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# Synthesis Of Indole Carboxamides As Modulators Of Cannabinoid 1 Receptors

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## Abstract

Cannabinoid 1 Receptors (CB1) are neuronal terminals, responsible for synaptic communication between nerves. They are essential for binding of ligands to form biologically active complexes and monitor G-protein functions. CB1 agonists are already being used clinically for therapeutic uses such as multiple sclerosis, inflammatory disorder, and cancer. Naturally occurring cannabinoids show high affinity for CB1 receptors, with the presence of an indole ring increasing binding affinity. Substitutions and their various positions on an indole ring system plays a crucial role in the binding to CB1 receptors. The synthesis of various substituted indoles by the Hemetsberger-Knittel method from a variety of substituted aldehydes is underway. 3,4,5 Trimethoxybenzaldehyde provided the best yield of vinyl azide 67% prior to thermolysis. The formation of ethyl azidoacetate using water and acetone as co-solvents, produced ethyl azidoacetate in greater yield than previously reported by literature and previous group members. Knoevenagel process and xylene mediated thermolysis under nitrogenous conditions produced vinyl azide and indole ester, respectively. Previous research suggests the presence of the carboxamide functionality at the 2 position of the indole is crucial for increased affinity to the CB1 receptors. Reduction of the indole ester to an indole acid will allow for a variety of indole-2-carboxamides to be synthesized using BOP reagent, triethylamine and N,N-dimethylformamide.

## 1. Introduction

### 1.1 Cancer Therapeutics

Natural product research has been a growing field in the search for new chemotherapeutic drugs. A variety of naturally occurring molecules have shown to be highly effective as anti-cancer treatments. Scientific evidence has shown cannabinoids to be active anti-cancer agents with anti-tumor and mitotic properties.[1] The cannabinoid, cannabis, is a relevant social and political topic today. The three most commonly known cannabinoids (Figure 1) all contain similar carbon ring pharmacophore structures with carbon chains assisting in optimization of binding. Since cancer is uncontrolled division of cells, finding a compound that inhibits the division of these mutated cells may lead to cancer treatments and previous studies show cannabinoids hold this potential. Studying the SAR, (structure activity relationship) of cannabinoid analogs with changed functional groups has provided insight for the controlled manipulation of natural compounds in therapeutic research.[1]

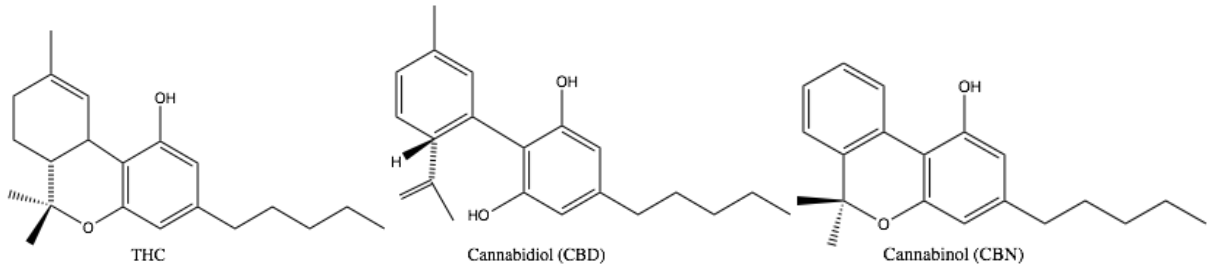



Figure 1. Commonly known naturally occurring cannabinoids

## 1.2 Cannabinoid CB1 Receptors

Cannabinoid 1 receptors at neuronal terminals monitor the release of neurotransmitters and are essential in the binding of ligands and protein functions. CB1 agonists are already being used clinically for therapeutic uses such as multiple sclerosis, inflammatory disorders, and multiple types of cancer.[2] Synthetic cannabinoids have shown anti-migratory and non invasive inhibition of cell replication that could slow down the metastasis of cancer significantly.[2]

Cannabinoids activate G-coupled protein receptors, which are essential in drug binding.[3] CB1 receptors are located in the central nervous system in abundance and therefore have accessibility to a wide variety of organs and tissues throughout the body.[4] The CB1 receptor shares the structure characteristic of all G-protein-coupled receptors, possessing seven trans membrane domains connected by three extracellular and three intracellular loops, an extracellular N-terminal tail, and an intracellular C-terminal tail (Figure 2). With a major and minor binding pocket, the variety of binding targets has allowed for research in binding affinities to be explored across analogs of synthetic cannabinoids.[5]

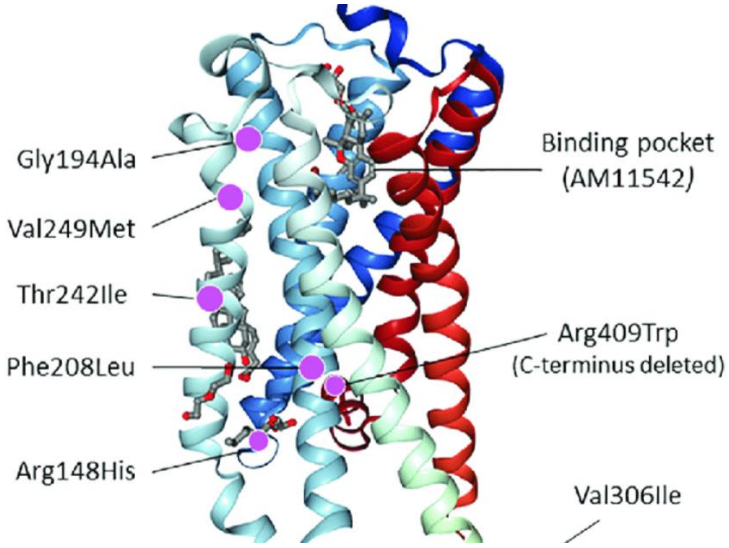



Figure 2. Cannabinoid 1 Receptor composed of seven transmembrane amino acid helixes.

## 1.3 Indole-2-Carboxamides

Francesco Piscitelli conducted a SAR study of CB1 allosteric modulators (molecules that indirectly affect protein functionality by binding to a distant location) in binding with indole carboxamides (Figure 3). The carbon chain elongation optimizes the affinity to CB1 receptors, as shown in naturally occurring cannabinoids and previous literature. Twenty-six different analogs were synthesized with varying substituents around the indole ring. The positions of the substituents around the indole plays a crucial role in the hydrophobic binding to cannabinoid CB1 receptors.[1]

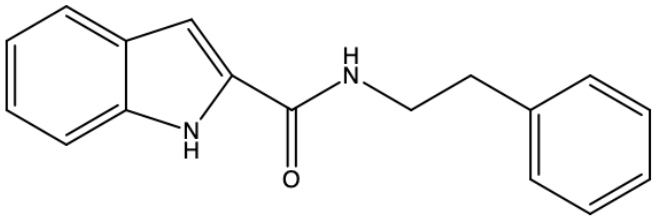


Figure 3. Indole carboxamide structure

There are four naturally occurring cannabinoids that include an indole ring within their structure (Figure 4). These cannabinoids naturally bind to CB1 receptors at the hydrophobic binding pocket, suggesting the indole structure has a high binding affinity to the receptors via hydrophobic interactions.

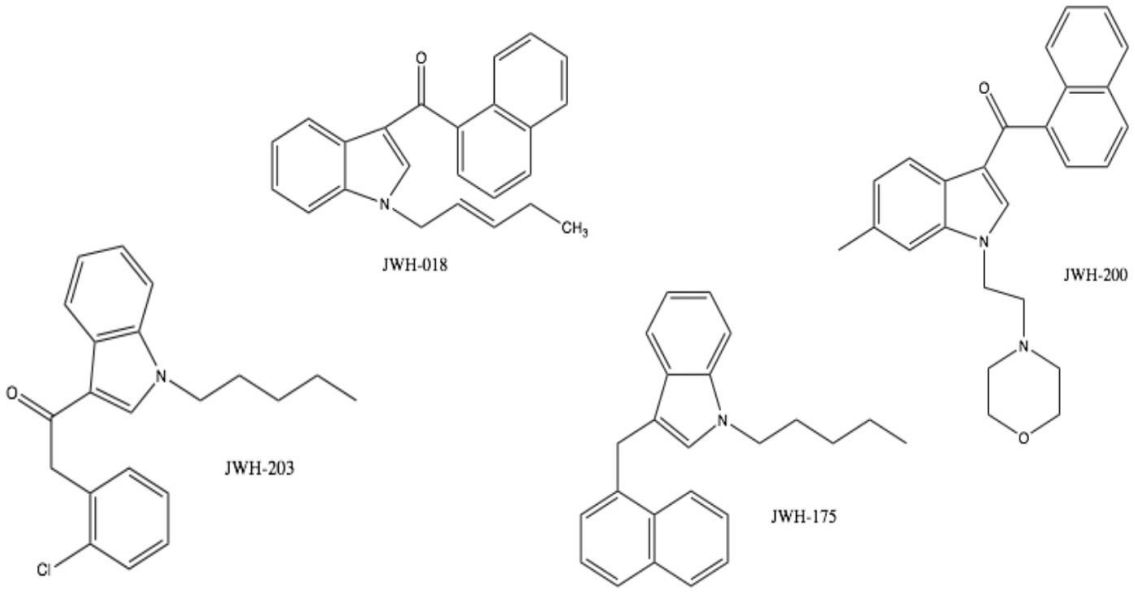



Figure 4. Naturally occurring cannabinoids JWH-203, JWH-018, JWH-175, and JWH-200 with indole ring structure

## 2. Results and Discussion

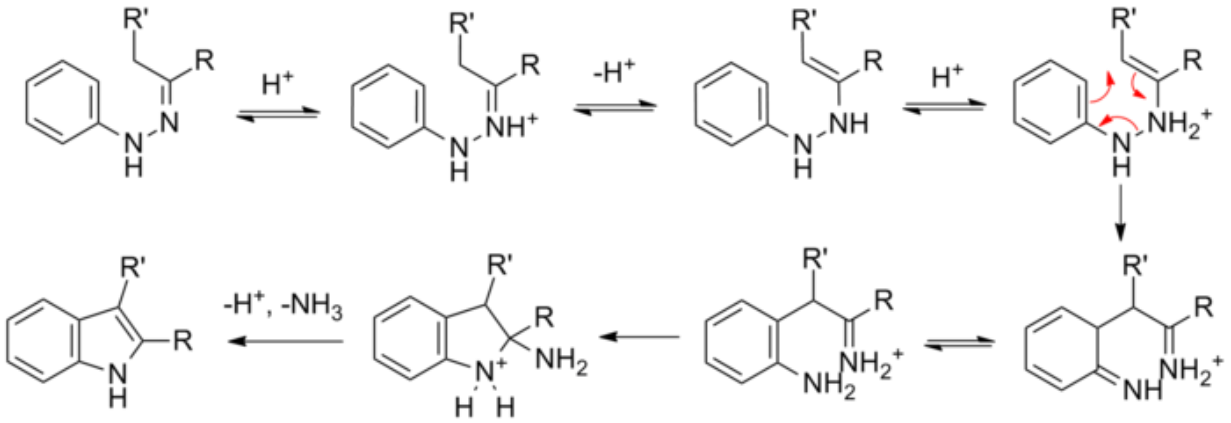
### 2.1 Indole Ring Synthesis

There are thirteen categories for the synthesis of indoles, which can be sigmatropic rearrangements to transition metal catalyzed cyclization.[6] Following are the explored methods pertinent to indole precursors for indole carboxamide synthesis. Computational and synthetic testing of indole carboxamide analogs will be used to analyze binding affinities.

#### *2.1.1 fischer indole synthesis*

The Fischer indole synthesis is a prolific route to indoles that has remained pertinent since its inception.[7] The mechanism of the Fischer indole cyclization is believed to involve a [3,3]-sigmatropic rearrangement of an

enehydrazine tautomer to a bis imino benzyl ketone. The subsequent cyclization and aromatization, accompanied by the expulsion of ammonia provides the desired indole product (Scheme 1).[8] Though Fischer indole synthesis remains dominant in research, extensive literature research did not present successful indole analogs with potential to be decarboxylated at the 2 position, thus, other methods were explored.




Scheme 1: Fischer Indole Synthesis

## *2.1.2 hemetsberger knittel indole synthesis*

The Hemetsberger Knittel method requires the use of azides and organoazides, like the azido phenyl acrylate.[9] Boger et al. used the route in the synthesis of the 5,6,7-trimethoxy indole component of duocarmycin, a DNA alkylating agent in 1990.[10] More recently, Condie et al. confirmed the viability of the Hemetsberger-Knittel route in their synthesis of dimethoxy indoles in 2005.[11] An Aldol reaction between benzaldehyde and methyl azidoacetate yields vinyl azide. The resulting vinyl azide is then added to refluxing xylenes to afford an azirine intermediate. This intermediate then rearranges to yield an indole ester due to the expulsion of nitrogen gas (Figure 5).[12]

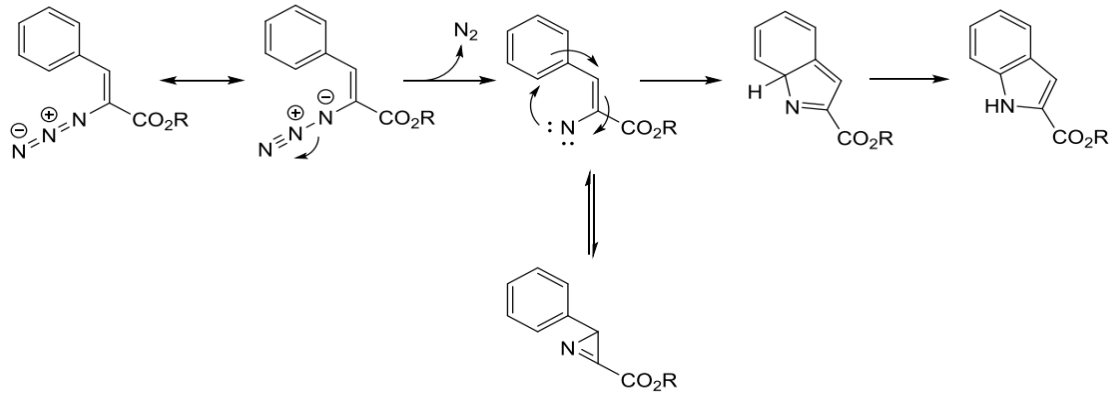



Figure 5. Mechanism of Hemesterberger indolization

Using previous research[1], a synthetic scheme was proposed to synthesize indole ester from ethyl azidoacetate using the Hemetsberger Knittel method. Synthesis of an alkyl azidoacetate, base promoted Knoevenagel condensation between azidoacetate and an aromatic aldehyde, and thermolysis of α-azido-β-arylacrylate resulted in cyclization to

produce an indole skeleton. Saponification of the indole ester to an indole acid will allow for the addition of a primary nitrogen, thus promoting carboxamide synthesis (Scheme 2).

Previously, Dr. Herman L. Holt and undergraduate students Teresa Rocha and Benjamin McDonald worked to synthesize indoles. Teresa made combretastatin A4 analogs (Figure 6) by decarboxylating synthesized indoles at the 2-position to subsequently add functionality at the 3-position. The Hemetsberger Knittel method inherently puts the carbonyl at the 2-position of the indole. Future work will focus on decarboxylating and substituting at the 2-position of the indole ring (Figure 7), with addition at the 2-position to replicate the desired indole carboxamide structure. Using the Hemetsberger Knittel method will allow for one-step synthesis of substituted indoles into 2-indole-carboxamides (Figure 8), as the aldehydes used in earlier synthesis will already be attached to the indole ring.

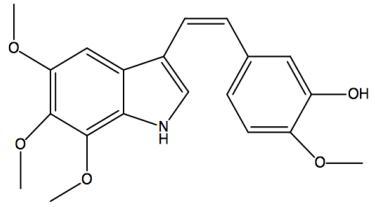



Figure 6.

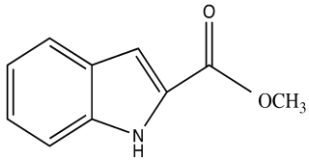



Figure 7.

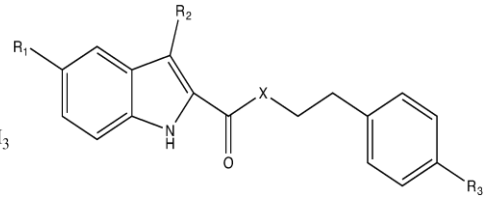
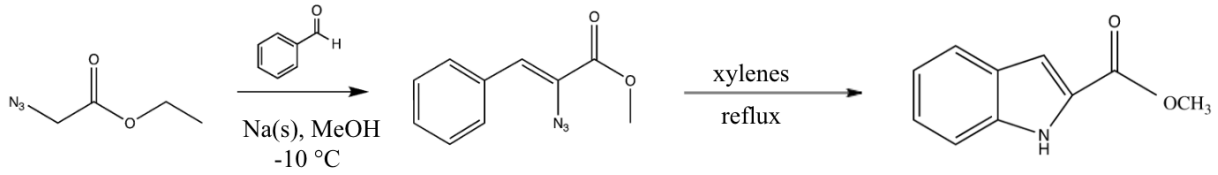



Figure 8.

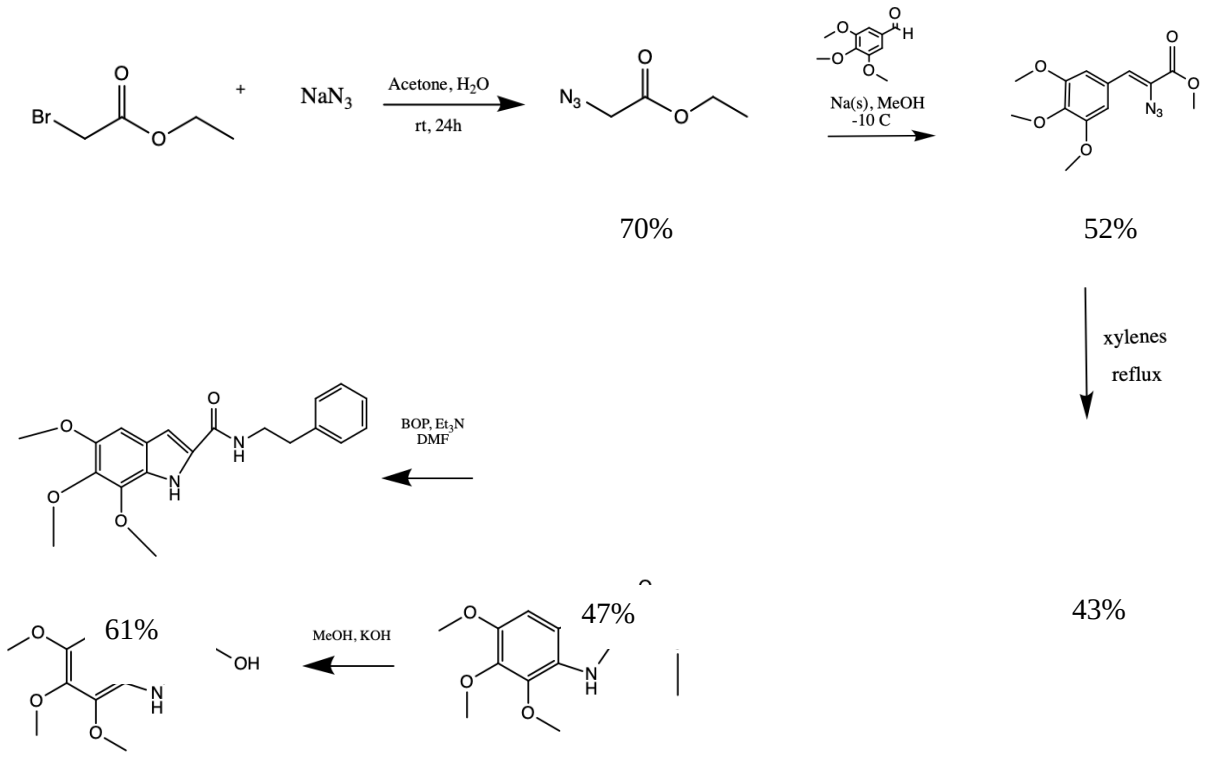
Indole esters was synthesized using the Hemetsberger- Knittel Method (Scheme 2) Ethyl azidoacetate was synthesized using water and acetone as co-solvents and then underwent a $SN_2$-reaction to yield the substituted vinyl azide with varying aldehyde groups attached. 3,4,5 trimethoxy benzaldehyde, 3,5 dimethoxybenzaldehyde, 3,5 dichloro benzaldehyde, and 3,5 dibromobenzaldehyde were all used due to previous research suggesting success in addition of these substituents.[1] After thermolysis, these azides produced the indole ester products, referred to as the Hemetsberger-Knittel method (Scheme 3).




Synthetic scheme of ethyl azidoacetate undergoing an $SN_2$ reaction to yield vinyl azide to allow for Hemetsberger Knittel synthesis of an indole

Research in this field will allow for more understanding of cannabinoids mode of inhibition of cell mutation and advance cancer research. A large population is affected by cancer, which makes it essential to explore treatments that are less invasive and less toxic. Chemotherapies today come with some undesired side effects, including the damage of healthy cells.[13] Being able to capitalize on synthetic cannabinoid binding to an already present receptor would change pharmaceutical research tremendously.

**Scheme 3.**




## 2.2 Indole Ring Synthesis

Hemetsberger-Knittel methodology was utilized for the synthesis of methyl 5,6,7-trimethoxy-1H-indole-2-carboxylate (Figure 9a), 5,7-dichloro-1H-indole-2-carboxylate (Figure 9b), and methyl 5,7-trimethoxy-1H-indole-2-carboxylate (Figure 9c), using varying aldehydes (Scheme 4).

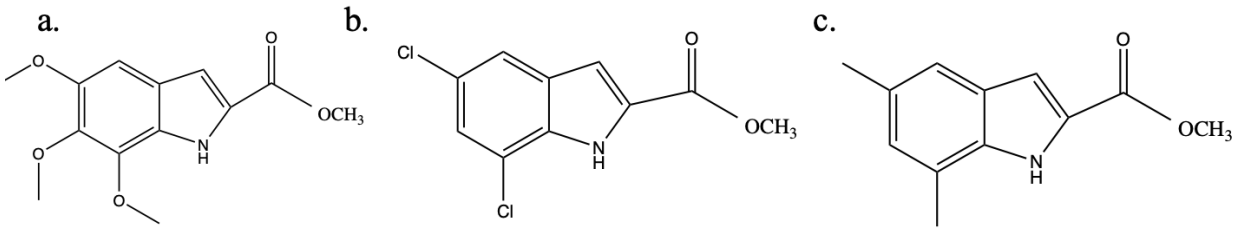
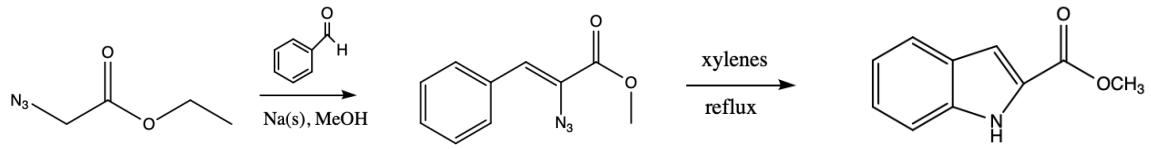



Figure 9. Indole ester analogs

Scheme 4. Hemestberger- Knittel Method

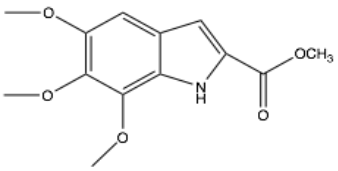



Ethyl azidoacetate Vinyl azide Indole ester

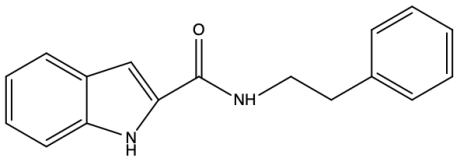
Ethyl azidoacetate (EAA) was generated in 70% yield (Scheme 3). An aldol condensation was then performed, with EAA and varying benzaldehydes producing methyl (Z)-2-azido-3-(3,4,5-trimethoxyphenyl)acrylate (49%), methyl(Z)-2-azido-3-(3,5-dichloro) acrylate (8.7%), and methyl (Z)-2-azido-3-(3,5-dimethoxyphenyl)acrylate (9.4%). It should be noted that sodium is very reactive and should not be exposed to air. The sodium used for this reaction was stored in an oil was used in excess in an effort to counter the mass of the oil coating the reagent. Refluxing the resulting vinyl azide in xylenes induced ring closure via thermolysis and produced methyl 5,6,7-trimethoxy-1H-indole-2- carboxylate (42.3%), 5,7-dichloro-1H-indole-2-carboxylate ( 35%), and methyl 5,7 -dimethoxy -1H -indole-2-carboxylate (5.0%). MeOH and KOH reduced the indole ester to an indole acid(47%), followed by a BOP reagent ((Benzotriazol-1-yloxy)tris(diethylamino)phosphonium hexafluorophosphate)) prompted reaction with triethylamine and phenylethylamine to yield a carboxamide (61%).

## 2.3 Computational Docking

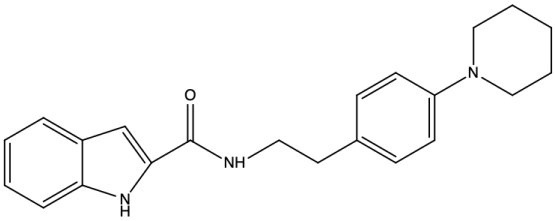
Computational docking of various carboxamides analogs using the Lamarckian algorithm in Autodock was used to analyze residue interactions and binding location of the compounds (Figure 10). Analog A was found to bind in the minor hydrophobic binding pocket of the cannabinoid 1 receptors (Figure 11). The greatest binding affinity computed was -6.5 kcal/mol. Phe237, Leu209, and Asp213 residues are all present in the binding pocket and show interactions with the docked molecule.



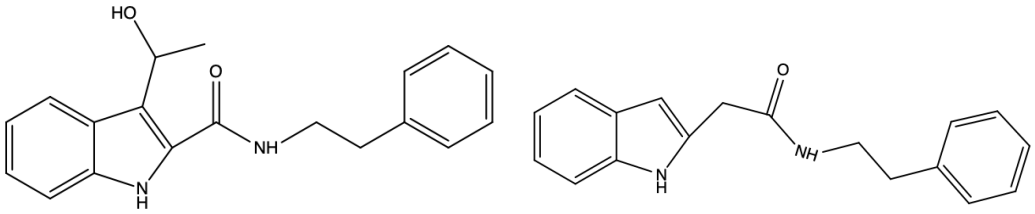

Analog A.



Analog B.

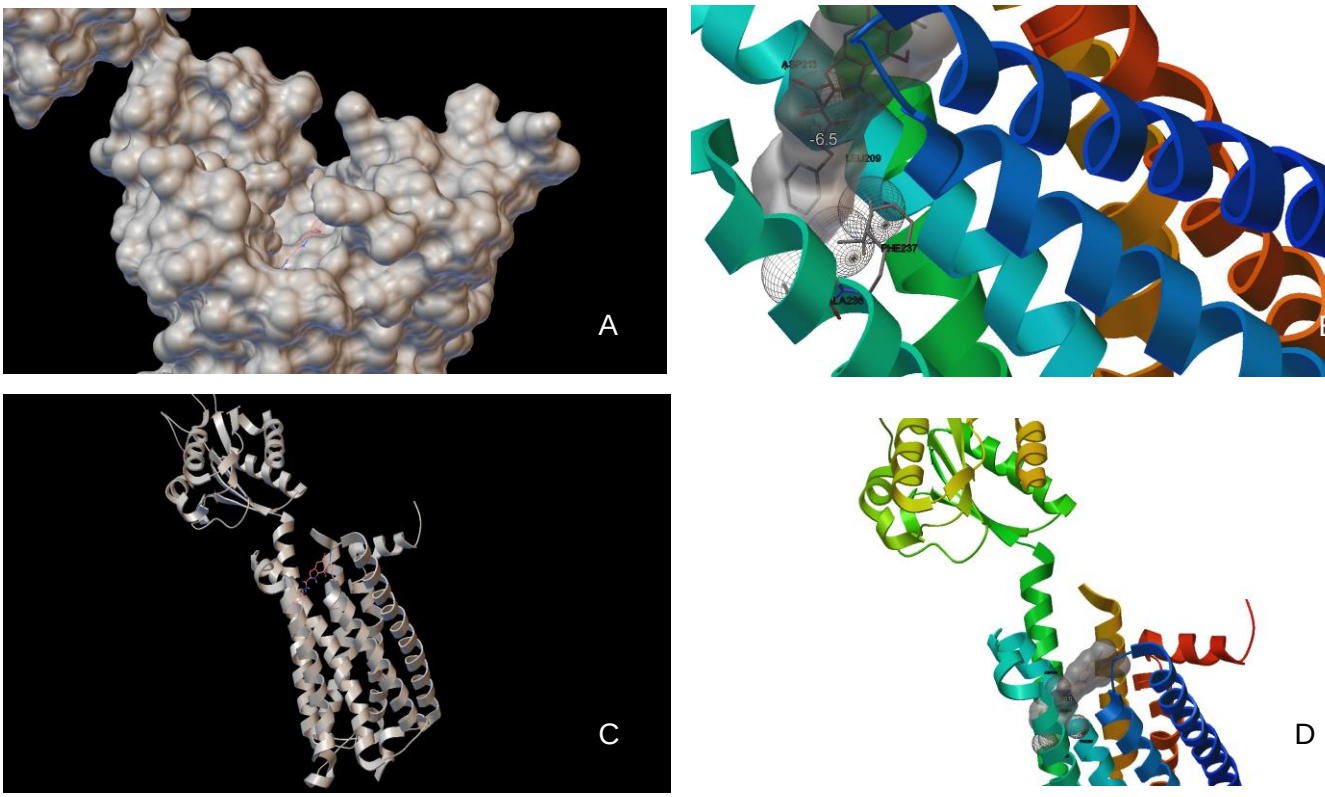


Analog C.




Analog D. Analog E.

Figure 10.




Computational docking using Autodock

Figure 11. Autodock computational docking of Analog A using Lamarckian algorithm. A) Confirmation of indole 2-carboxamide binding in the minor hydrophobic region of the CB1 Receptors. B) Indole-2-carboxamide residue interactions in the CB1 minor pocket. C) visual of major and minor binding pocket, with major being upper bundle of helixes and minor being lower bundle. D) CB1 amino acid residues with indole-2-carboxamide bound.

Lamarckian Algorithm suggests the indole-2-carboxamide would bind in the minor hydrophobic binding region on the CB1 Receptors. Interactions with Leu209, Asp232, and Phe237 will allow for optimizations of substituents around the carboxamide due to their hydrophobic binding properties, ideal for carboxamide interactions. The indole-2-carboxamide bound with a -6.9 kcal/mol binding efficacy to the CB1 receptor protein, from the International Protein Database. A negative kcal/mol binding energy is ideal, as this means it will not require energy for binding.

Analog B was docked to show the importance of the trimethylation on the indole ring (Figure 12). The binding affinity decreased to -9.9 kcal/mol and the analog docked in the major hydrophobic region of the CB1 receptor. This

implies the methoxy additions are essential in binding the compound to the minor region, and provide hydrogen bonding with residues in said region.

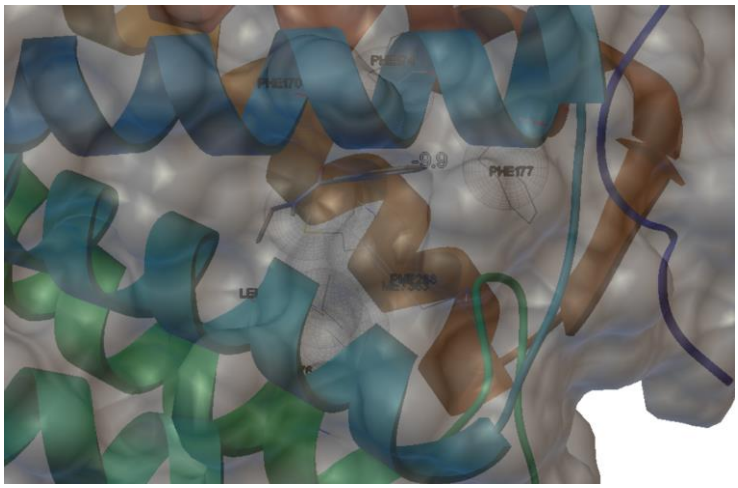



Figure 12. Autodock computational docking using Lamarckian algorithm. Analog B binding in the major hydrophobic region of the CB1 Receptors, with -9.9 kcal/mol binding energy.

A 6-member nitrogenous ring at the $R_3$ position of indole-2-carboxamide (Analog C) showed a lower binding affinity of -8.6 kcal/mol, with interactions mostly present in SER152 and ALA236 (Fig 12). With a less negative binding affinity compared to the analog while the nitrogenous ring contributes hydrogen binds to surrounding residues. This analog was found to bind further down in the minor binding pocket of the CB1 receptor.

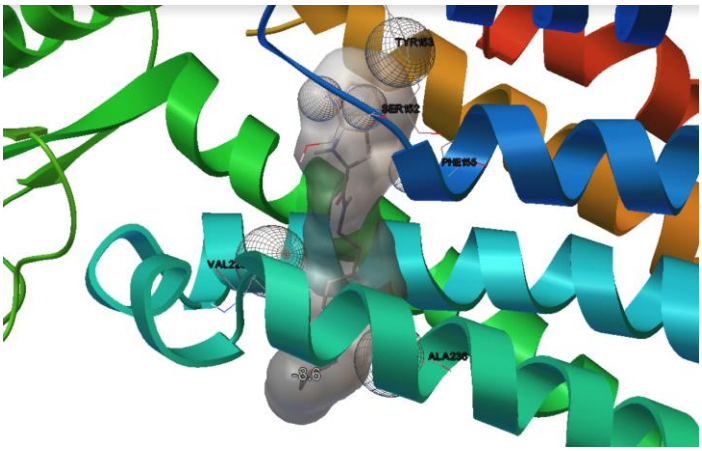



Figure 13. Autodock computational docking using Lamarckian algorithm. Analog C binding in the minor hydrophobic region of the CB1 Receptors.

Analog D shows the addition of an alcohol containing group at the $R_2$ position, labeled in the Piscetelli paper (Figure 14). Though Analog D did not dock in the minor hydrophobic region, the efficacy of -7.0 kcal/mol, was improved from all other analogs, excluding analog A. This suggests additional hydrogen bonding substituents in the hydrophobic region are beneficial in overall binding energy.

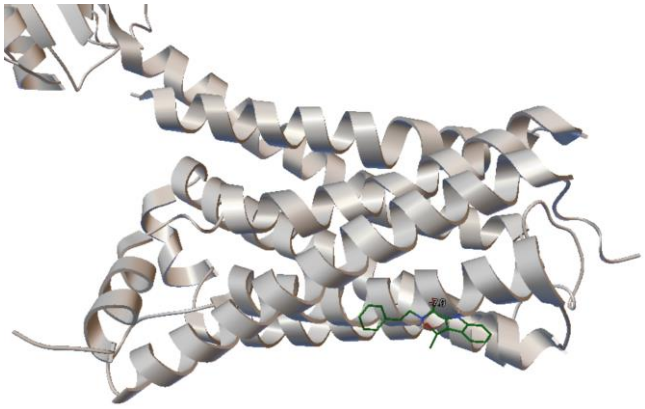


Figure 14. Autodock computational docking using Lamarckian algorithm. Analog D binding in the major hydrophobic region of the CB1 Receptors with -7.0 kcal/mol binding energy.

## 2.4 In Vitro Docking

In a 96 well plate, approximately 5,000 cancer cells/well were treated with indole-2-carboxamides in varying concentrations of: 1 mM, 100 µ M, 10µ M, 1 µ M, 100 nM. Next, 20 microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide , abbreviated MTT, were added per well. Concentration of indole-2-cacrboxamide decreased as the columns read left to right. MMT assays introduced a tetrazolium salt dye. Living cells can reduce MMT to formazan via reduction in the mitochondria. This produced purple formazan crystals in varying transparencies, as cell death varied. After 4 hours the purple crystals were dissolved with acidic solution, to produce a yellow fading gradient, with highest concentrations of indole-2-carboxamide being most effective. A plate reader, used to measure relative absorbance of each well in in vitro studies, provided the readings seen in Table 1. Indole-carboxamide at 1 mM concentration killed approximately 50% of all cancer cells, while % of living cells increased as concentration of drug decreased. 1mM concentrations produced 51.2% cell vitality, meaning only 50% of cells survived. The percent of living cells increases as the drug concentration decreases, showing that higher concentrations of drugs were more successful. The same docking process was done for non-methylated indole-2-carboxamide, seen in Table 3. At 1 mM concentration, cell viability was 69.9%, with increasing viability as concentration of drug decreased.

Table 1. Absorbance (nanomolar, nm) of each well taken with a 630 $cm^{-1}$ background reading.

| 1 mM | 100 uM | 10 uM | 1 uM | 100 nM | w/ DMSO | 0 DMSO | |
|---|---|---|---|---|---|---|---|
| 0.195 | 0.248 | 0.262 | 0.273 | 0.288 | 0.267 | 0.326 | 570 |
| 0.129 | 0.152 | 0.164 | 0.173 | 0.181 | 0.171 | 0.205 | background:630 |
| 0.168 | 0.227 | 0.269 | 0.274 | 0.266 | 0.249 | 0.352 | 570 |
| 0.112 | 0.146 | 0.174 | 0.18 | 0.175 | 0.157 | 0.232 | background:630 |
| 0.215 | 0.242 | 0.255 | 0.283 | 0.29 | 0.275 | 0.329 | 570 |
| 0.147 | 0.152 | 0.162 | 0.185 | 0.191 | 0.184 | 0.199 | background:630 |

Table 2. Absorbance of each well with 630 $cm^{-1}$ background removed, showing percent of cells remaining per concentration. Rows B, C, D remain constant, with concentration of drug (columns) being the only variable changing.

| Concentration | 1 mM | 100 uM | 10 uM | 1 uM | 100 nM | w/ DMSO | 0 DMSO |
|---|---|---|---|---|---|---|---|
| B | 0.066 | 0.096 | 0.098 | 0.1 | 0.107 | 0.096 | 0.121 |
| C | 0.056 | 0.081 | 0.095 | 0.094 | 0.091 | 0.092 | 0.12 |
| D | 0.068 | 0.09 | 0.093 | 0.098 | 0.099 | 0.091 | 0.13 |
| Average | 0.0633 | 0.0890 | 0.0953 | 0.0973 | 0.0990 | 0.0930 | 0.1237 |
| % viabillity | 51.2129380 | 71.9676549 | 77.0889487 | 78.7061994 | 80.0539083 | 75.2021563 | 100 |

Table 3. Non methylated indole-2-carboxamide Absorbance (nanomolar, nm) of each well taken with a 630 $cm^{-1}$ background reading.

| 1 mM | 100 uM | 10 uM | 1 uM | 100 nM | DMSO | no DMSO | |
|---|---|---|---|---|---|---|---|
| 0.282 | 0.346 | 0.294 | 0.349 | 0.387 | 0.409 | 0.391 | 570wavelength |
| 0.222 | 0.289 | 0.25 | 0.29 | 0.315 | 0.343 | 0.289 | 630 background |
| 0.236 | 0.323 | 0.345 | 0.345 | 0.427 | 0.428 | 0.386 | 570wavelength |
| 0.189 | 0.277 | 0.302 | 0.291 | 0.356 | 0.362 | 0.283 | 630 background |
| 0.302 | 0.314 | 0.318 | 0.334 | 0.369 | 0.396 | 0.396 | 570wavelength |
| 0.235 | 0.26 | 0.261 | 0.269 | 0.307 | 0.323 | 0.331 | 630 background |
| 69.9062233 | 83.8022165 | 81.5856777 | 87.6385336 | 100.852514 | 105.115089 | 100 | %Cell Viability |

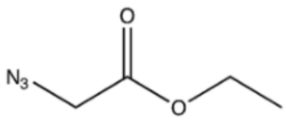
## 4. Future Directions

Understanding the ways in which synthetic cannabinoids and their substituents interact with cannabinoid CB1 binding sites will help determine their ability as cancer treatments. Synthesis of 2-indole-carboxamides will occur with indole acids. Computational docking of indole-carboxamides will help narrow down the substituents via analysis of cannabinoid binding site interactions. HELA and cancer cells will be treated with purified carboxamides for further analysis of products. Manipulation of substituents around the indole ring and the carboxamide region of the indole carboxamide to find which positions are most crucial to binding, will allow for a better understand and narrow the scope of research for CB1 studies.

## 5. Conclusion

There is a vast need for new cancer treatments and the search for new chemotherapeutic drugs. Cannabinoid 1 (CB1) receptors are essential in the binding of ligands and protein functions. CB1 agonists are already being used clinically for therapeutic uses such as multiple sclerosis, inflammatory disorder, and cancer. Naturally occurring cannabinoids show high affinity for CB1 receptors, with the presence of an indole ring increasing binding affinity. Studied here, substituents and their various positions on an indole ring system played a crucial role in the binding to CB1 receptors. Computational Docking using Autodock showed amino acid residue interactions with the bound compounds, allowing for optimization of substituents. A trifecta of substituted indole esters were produced using the Hemetsberger Knittel method, with yields ranging from 19%-35%. 3,4,5-Trimethoxybenzaldehyde provided the best yield of vinyl azide 67% prior to thermolysis. Ethyl azidoacetate was formed using water and acetone as co-solvents, produced ethyl azidoacetate in greater yield than previously reported by literature and previous group members. Knoevenagel process and xylene mediated thermolysis under nitrogenous conditions produced vinyl azide and indole ester, respectively. Reduction of the indole ester to an indole acid will allow for a variety of indole-2-carboxamides to be synthesized using BOP reagent, triethylamine and N,N-dimethylformamide. Trimethylated indole-2-carboxamide was able to produce a 51.2% cell vitality, or 49.8% cell death in MTT docking analysis using HELA cells. The combined methods of computational, synthetic, and cell death assay results provide promising insight into the relationship of CB1 receptors and cancer.

## 6. Experimental

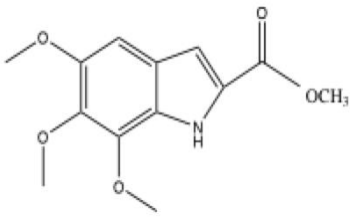



### 6.1 Ethyl Azidoacetate

A 100 mL, 3-neck round bottom flask (RBF) was fitted with a stir bar, condenser,Addition funnel, thermometer adapter and thermometer and the system was placed under Nitrogen. $NaN_3$ (1.30 g, 0.0200 mol, 2 eq) was added into the flask and rinsed with a small amount of water. Fifteen milliliters of water was then added with stirring to make a tan solution. Using a glass syringe, 1.1 mL of ethyl bromoacetate (0.010 mol, 1 eq) were syringed into the addition funnel. Using a graduated cylinder, 50.0 mL of acetone and 50.0 mL of $H_2O$ were added. The solution was allowed to stir overnight, producing a pale yellow liquid.

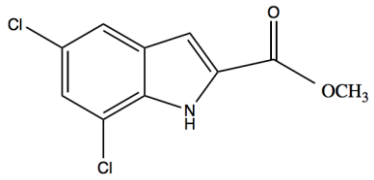
The solution was reduced through rotary evaporation. The resulting oil was treated with 20.0 mL of water and then extracted with ethyl acetate (3x15mL). The combined organic layers were washed with 25.0 mL of brine and dried with MgSO4. The organic solution was filtered and concentrated to yield 0.90 g of a yellow oil (70% yield).

Identification was obtained through proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy. $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.276 (t, 3H), 3.652 (s, 2H), 4.244 (q, 2H).



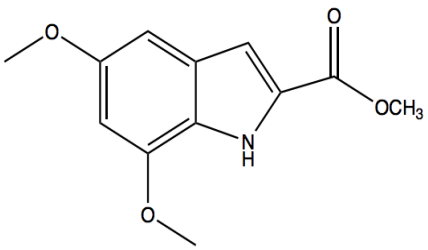
### *6.1.1 Methyl (Z)-2-azido-3-(3,4,5-trimethoxyphenyl) acrylate*

A 100 mL 2-neck RBF was fitted with a stir bar, addition funnel and an air inlet adapter, closing the system under N2 gas. A 50 mL 1-neck RBF was closed with a septum and placed under nitrogen using a nitrogen balloon. Using a glass syringe, 3.8 mL of dry MeOH were added to the 2-neck RBF. The 2-neck RBF was then placed into a dewer of dry ice and wet MeOH that was kept at -10 °C and 0.202 g of Na(s) (0.008 mol, 3.5 eq) was added piecewise while stirring to dissolve sodium in the MeOH. Into the 1-neck RBF, 0.33 g of 3,4,5- trimethoxybenzaldehyde (0.002 mol, 1 eq), 0.5 mL of ethyl azidoacetate (0.0062 mol, 3 eq) and 1.48 mL of dry MeOH were added. The benzaldehyde and ethyl azidoacetate solution was then swirled until all solid had dissolved, making a clear, yellow solution. The contents of the 1-neck RBF were then syringed into the addition funnel. This solution was added dropwise via addition funnel to the sodium/methanol mixture over a period of one hour. Once the entire contents of the addition funnel had been added to the 2-neck round bottom flask, the system was covered in foil and allowed to warm to room temperature and react overnight. A 250 mL beaker was filled to the 100 mL mark with crushed ice and saturated NH4Cl. The reaction solution was then poured into the saturated NH4Cl and ice mixture and allowed to stir for ~1.5 hr at which point a precipitate formed. The dark yellow precipitate was collected through vacuum filtration and 0.28 g (48.2% yield) was produced. Identification of precipitate as desired product was indicated positively through $^{1}$H-NMR spectroscopy. $^{1}$H-NMR (400 MHz, CDCl3): δ 3.067 (s, 3H), 3.081 (s, 6H), 3.181 (s, 3H), 5.998 (s, 1H), 6.575 (s, 2H).



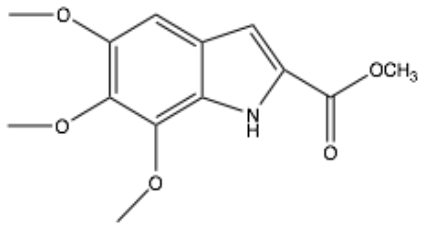

### *6.1.2 Methyl(Z)-2-azido-3-(3,5-dichloro) acrylate*

A 100 mL 2-neck RBF was fitted with a stir bar, addition funnel and an air inlet adapter, closing the system under N2 gas. A 50 mL 1-neck RBF was closed with a septum and placed under nitrogen using a nitrogen balloon. Using a glass syringe, 48.0 mL of dry MeOH were added to the 2-neck RBF. The 2-neck RBF was then placed into a dewer of dry ice and wet MeOH that was kept at -10 °C and 2.14 g of Na(s) (0.093 mol) was added piecewise while stirring to dissolve sodium in the MeOH. Into the 1-neck RBF, 4.91 g of 3,5-dichlorobenzaldehyde (0.002 mol, 1 eq), 7.5 mL of ethyl azidoacetate (0.062 mol, 3 eq) and 16.0 mL of dry MeOH were added. The benzaldehyde and ethyl azidoacetate mixture was then swirled until all solid had dissolved, making a clear, yellow solution. The contents of the 1-neck RBF were then syringed nto the addition funnel. This solution was added dropwise via addition funnel to the sodium/methanol mixture over a period of one hour. Once the entire contents of the addition funnel had been added to the 2-neck round bottom flask, the system was covered in foil and allowed to warm to room temperature and react overnight. A 250 mL beaker was filled to the 100 mL mark with crushed ice and saturated NH4Cl. The reaction solution was then poured into the saturated NH4Cl and ice mixture and allowed to stir for ~1.5 hr at which point a precipitate formed. The tan precipitate was collected through vacuum filtration and 0.90 g (9.4% yield) was produced. Identification of precipitate as desired product was indicated positively through $^{1}$H-NMR spectroscopy. $^{1}$H-NMR (400 MHz, CDCl3): δ 3.067 (s, 3H), 3.081 (s, 6H), 3.181 (s, 3H), 5.998 (s, 1H), 7.172 (s, 2H).



### *6.1.3 methyl (z)-2-azido-3-(3,5-dimethoxyphenyl)acrylate*

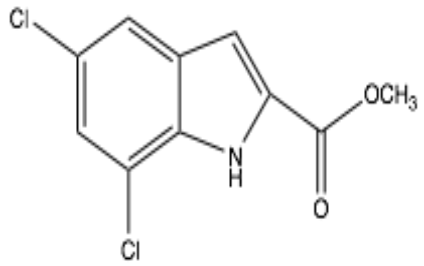
A 100 mL 2-neck RBF was fitted with a stir bar, addition funnel and an air inlet adapter, closing the system under N2 gas. A 50 mL 1-neck RBF was closed with a septum and placed under nitrogen using a nitrogen balloon. Using a glass syringe, 48.0 mL of dry MeOH were added to the 2-neck RBF. The 2-neck RBF was then placed into a dewer of dry ice and wet MeOH that was kept at -10 °C and 2.14 g of Na(s) (0.093 mol) was added piecewise while stirring to dissolve sodium in the MeOH. Into the 1-neck RBF, 04.91 g of 3,5- dichlorobenzaldehyde (0.002 mol, 1 eq),7.5 mL of ethyl azidoacetate (0.062 mol, 3 eq) and 16.0 mL of dry MeOH were added. The benzaldehyde and ethyl azidoacetate solutions was then swirled until all solid had dissolved, making a clear, yellow solution. The contents of the 1 neck RBF were then syringed into the addition funnel. This solution was added drop wise via addition funnel to the sodium/methanol mixture over a period of one hour. Once the entire contents of the addition funnel had been added to the 2-neck round bottom flask, the system was covered in foil and allowed to warm to room temperature and react overnight. A 250 mL beaker was filled to the 100 mL mark with crushed ice and saturated NH4Cl. The reaction solution was then poured into the saturated NH4Cl and ice mixture and allowed to stir for ~1.5 hr at which point a precipitate formed. The tan precipitate was collected through vacuum filtration and 0.90 g (9.4% yield) was produced. Identification of precipitate as desired product was indicated positively through $^1$H-NMR spectroscopy. $^1$H-NMR (400 MHz, CDCl3): δ 3.067 (s, 3H), 3.081 (s, 6H), 3.181 (s, 3H), 5.998 (s, 1H), 7.231 (s, 2




### *6.1.4 5,6,7-trimethoxy-1H-indole-2- carboxylate*

A 500 mL 3-neck RBF was fitted with a stir bar, air inlet adapter, condenser, thermometer adapter, thermometer and addition funnel and the system closed and placed under N2 (g). A 100 mL 1-neck RBF was closed using a septum and placed under N2(g) through use of a nitrogen balloon. To the 3-neck RBF 106.0 mL of dry xylenes was added and brought to reflux. An additional 40.0 mL of dry xylenes was added to 2.20 g of vinyl azide in the 1-neck RBF while swirling. Heat was applied to the 1-neck flask to help dissolve any remaining solid. Once the xylenes in the 3-neck RBF started refluxing, the vinyl azide solution was transferred to the addition funnel and then added to the 3-neck flask drop wise. The mixture was allowed to reflux overnight. TLC was performed using 80/20 Hex/EA to monitor the reaction.

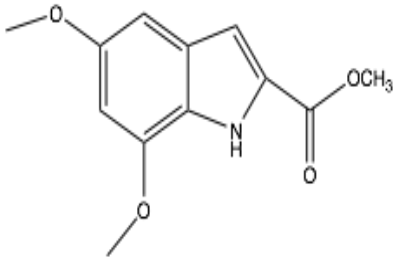
The solution was cooled to 80 °C and then transferred to a 500 mL 1-neck RBF. Through rotary evaporation, the solution was concentrated and then 50.0 mL MeOH was heated and then added to the concentrated solution. A crystalline solid formed and then the flask was placed in the freezer overnight for further recrystallization. The resulting light yellow crystals, 0.24 g (42.3 % yield), were collected via vacuum filtration. High vacuum was attached to the flask of crystals to remove any remaining solvent and the desired product was confirmed through $^1$H-NMR spectroscopy.$^1$H-NMR (400 MHz, CDCl3): δ 3.910 (s, 3H), 3.937 (s, 3H), 3.987 (s, 3H), 4.082 (s, 3H), 6.829 (s, 1H), 7.112 (s, 1H), 8.895 (s, 1H).



### *6.1.5 5,7-dichloro-1H-indole-2-carboxylate*

A 500 mL 3-neck RBF was fitted with a stir bar, air inlet adapter, condenser, thermometer adapter, thermometer and addition funnel and the system closed and placed under N2 (g). A 100 mL 1-neck RBF was closed using a septum and placed under N2(g) through use of a nitrogen balloon. To the 3-neck RBF 9.68 mL of dry xylenes was added and brought to reflux. An additional 3.42 mL of dry xylenes was added to 0.20 g of vinyl azide in the 1-neck RBF while swirling. Heat was applied to the 1-neck flask to help dissolve any remaining solid. Once the xylenes in the 3-neck RBF started refluxing, the vinyl azide solution was transferred to the addition funnel and then added to the 3-neck flask drop wise. The mixture was allowed to reflux overnight. TLC was performed using 80/20 Hex/EA to monitor the reaction.

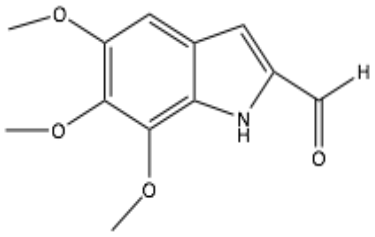
The solution was cooled to 80 °C and then transferred to a 500 mL 1-neck RBF. Through rotary evaporation, the solution was concentrated and then 50.0 mL MeOH was heated and then added to the concentrated solution. A crystalline solid formed and then the flask was placed in the freezer overnight for further recrystallization. The resulting yellow crystals, 0.32 g (35 % yield), were collected via vacuum filtration. High vacuum was used to remove any remaining solvent and the desired product was confirmed through $^{1}$H-NMR spectroscopy.




### *6.1.6 Methyl 5,7-dimethoxy-1H-indole-2-carboxylate*

A 500 mL 3-neck RBF was fitted with a stir bar, air inlet adapter, condenser, thermometer adapter, thermometer and addition funnel and the system closed and placed under N2 (g). A 100 mL 1-neck RBF was closed using a septum and placed under N2(g) through use of a nitrogen balloon. To the 3-neck RBF 5.00 mL of dry xylenes was added and brought to reflux. An additional 5.2 mL of dry xylenes was added to 0.10 g of vinyl azide in the 1-neck RBF while swirling. Heat was applied to the 1-neck flask to help dissolve any remaining solid. Once the xylenes in the 3-neck RBF started refluxing, the vinyl azide solution was transferred to the addition funnel and then added to the 3-neck flask drop wise. The mixture was allowed to reflux overnight. TLC was performed using 80/20 Hex/EA to monitor the reaction.

The solution was cooled to 80 °C and then transferred to a 500 mL 1-neck RBF. Through rotary evaporation, the solution was concentrated and then 50.0 mL MeOH was heated and then added to the concentrated solution. A crystalline solid formed and then the flask was placed in the freezer overnight for further recrystallization. The resulting brown/yellow crystals, 0.05 g (5.03 % yield), were collected via vacuum filtration. High vacuum was used to remove any remaining solvent and the desired product was confirmed through $^{1}$H-NMR spectroscopy.

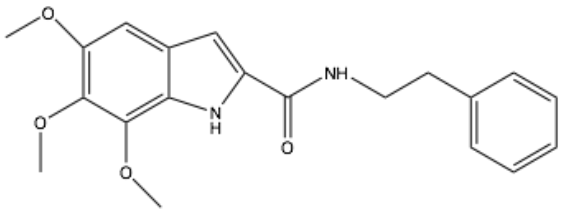



## 6.2 Indole Acid

A 2-neck, 50 mL RBF was fitted with a condenser, stir bar, thermometer adapter and thermometer and was placed

under $N_2$(g). To the 2-neck RBF 13.0 mL MeOH and 0.500g (1.88mmol, 1 eq) of indole ester were added, stirring to form a yellow solution. Five milliliters of 3M KOH was added to the flask. Upon addition of the KOH condensation formed on the sides of the condenser indicating an exothermic reaction. Heat was applied and the solution refluxed 1 hour.

The solution was concentrated through rotary evaporation and 1M HCl was added drop wise until the pH reached 2, forming a precipitate. The precipitate was then collected through vacuum filtration yielding 0.201g (45% yield) of product. $^1$H-NMR (400 MHz, CDCl3): δ 2.693 (s, 3H), 3.421 (s, 3H), 3.784 (s, 3H), 3.801 (s, 3H), 3.896 (s, 3H), 6.928 (s, 1H), 7.019 (s, 1H), 11.622 (s, 1H).




### *6.2.1 indole carboxamide*

Indole acid (0.89 g), BOP reagent (0.43 g), 2-phenylalanine (0.20 ml), triethylamine (0.60 ml), and DMF (5.0 ml) were added to a 250 RBF, while stirring. Solution was stirred at room temperature for 16 hours and diluted with water. Liquid extraction was performed with ethyl acetate, washing the organic layers with brine. The organic layers were dried in a desiccator overnight. The solution was concentrated through rotary evaporation, producing yellow oil.

## 7. Endnotes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