiosis within the intestinal environment.

From the environmental perspective, various things from one's environment can increase the risk of IBD within a person. Environmental factors such as diet and use of antibiotics have been studied and found to affect the microbiota composition of the gut and led a change in the gut's immune response (Abegunde et al., 2016). Other factors like air and water pollution have been indicated in the possible exacerbation of flare-ups as exposure from certain chemicals could trigger an unwanted chemical reaction within the body (Abegunde et al., 2016). In early studies, smoking was often cited as one of the largest risk factors, but later studies found that while smoking worsened CD, it acted as a protective agent in the development of UC but the underlying explanation for this response is still being studied upon. Because of the complexity of

isolating each environmental factor, most of these results thus far have only been suggestive and not definite.

Nonetheless, scientists have agreed it is through the contribution of one or more of these factors that lead to a defect of the mucosal epithelial lining within IBD patients. In a normal, healthy patient, the mucosal layer of the epithelium acts as a selective barrier by regulating the passage of specific substances and guarding the entry against invasive microbes (Antoni et al., 2014). In IBD patients, the tight junctions which regulate the permeability of the mucosal layer become compromised, allowing for the invasion of harmful bacteria (Antoni et al., 2014). This unwanted entry then activates the immune response of the gut, leading to cytokine production and the resulting inflammation within the gut.

It is unclear at this moment whether the constant inflammation is causing thinning of the mucosal lining or the dysregulation within the mucosal barrier leads to inflammation (McDowell et al., 2023). Yet, what is certain that constant inflammation in the gut over time can have detrimental effects on a person's health. Due to the chronic inflammation, IBD-affected patients are at a higher risk of developing some form of colitis-associated cancer (CAC), and this risk exponentially increases by each decade (Axelrad et al., 2016).

1.2 Treatments for IBD

There is no cure to IBD, and as a result, the primary goal for treatment is to "… reduce inflammation, maintain remission, and improve quality of life" (Xu et al., 2022). Current treatments are insufficient in achieving this goal though. Usually, patients begin treatment with aminosalicylates, followed by corticosteroids and immunomodulators, or a mixed combination of the three (McDowell et al., 2023). As the severity of the disease progresses, patients may need to resort to alternative forms of treatments, such as surgery or biologic therapy. In most cases, patients with worsening progression of the disease will need to undergo at least 1-2 surgeries within their lifetime.

Fortunately, due to the increased use of biologics, rates of surgical procedures in IBD (CD and UC) patients have decreased by 2% in elective procedures and 7% in urgent procedures between the years of 2010-2017 (Lowe et al., 2020). Biologic therapy is one of the most recently developed treatments for IBD and has shown to be a great alternative treatment as it targets against pro-inflammatory cytokines, such as TNF-alpha and IL-12/13 (Cai et al., 2021). Despite its success during pre-clinical trials, biologics still fall short of being a reliable form of treatment as many patients show no response to the initial treatment or after successive rounds or have experienced adverse effects from the use of biologics (e.g., opportunistic infections) (Amiot & Peyrin-Biroulet, 2015).

1.2.1 Drug Delivery

To reduce the potential of side effect aversion, researchers have turned to the possibility of utilizing nanoparticles as an alternative drug delivery system. Unlike traditional medicine (i.e., oral pills or intravenous injections), nanoparticles do not affect the entire body but solely the area of concern (Patra et al., 2018). While traditional medicine disperses its effects throughout the entire body, nanoparticles are designed to activate upon arrival at the target area. Upon release, nanoparticles can spread out and work with a larger surface area of the affected region. This method reduces toxicity to other organs of the body while directing the full treatment to the affected area (Patra et al., 2018).

The encapsulated shell of nanoparticles can be made of inorganic or organic materials. As inorganic materials add another layer of toxicity, researchers investigated the benefits and viability of using organic materials instead, specifically plant-derived edible nanoparticles (PDNPs). Plant-derived edible nanoparticles are non-toxic to the human body as when absorbed, they break down into organic compounds (Yang et al., 2018) Moreover, plant-derived edible nanoparticles have been shown to display efficient uptake and compatibility to hold various types of drugs and are sustainable option compared to synthetic materials. (Yang et al., 2018).

1.2.2 Ginger-Derived Nanoparticles and 6-shogaol

One study in particular analyzed the use of ginger lipids as a potential candidate in the development of nanoparticles for the treatment of IBD (Sung et al., 2019). Ginger (*Zingiber officinale*) has traditionally been associated as an herbal spice known to relieve nausea, pain, and motion sickness (Anh et al., 2020). Through research, ginger has demonstrated to exhibit anti-cancerous and anti-inflammatory benefits, to which the latter can be attributed to ginger's active component, 6-shogaol (Zhang et al., 2016) (**Figure 1)**. These combination of characteristics in addition to ginger being a readily available organic resource, makes it a good candidate in the development of nanoparticles for IBD treatment.

To test the efficacy of this, a research study orally administered ginger-derived nanoparticles (GDNPs) in vivo to dextran sulfate sodium (DSS) – induced acute colitis mice models (Zhang et al., 2016). By the end of the study, the induced mice showed lower concentrations of lipocalin-2 (Lcn-2) within their feces and lower MPO activity in comparison to the control groups (Zhang et al., 2016). In addition, within the same study, they also tested the GDNP-2's effect upon wound healing. In comparison to the other groups, the group treated with GDNP-2s showed the fastest recovery rate approximately 10 hours after being wounded. This indicated that GDNPs were effective in reducing the overall inflammation in the colitis-induced mice and were beneficial in promoting wound healing.

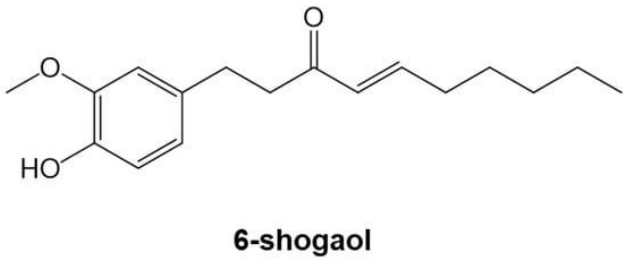


Figure 1 Structure of 6-shogaol.
6-shogaol is the active component of ginger that is responsible for its anti-inflammatory properties (Kawase et al., 2023).

When metabolized, 6-shogaol is conjugated into the hydrophilic compound M13 (**Figure 2**, glutathione-conjugated metabolite). M13 has been demonstrated to have greater anti-inflammatory properties and chemical stability than 6-shogaol when loaded into a nanoparticle, thus making the former a better pharmaceutical candidate for therapeutics (Long et al., 2023).

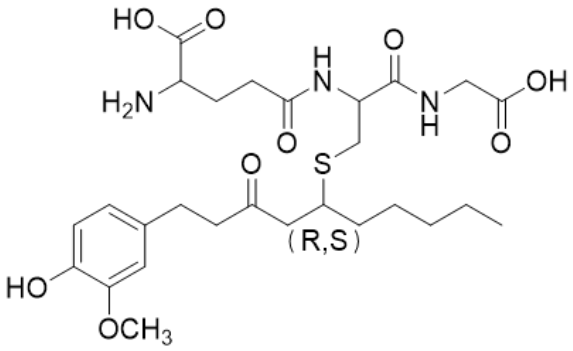


Figure 2 Structure of M13.
M13 is a phase II metabolite (Glutathione conjugated) of 6-shogaol.

1.3 Focus of Study

This current study focused on improving M13 anti-inflammatory properties through modification of its amino acids. Referencing a study conducted by Dr. Duan and others in 2021, the researchers at the time synthesized a group of M13 analogs with increased anti-cancerous activity. In this study, we wanted to use different M13 analogs and analyze their potential anti-inflammatory properties and potential therapeutic effects in comparison to M13.

2. METHODS

All experiments conducted within this research utilized cultures from colonic epithelium cells (Caco-2/BBE cell line), which was purchased from ATCC from Manassass, VA. L-Glutathione reduced, $NaHCO_3$, ethanol (200 proof), methanol, and dichloromethane) were purchased from Sigma-Aldrich (St. Louis, MO). The phosphate buffer saline (Corning TM PBS, 1x) was obtained from Fisher Scientific (Hampton, NH). Fetal bovine serum (FBS) was obtained

from Atlanta Biologicals (R&D Flowery Branch, GA). Ultrapure deionized water was supplied by a Milli-Q water system (Millipore, Bedford, MA). Fifteen M13 analogs (**Figure 4**) were synthesized and purified using a method that had been previously published (Zhu et al., 2013). Unless otherwise specified, the cell lines were cultured in a DMEM solution consisting of 10% FBS and 1% penicillin. All cultures and plates were constantly incubated under conditions of 37 ° C and 5% CO_2.

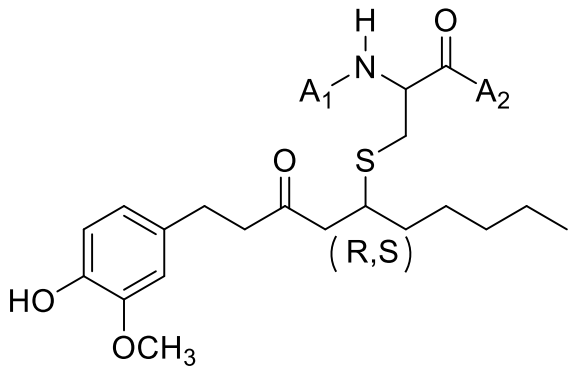
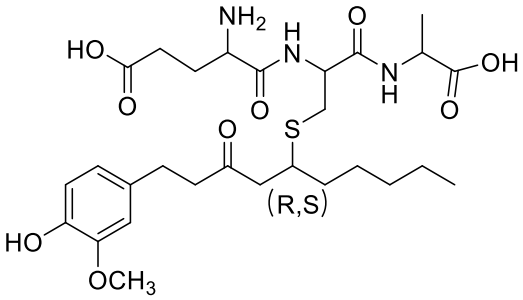


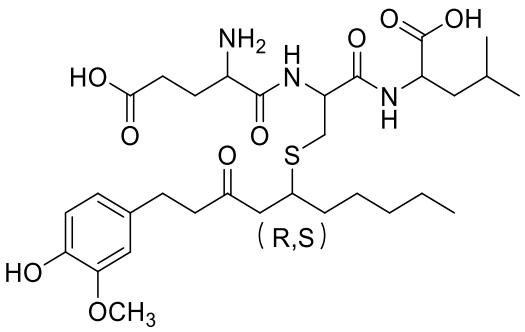
Figure 3 General Structure of Synthesized Analogs.
Two groups (A_1 and A_2) on this M13 structure were modified with varying amino acids, and the resulting synthesized compounds are listed in **Table 1**.

Table 1 List of M13 Analogs

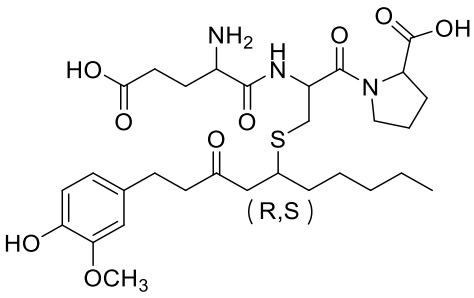
Name of Derivative	A1	A2	Structure
MLY-1	Glu	Ala	Glu(alpha)-Cys(6-shogaol)-Ala
MLY-2	Glu	Leu	Glu(alpha)-Cys(6-shogaol)-Leu
MLY-3	Glu	Pro	Glu(alpha)-Cys(6-shogaol)-Pro
MLY-4	Glu	Trp	Glu(alpha)-Cys(6-shogaol)-Trp
MLY-5	Glu	Tyr	Glu(alpha)-Cys(6-shogaol)-Tyr
MLY-6	Asp	Phe	Asp(alpha)-Cys(6-shogaol)-Phe
MLY-7	Asp	Leu	Asp(alpha)-Cys(6-shogaol)-Leu
MLY-8	Asp	Met	Asp(alpha)-Cys(6-shogaol)-Met
MLY-9	Asp	Pro	Asp(alpha)-Cys(6-shogaol)-Pro
MLY-10	Asp	Val	Asp(alpha)-Cys(6-shogaol)-Val
MLY-11	Asp	Trp	Asp(alpha)-Cys(6-shogaol)-Trp
MLY-12	Asp	Tyr	Asp(alpha)-Cys(6-shogaol)-Tyr
MLY-13	Glu	Met	Glu(alpha)-Cys(6-shogaol)-Met
MLY-14	Asp	IIe	Asp(alpha)-Cys(6-shogaol)-Ile
MLY-15	Glu	Phe	Glu(alpha)-Cys(6-shogaol)-Phe



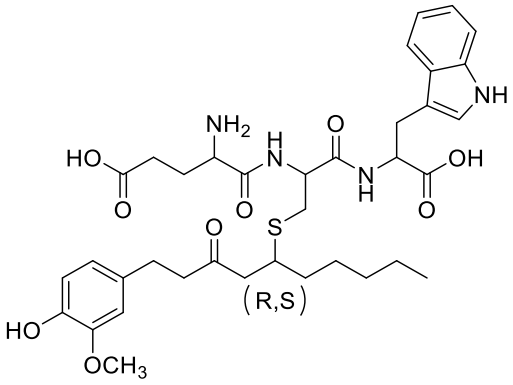
MLY-1



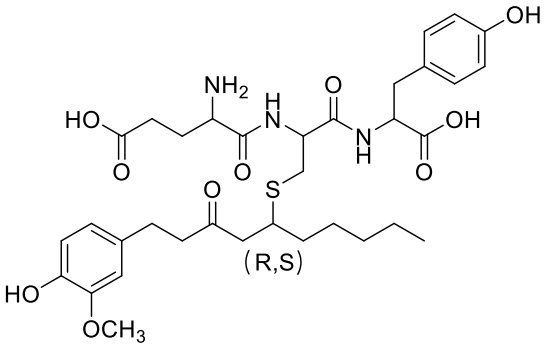
MLY-2



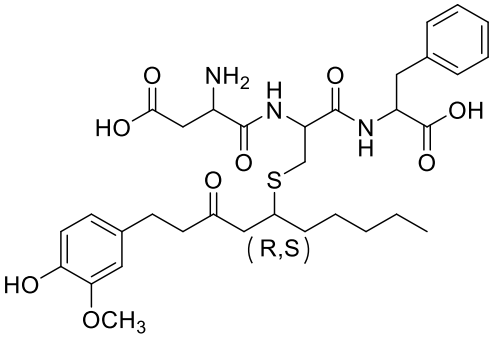
MLY-3



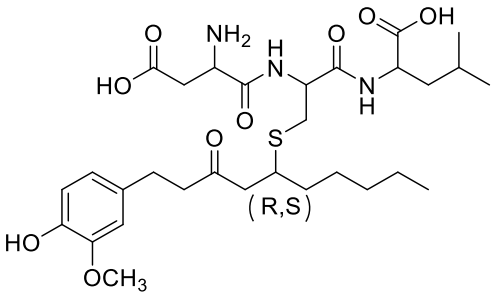
MLY-4



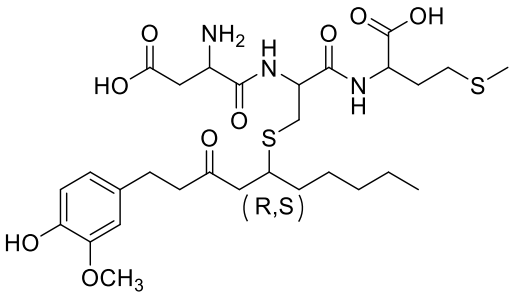
MLY-5



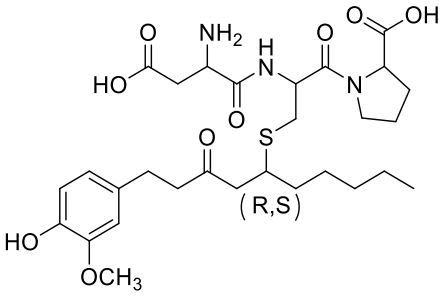
MLY-6



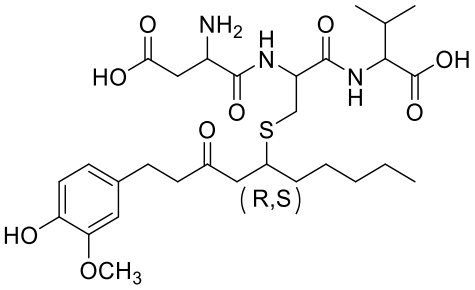
MLY-7



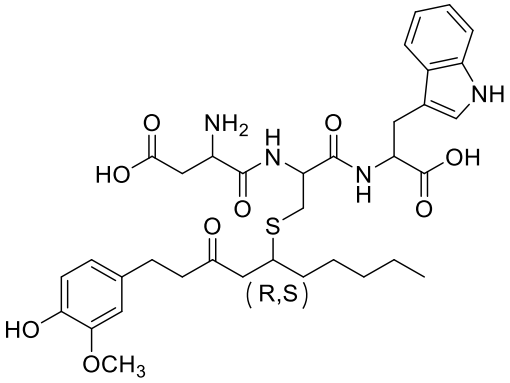
MLY-8



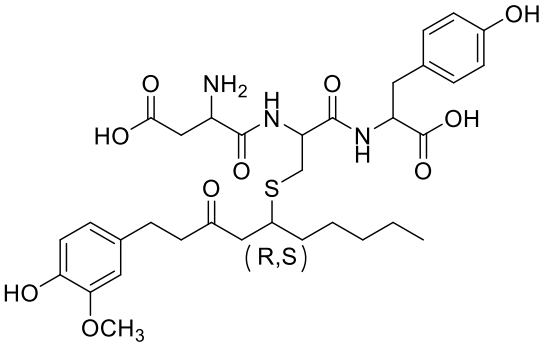
MLY-9



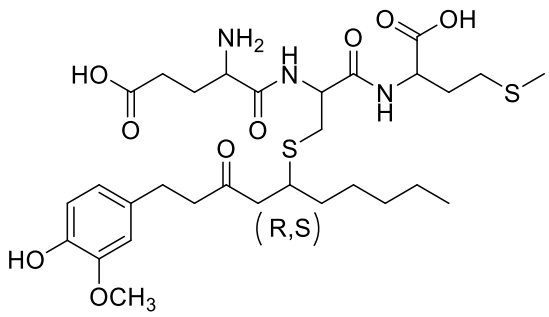
MLY-10



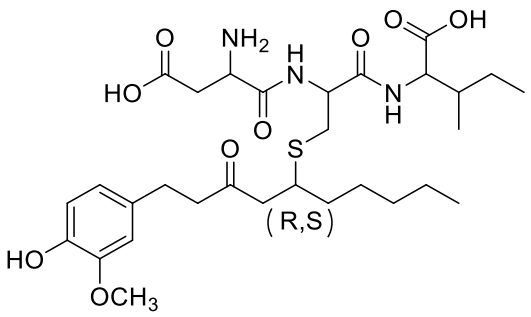
MLY-11



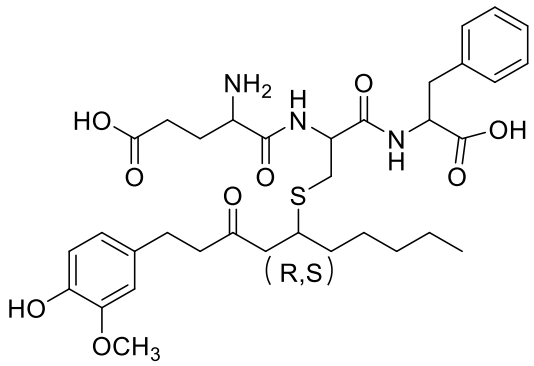
MLY-12



MLY-13



MLY-14



MLY-15

Figure 4 Structures of Fifteen M13 Analogs.
M13 is a phase II metabolite (Glutathione conjugated) of 6-shogaol.

2.1 Evaluation of Drug Sensitivity

Cultures of Caco-2/Bbe cells were subcultured and pipetted into 96-wells plate reader trays. Each well within the plate reader contained on average 5000 cells per mL, and the plates were left to incubate until cell attachment was seen. Solutions of 80 uM, 40 uM, 20 uM, 10 uM, 5 uM, and 2.5 uM were created for each tested drug analog and pipetted into the wells, respectively. Quadruplicates of each concentration per drug analog was performed. Quadruplicates of DMEM and DMSO 0.01% solution were also added to separate wells to serve as a control. After the addition of the drug analogs, the plate reader was incubated for 24 hours. At 24 hours, the cell counting dye was added into each well, and the plate was left to incubate for

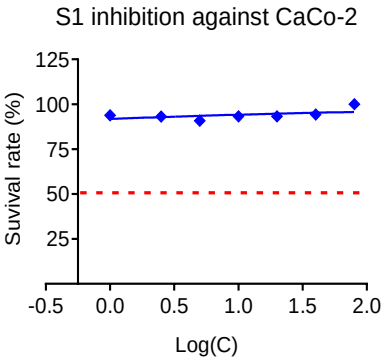
72 more hours before analysis of the tray in the plate reader at a wavelength of 460 nm. This process was repeated for all tested drug analogs.

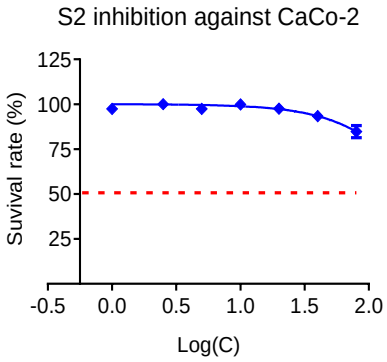
2.2 Wound Healing Assay

The protocol listed in CytoSelect™ 24-Well Wound Healing Assay was followed in this experiment. A cell toxicity test was conducted prior to this experiment and the determined safe concentration level of the drugs analogs was 5 uM. After development of a monolayer, the inserts were removed, and duplicates of each 5-uM drug analog solution were added to the wells. DMEM and DMSO 0.01% were added to separate wells to serve as a control. NucBlue™ Live ReadyProbes™ Reagent was then added to each well, and after 15-20 minutes, the 24-well plate was analyzed and captured under a Keyence Microscope. The plate was placed back into the incubator to be incubated for another 72 hours and taken out afterwards to be captured under the Keyence Microscope again. This process was repeated for all tested drug analogs.

3. RESULTS

After collecting the data, the survival rates of the cells from each drug analog were compiled into a chart for visualization and comparison. Majority of the drug analogs tested showed a trend of greater cell inhibition with increasing drug concentration, except for drugs drug candidates 1 and 15 (**Figure 4)**. Whereas drug candidate 1 showed little to no change at varying drug concentrations, drug candidate 15 appeared to possibly promote growth instead of inhibition of the cells. While charting the drug efficacy of all the analogs, drug candidates 4 and 13 showed the highest potential of killing off cancer cells (**Figure 5**).


S1 inhibition against CaCo-2
Suvival rate (%)
125
100
75
50
25
-0.5
0.0
0.5
1.0
1.5
2.0
Log(C)


S2 inhibition against CaCo-2
Suvival rate (%)
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75
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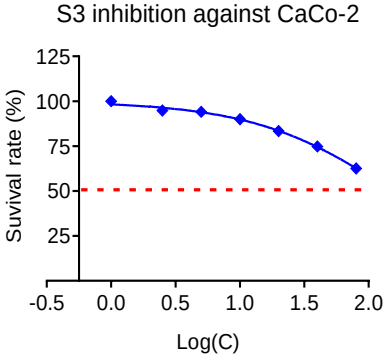
0.5
1.0
1.5
2.0
Log(C)


S3 inhibition against CaCo-2
Suvival rate (%)
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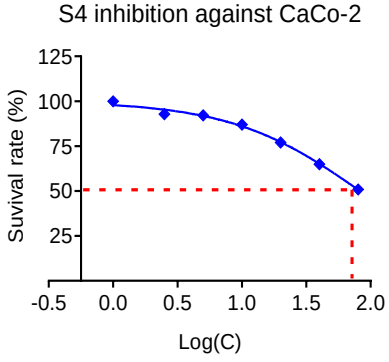
1.5
2.0
Log(C)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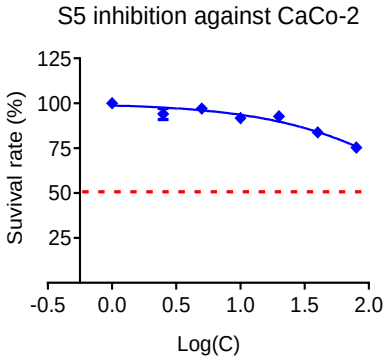
0.5
1.0
1.5
2.0
Log(C)


S5 inhibition against CaCo-2
Suvival rate (%)
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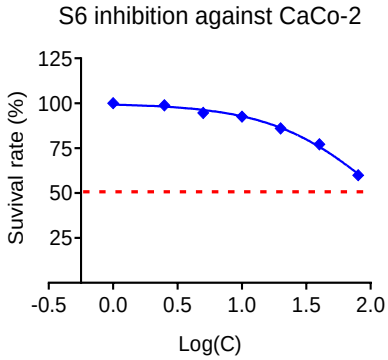
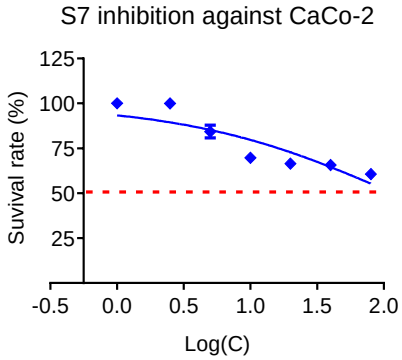
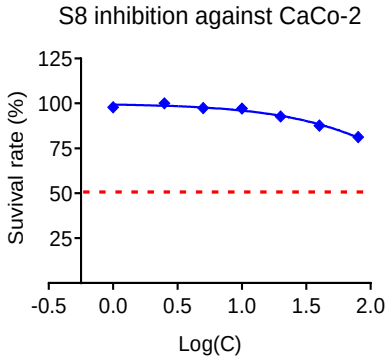
100
75
50
25
-0.5
0.0
0.5
1.0
1.5
2.0
Log(C)


S6 inhibition against CaCo-2
Suvival rate (%)
125
100
75
50
25
-0.5
0.0
0.5
1.0
1.5
2.0
Log(C)


S7 inhibition against CaCo-2
Suvival rate (%)
125
100
75
50
25
-0.5
0.0
0.5
1.0
1.5
2.0
Log(C)


S8 inhibition against CaCo-2
Suvival rate (%)
125
100
75
50
25
-0.5
0.0
0.5
1.0
1.5
2.0
Log(C)

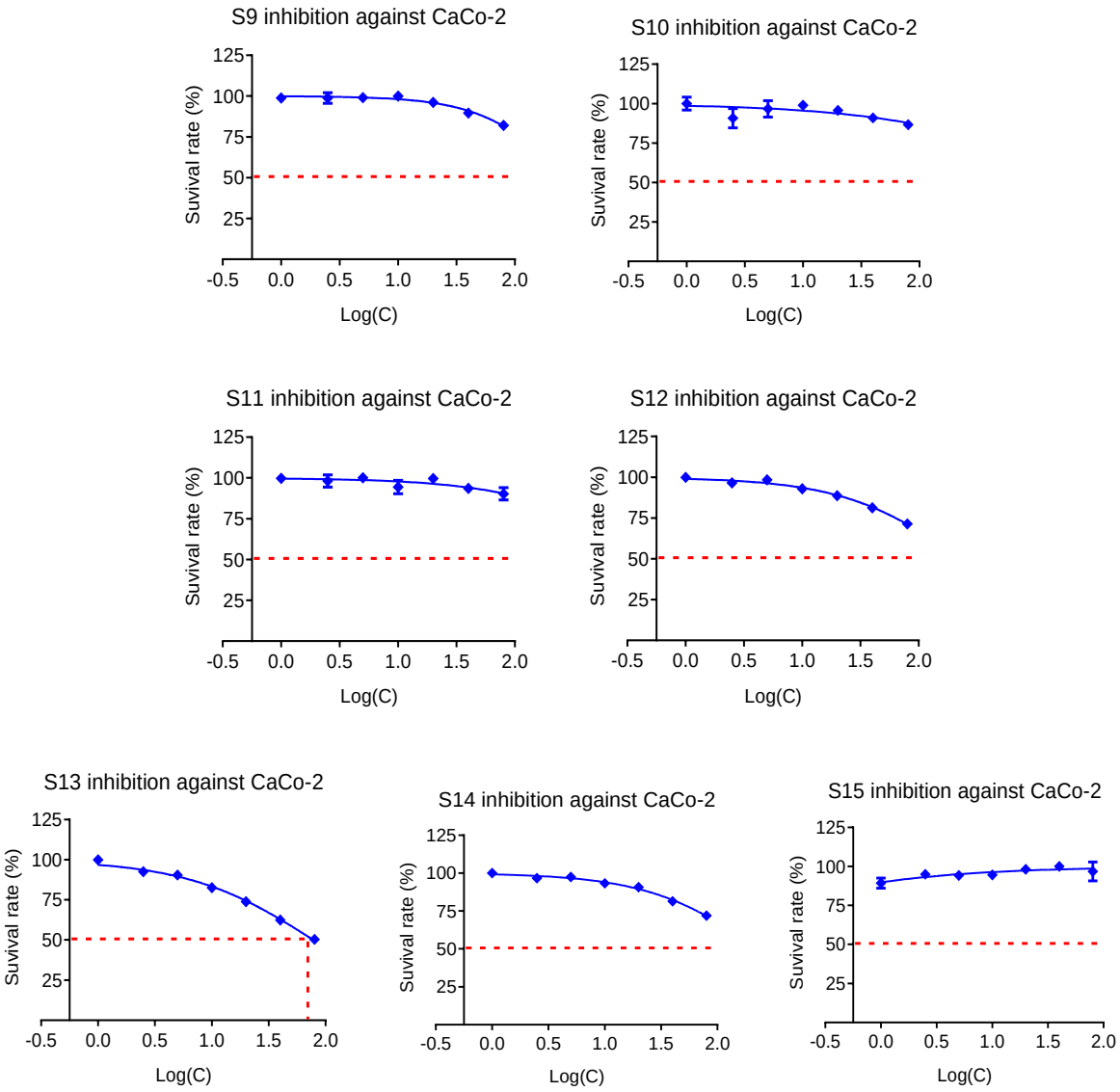


Figure 5 M13 Analogs Inhibition Against Caco-2/Bbe.
All drug analogs were tested at varying concentrations (i.e. 80 uM, 40 uM, 20 uM, 10 uM, 5 uM, and 2.5 uM) against Caco-2/Bbe cells. Out of all the analogs, drug candidates 1 and 15 showed poor inhibition against Caco-2 cells at each concentration. All other analogs showed a trend of increasing inhibition as the drug concentration increased.

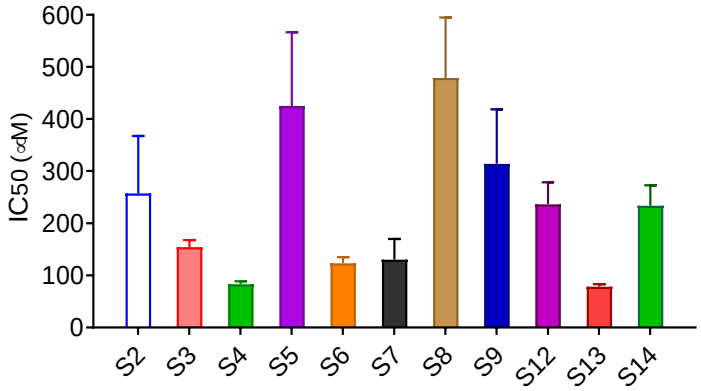


Figure 6 Drug Concentration Efficacy of M13 Analogs.
After 3 days of incubation, the plate readers with the drug analogs were analyzed via a plate reader set to an absorbance of 460 nm. In this set of data, drug analog 8 had the highest survival rate of Caco-2/Bbe cells and required a larger concentration to be effective in reducing the cell count. Both drug analogs 4 and 13 showed the same effectivity as the other analogs but at a lower drug concentration.

4. DISCUSSION

The major reasons for failure in drug development are poor biopharmaceutical properties, which include low aqueous solubility and chemical instability and its toxicity, therefore it is crucial to find a safe and effective drug candidate that also has good biopharmaceutical properties in the early stage of drug discovery. M13, a phase II metabolite of potent anti-inflammatory drug candidate 6-shogaol, is a detoxified compound that maintains strong bioactivity. Our groups' previous studies showed that M-13 demonstrates good biopharmaceutical properties. M13 (Molecular Weight, MW: 583.26) is very stable at various pH levels (pH 2 to pH 10). Also, we found that M13 has high aqueous solubility as determined by: (i) the partition of M13 between and organic solvent (octanol) and aqueous buffer with a LogD7.4 of -1.75; (ii) the kinetic solubility, the maximum solubility of the fastest precipitating species of M13, which was found to be >200 μM, and (iii) the thermodynamic (or equilibrium) solubility, and assessment of the solubility of M13 as a saturated solution in equilibrium, which was found to be 1390.93 μM.

The pKa of a drug influences lipophilicity, solubility, protein binding, and permeability, which in turn directly affects pharmacokinetic (PK) characteristics such as absorption, distribution, metabolism, and excretion. It was found that M13 at physiological pH levels (pH 6 to pH 7) is not ionized, given the observations that M13 pka1 = 3.24; M13 pka2 = 9.12, and M13 pka3 = 10.42 as determined by UV metric at pH 2 to pH 12. Together these M13 physiochemical properties suggest that M13 exhibits a good balance between solubility and has low metabolic liability.

The safety of M-13 was demonstrated by its low mutagenic potential, which was assessed using the Ames test. The Ames test is a rapid and convenient assay to estimate the carcinogenic potential because standard assays in pre-clinical trials are time-consuming (~2 to3 years) and are more expensive. A positive result on the Ames test indicates that the tested chemical is mutagenic and may therefore act as a carcinogen, since cancer is often linked to genetic mutation in an oncological study. We found that M13 did not demonstrate an increase in revertant colony numbers, and the mutation factor was less than ~2.0 for all concentrations in all strains (Long et al., 2023b).

Given that M13 demonstrates good biopharmaceutical properties as an drug lead compound, our group synthesized M13 analogs to discover more potent drug candidate to treat IBD and colitis-associated cancer. Thus far, several of the M13 analogs showed potential to have, at minimum, a similar effect to that as M13. Like M13, these analogs were effective in targeting against colon cancer cells. Through the experimentation described previously, these analogs were capable of reducing the amount of colon cancer cells over a period of time. In terms of wound-healing assay, a toxicity screening was done and identified a concentration of 5 μM as a safe limit.

It is important to mention that these results are still very limited and would require additional studies to be done. Though this study focused on the effectiveness of the analogs as a

drug, an additional study being performed in the lab recently examined the anti-inflammatory effects of the M13 analogs, and the results showed how certain analogs had greater anti-inflammatory properties than M13 alone (Long et al., 2023b). In addition, the wound healing assays for the drugs analogs are still being conducted, and as of right now, it is still hard to determine whether these drug analogs also have additional therapeutic effects, such as increasing the rate of wound healing.

Taking all of this into consideration, we can presume that these M13 analogs can still serve as a potential therapeutic therapy for many diseases, such as autoimmune disorders, cancer, and more specifically IBD. As stated previously, IBD is a chronic condition which persistently worsens over time. IBD can in turn cause additional complications for patient such as colitis-associated cancer (CAC). As the present data on M13 analogs showed effectiveness in reducing colon cancer cell counts, utilization of M13 analogs as a form of treatment can potentially help to eliminate any tumors forming because of the constant inflammation from IBD. More studies will be done, but the data thus far show promising hopes for M13 analogs.

For future studies, it would be important to continue studying the effects of the M13 analogs on wound healing. As we know, within IBD-affected patients, the chronic inflammation leads to thinning of the intestinal mucosal layer and causes this vicious cycle of invasion of pathogens and constant inflammation. Within these individuals, it is important to improve the mucosal healing rate of their epithelium in order to promote recovery and lower the risks of continuous flare-ups. Once this study is conducted, it will be important to follow-up with further assessments and investigations in vivo.

Another possible future study to endeavor would be to compare the activities of these M13 analogs in comparison to KPV. KPV is a naturally occurring peptide within the human body which is responsible for anti-inflammatory properties. In a previous study, it was found that when uptaken by PepT-1, KPV assisted led to a decrease of inflammation within the intestinal

tracts (Dalmasso et al., 2007). Since M13 analogs are also tripeptides, would they also be uptaken by PepT-1? Between KPV and M13, which would shower higher anti-inflammatory properties? These are possible questions and pursuits we can investigate further into the future.

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- Mercer University Dean's List
  - Fall of 2020

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  - Course: Research in Biology
  - Partner: Raj Patel
- Analysis and Comparison of Phosphomimetic Mutation of optimized E. coli OGA and mammalian OGA (*Fall 2021*)
  - Instructor: Dr. Garland Crawford
  - Course: Biochemistry Capstone
  - Partner: N/A
- Investigation of Roc1a in *Drosophila virilis* (*Fall 2021*)
  - Instructor: Dr. John Stanga
  - Course: Molecular Genetics
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