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# Synthetic Strategies Towards Indole Analogues of a Tamoxifen® Metabolite

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## Abstract

The target indole analogue of 4-hydroxy Tamoxifen® is of interest due to its theoretically increased because it has been hypothesized to have binding affinity for the estrogen receptor relative to the popular breast cancer drug, Tamoxifen®. The Hemetsberger-Knittel indole synthesis methodology has proven to be an effective and efficient procedure to produce substituted indoles from vinyl azides. The use of substituted benzophenones in producing vinyl azides would provide the most efficient route to aryl substituted indoles and the series of analogs of 4-hydroxy Tamoxifen® desired in this project. Unfortunately, the low reactivity and greater steric hindrance of benzophenone compared to benzaldehydes hindered this process. The Hemetsberger-Knittel indole methodology was then employed to generate the desired methoxyindole core followed by bromination at the 3-position of the indole. This would allow for the addition of the aryl group via a Kumada coupling. The alternative but efficient route will be discussed along with the progress toward an indole analogue of Tamoxifen®.

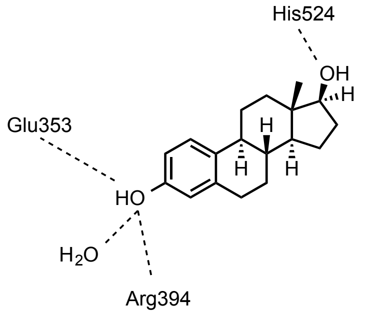
## 1. Introduction

### 1.1. Breast Cancer and Drug Discovery

Scientific American stated in 1912, "the beginning of the end of the cancer problem is in sight." The editorial was referring to Professor Paul Ehrilch's work on Salvarsan, the first chemical therapy, found to treat patients with Syphilis. Ehrlich was also the first to devise the idea that synthesized chemicals could be used to fight disease-causing bacteria, and develop the standard protocol for pharmaceutical drug discovery.[1]

In the United States breast cancer ranks second most prevalent cancer behind lung cancer. It was estimated by Howlander et al. in 2012 that 39,510 of 275,370 American women that will die from cancer will die from breast cancer.[2] There is a definite need for synthetic medicinal chemistry research to develop a more efficient preventative breast cancer drug for high risk patients, and patients who have undergone mastectomy or lumpectomy.

Breast cancer has been found to have both genetic and environmental causes. Women with high risk of breast cancer can be identified by the over-expression of the oncogene HER2, which plays an important role in the development and proliferation of breast cancer. Oncogenes are mutated genes that become permanently active, causing the cell to proliferate.[3] Besides genetic factors, an environment rich with endocrine disruptors and estrogen mimicking molecules increases the risk of estrogen receptor positive breast cancer.[4] These breast cancer cells proliferate from estrogen.



**1**

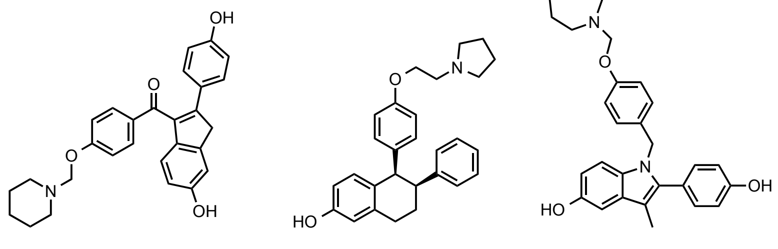
Figure 1. Simplified version of 17β-estradiol (**1**) binding to both human estrogen receptor α (ERα) . The lines depict the intermolecular interactions occurring between three amino acid residues and a water molecule, locking in the natural substrate.[5]

The Women's Health Initiative (WHI) was established by the U.S. National Institutes of Health in 1991. This initiative consisted of two clinical trials and an observational study consisting of 161,808 'healthy' women. The focus was to test the effects of postmenopausal hormone therapy, diet modification, calcium and vitamin D supplements, all environmental factors, on heart disease, fractures, and breast and colorectal cancer.[6] In 2002, the results were published for the WHI trials and it was found that 26% of the subjects who received hormone therapy increased the incidence of developing breast cancer. After a follow up study, the Million Women Study, it was concluded that post-menopausal women who participated in hormone replacement therapy were more likely to develop breast cancer than women who had never participated.[7]

## 1.2. Selective Estrogen Receptor Modulators (SERMs)

Throughout decades of research, clinical trials and patient results, selective estrogen receptor modulators (SERMs) have been found to decrease the proliferation of breast cancer cells by targeting the estrogen receptor. A synthetic drug competes with the natural substrate 17β-estradiol (**1**) to inhibit the production of estrogen, by binding to the human estrogen receptor. These molecules have been used and studied as short term (approximately 5 years) preventative treatment for women with high risk of developing breast cancer and for women who have already completed a single, or double, breast mastectomy to ensure that the cancer does not return.

SERMs can bind to the estrogen receptors and confer either estrogen-agonistic (estrogen-like) or estrogen-antagonistic (anti-estrogen-like) actions in various estrogen target tissues and cells.[8] The most desired SERMs are those that act agonistically in the central nervous system and bone, while acting antagonistically in breast and uterine tissue, but unfortunately this ideal SERM has yet to be developed.[9] This ideal SERM's antagonist activity would starve breast and uterine tissue of estrogen, decreasing potential carcinogenic masses. The estrogen agonist activity would decrease the risk of osteoporosis in the bone matrix and provide neuroprotection to the central nervous system. SERMs of interest to this research include Tamoxifen®, Raloxifene®, Bazedoxifene, and Lasofoxifene (Figure 2). These SERMs are all currently on the market in the US. All of these current SERMs, except Tamoxifen®, are majorly prescribed for the treatment of osteoporosis and prescribed off label for hormone dependent disorders.



**2** **3** **4**

Figure 2. Examples of selective estrogen receptor modulators. Raloxifene® (**2**), Lasofoxifene (**3**) and Bazedoxifene (**4**).

To understand the agonist mechanism of 4-hydroxy Tamoxifen on the estrogen receptor, one must analyze the binding studies done by Shiau et al. Their results described the significant event of agonist binding as the repositioning of helix 12 (magenta) to seal diethylstilbestrol, the natural substrate, in the hydrophobic pocket of ERα (Figure 3). This event allows the ERα ligand binding domain to signal coactivators to initiate transcription. The ribbon diagram in Figure 3 depicts the different regional conformations helix 12 has for agonist and antagonist binding. The nitrogen containing side chain inhibits the agonist conformation of helix 12. When diethylstilbestrol is present in ERα, hydrogen bonding occurs between His 524, Glu 353, Arg 394, and a water molecule. During the binding of 4-OHT the unsubstituted benzene ring inhibits the hydrogen bonding of His 524 and ligand binding domain adopts the antagonist conformation, leading to a lack of estrogen production.[10]

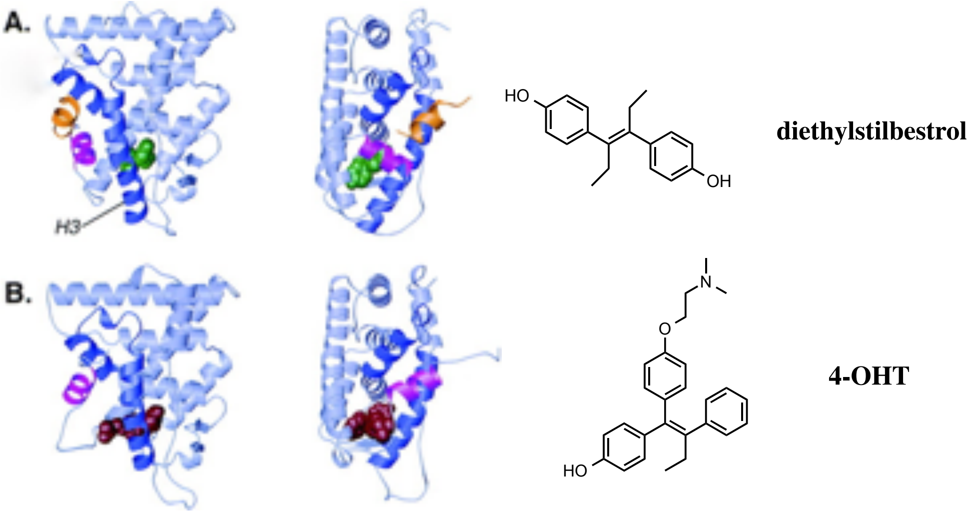
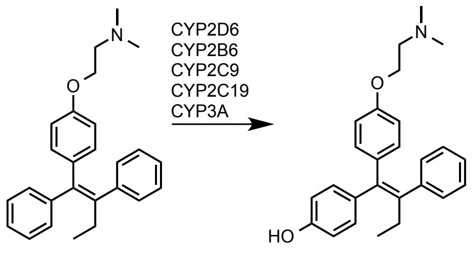


Figure 3. Ribbon diagram of diethylstilbestrol (**A**), an agonist, and 4-hydroxy Tamoxifen® (**B**), an antagonist, binding to the ligand binding domain of ERα in two orthogonal views. Helix 12, found in both ERβ and ERα, is shown in magenta.[10]

Osteoporosis, coronary heart disease, strokes, and endometrial cancer are other health concerns related to the estrogen receptor and have been shown to be affected by SERMs. If continued to be developed and explored, this multifunctional drug family will benefit a variety of the population with estrogen receptor related illnesses and diseases.

## 1.3 Tamoxifen®

Tamoxifen® (**TAM**) (Figure 4) was the first compound identified as SERM and has been used for over two decades in the treatment of hormone-dependent breast cancer. TAM is a prodrug, meaning that the drug once taken is converted to the active metabolite 4-hydroxy Tamoxifen® (**4-OHT**), and other inactive metabolites throughout metabolism. In 1996, Klinge et al. studied TAM and its metabolite, 4-OHT. It was found that 4-OHT binds to the estrogen receptor with an affinity 3-fold higher than estradiol.[11] In 1978 the multifunctional use of TAM was found by Jordan et al. at Northwestern University reporting the compounds ability as a treatment for post-menopausal osteoporosis and breast cancer.[12] More recently, in 1996 Saarto et al. observed TAM to decrease circulating LDL-cholesterol by increasing the LDL receptors in the liver. This effect on LDL-cholesterol is another advantage for older patients with cardiovascular concerns.[13] TAM also displays estrogen-agonist activity in the uterus, which can lead to cancer of the uterus if used over a long period of time. Long periods of treatment with TAM has also been found to cause SERM resistant breast cancer.[7]



**TAM** **4-OHT**

Figure 4. The structure of Tamoxifen® and it's active metabolite via multiple liver enzymes.[14]

## 1.4 Indole Analogue of Tamoxifen®

Indoles (see Figure 5) are important biologically as they are stable to metabolic enzymes and they reduce the electron density by adding heteroatoms to the ring.[15] Indoles have also been known to increase water solubility, which can be seen in the hydrophilic activity of the amino acid tryptophan. Bazedoxifene (Figure 2), one of the SERMs of interest, has an indole core and is administered to patients orally as a pill. Orally-administered drugs are ideal for patients. It is clear that indoles play a significant role in pharmaceuticals, by utilizing an indole as the core of some well-known drugs, such as Tamoxifen biological activity could be enhanced.

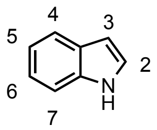
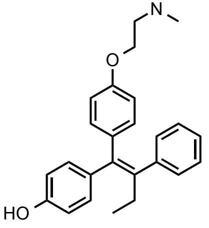
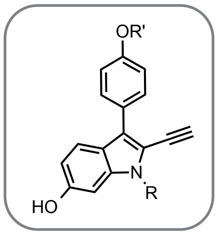


Figure 5. Indole molecular structure with numbered carbons.

The goal of this research project was to synthesize a an indole analogue of 4-OHT (see Figure 6) with the Hemetsberger-Knittel method to synthesize the indole core of this indole analogue. Dr. Holt's research group investigates heterocyclic organic synthesis of biologically active molecules, such as Combretastatin A-4, phenstatins, and chalcones. This project implements research of previous students' synthesis techniques and routes of other heterocyclic indole-core structures to create a more effective derivative of the current preventative treatment for breast cancer. The target molecule was chosen through a structure-based approach. The target has structural similarities to **4-OH TAM**, such as the aromatic backbone, alkene and para-substituted oxygens, but incorporates the indole core to improve physical properties.

The characteristics that make this molecule different, and which might improve SERM activity is the indole core. The presence of the alkyne group on the indole is to increase potency due to possible hydrophobic interactions and to decrease metabolism. The alkyne functionality can be used later for coupling reactions. The first target of this project was to synthesize the 4-OHT analogue target molecule (**5**). Derivatives of this molecule could then be synthesized by utilizing the alkyne moiety for coupling reactions. These derivatives could then be analyzed for estrogen activity.





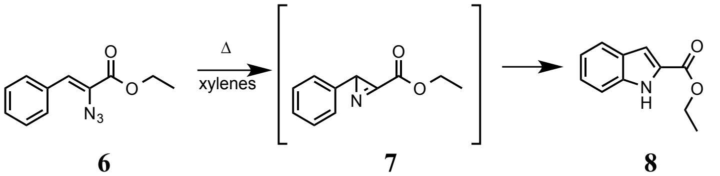
**4-OH TAM** 4-OHT indole analogue target (**5**)

Figure 6. The metabolized version of **TAM** and this research project's 4-OHT indole analogue target molecule (**5**).

## 2. Experimental Design of an Indole Analogue of Tamoxifen®

### 2.1. Hemetsberger-Knittel Indole Synthesis

The Hemetsberger-Knittel indole synthesis method involves transforming a vinyl azide into an indole ring (Scheme 1). Sodium azide and ethyl bromoacetate were used to create ethyl azidoacetate, which was then reacted with a variety of aromatic ketones and benzaldehydes to create the vinyl azide (**6**) to then be converted into the an indole ring via thermolysis.

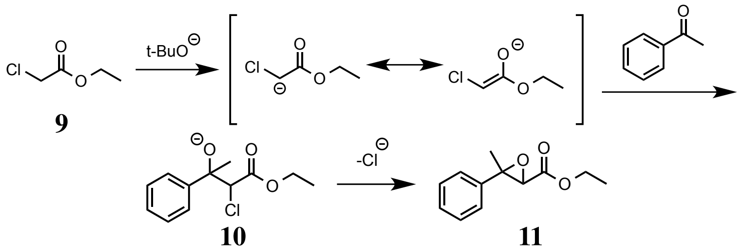


**6** **7** **8**

Scheme 1. Hemetsberger-Knittel Indole Synthesis: The azirine intermediate (**7**) is created from the vinyl azide (**6**) before going to the indole ester (**8**).

#### *2.1.1. the darzen reaction*

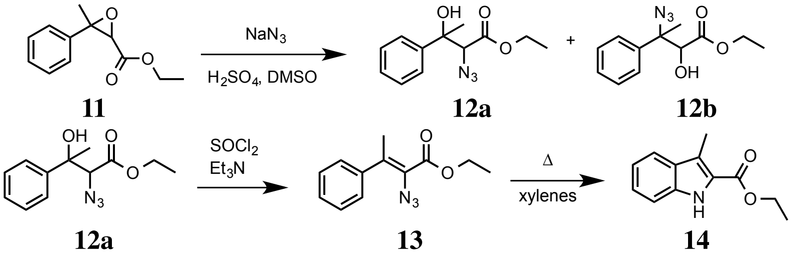
In 1953, a new method to produce the vinyl azide of the Hemetsberger-Knittel was found to be very efficient. Acetophenone was treated with ethylchloroacetate and potassium *tert*-butoxide to create a racemic epoxide, this is described as the Darzen reaction.[16]



**9** **10** **11**

Scheme 3. Darzen's Glycidic Ester (**11**) Condensation Reaction[17]

Murakami et al. then proceeded to open the epoxide with sodium azide and sulfuric acid in dimethyl sulfoxide to yield a 2:1 ratio of azidoalcohol isomers (**12a** and **12b**). By treating the isomers with thionyl chloride and triethylamine in dichloromethane the E and Z isomers were produced and ready to be separated using column chromatography. Due to the SN2 nature of this reaction, the E isomer is the ideal isomer for the indole ring formation, although Z isomer has been found to create the indole as well. The indole ring can then be created under reflux of xylenes for approximately two hours.[18]



**11** **12a** **12b**

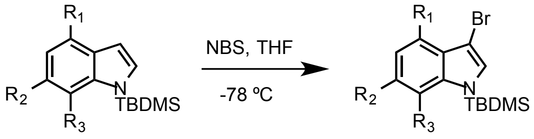
**12a** **13** **14**

Scheme 4. Indole ring formation using method by Murakami et al.[18]

The nitrogen of the indole will need to be protected for future reactions requiring basic conditions. Previous group members have seen positive results when protecting the indole with a methyl or *tert*-butyloxycarbonyl (Boc) protecting group, although the methyl group is difficult to remove if desired, and the Boc group has been removed during column chromatography. Trial reactions could be run to protect the indole with either a benzyl group, formyl, or allyl group. These protecting groups might affect binding to the ER.

## 2.2. Indole Bromination

Utilizing the Hemetsberger-Knittel method, a 3-position brominated indole is the next necessary step to allow for addition of the second aryl group in the target analogue. Yang et al. provided the experimental procedures of brominating a tert-butyldimethylsilyl ether (TBDMS) protected indole with three different aromatic substitution patterns.[19] N-bromosuccinimide (NBS), in comparison to other brominating agents is safe and easy to use. The results provided by Yang support future work with protected indole species. Once the 3-position brominated indole is isolated, a Kumada coupling reaction will result in the diphenyl indole of interest.



**15a-c** **16a-c**

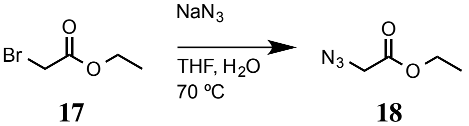
Scheme 5. Bromination of a protected indole (**15a-c**) via NBS at -78 ºC to create the brominated species (**16a-c**); **a**: R1=R2=R3=H; **b**: R1=R3=H, R2=Br; **c**: R1=Br, R2=H, R3=OMe.

## 3. Results and Discussion

All syntheses were completed under inert atmosphere. Throughout each step of the synthesis process $^1$H-NMR spectroscopy, IR, and thin layer chromatography were used to identify products and assess progress. Flash chromatography was carried out on silica gel (0.040-0.063 mm particle size).

## 3.1. Ethyl Azidoacetate

Commercially purchased ethyl bromoacetate (**17**) and sodium azide were combined in a 1:1.1 molar ratio with tetrahydrofuran (THF) and water with a fitted heating mantle to 70 ºC to yield ethyl azidoacetate (**18**). In earlier reactions and by past group members, this synthesis was run with methanol instead of THF as the solvent, the yields were low (33-46%). After changing the solvent to THF, yields increased to 92% an average. The other advantage to this method is the conserved ethyl group of the ester. When this reaction is run in methanol with methoxide, the ethyl is exchanged for a methyl, which can convolute NMR spectra data later in the synthesis when using methoxy substituted benzenes.



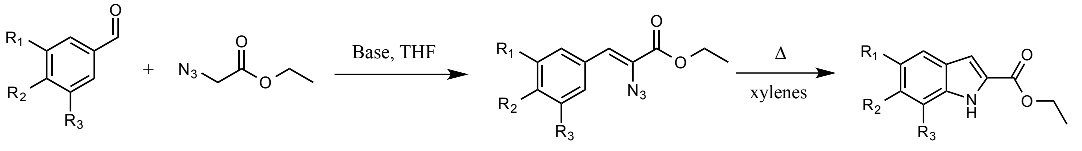
**17** **18**

Scheme 6. Synthesizing ethyl azidoacetate (**18**) from ethyl bromoacetate (**17**) and $NaN_3$ in THF.

## 3.2. Indole

### *3.2.1. aldol condensation and thermolysis*

The ethyl azidoacetate was then used for an aldol condensation with a carbonyl. Ideally, the starting material for this project would be benzophenone, yet benzophenones have low reactivity under the experimental conditions compared to benzaldehydes. After many failed reactions with benzophenone, benzaldehydes (**19**) were used to optimize Aldol Condensations reaction conditions. Due to the electron donating groups by resonance nature of the methoxy groups, making the carbonyl a better electrophile, the condensation was able to form the vinyl azide (**20**). Thermolysis of the vinyl azide in xylenes yielded the desired indole ring system (**21a-c**).



**19** **18** **20** **21a-c**

Scheme 6. Aldol condensation of benzaldehyde and ethyl azidoacetate (**18**) to create the desired vinyl azide (**20**) which was refluxed with xylenes to produce the indole ester (**21a-c**). Reaction conditions found in Table 1.

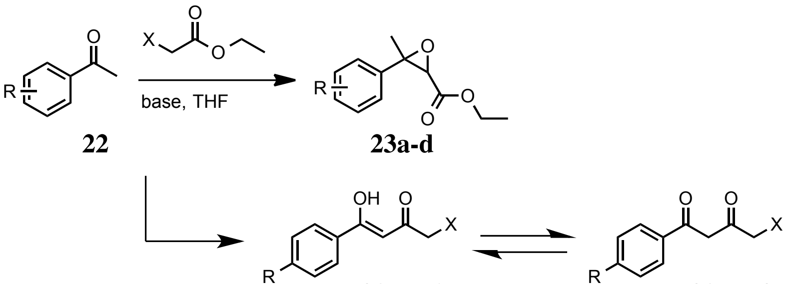
Table 1. Aldol Condensations Conditions

| Reaction | $R_1$ | $R_2$ | $R_3$ | Base | Temperature |
|---|---|---|---|---|---|
| **21a** | **$OCH_3$** | **$OCH_3$** | **$OCH_3$** | **t-BuOK** | **-78 ºC** |
| **21b** | **H** | **$OCH_3$** | **H** | **t-BuOK** | **-78 ºC** |
| **21c** | **H** | **$OCH_3$** | **H** | **NaH** | **0 ºC** |

Table 1. Aldol condensation reaction attempted with various benzaldehydes (**19**) and bases to determine ideal conditions of creating the vinyl azide (**20**). All reactions were completed in dry THF and proceeded to thermolysis to create the indole ester (**21a-c**).

## 3.3. The Darzen Reaction

Commercially purchased ethyl chloroacetate and acetophenone were reacted with potassium *tert*-butoxide and THF under cold conditions to produce the epoxide. Ethyl bromoacetate was used initially instead of ethyl chloroacetate due its ready availability. Although, both halo-esters were found to produce the desired vinyl azide, ethyl chloroacetate was used by Murakami et al. to produce higher yields at a lower cost. Ethyl chloroacetate was later acquired for further reactions. Four main reactions were completed to optimize reaction conditions (Table 1). Thin layer chromatography showed that at least four products were being produced. Even after flash chromatography, the separations were not pure based on $^1$H-NMR spectroscopy. It was found that increasing the molar ratio to 1:2 and adding a methoxy group to the ketone to assisted in cleaning up the reaction (**23d**) based on H$^1$-NMR. According to the spectral data, side products theorized to be forming are species (**23a-d 1-2**) shown below existing in the keto-enol tautomerism equilibrium.



**22** **23a-d** **23a-d 1** **23a-d 2**

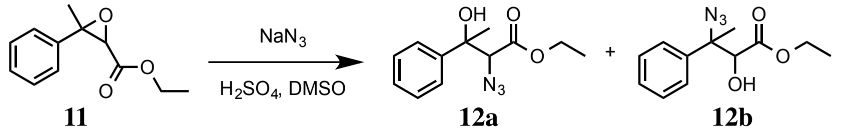
Reaction 4. Darzens reaction of a halogen containing ester and ketone to produce the glycidic ester product (**23a-d**). Reaction conditions found in Table 2.

Table 2. Darzen Reactions Conditions

| Reaction | Ratio | R | X | Base | Temperature |
|---|---|---|---|---|---|
| **23a** | **1:1** | **H** | **Br** | **t-BuOK** | **10-15 ºC** |
| **23b** | **1:1** | **H** | **Cl** | **t-BuOK** | **10-15 ºC** |
| **23c** | **2:1.5** | **H** | **Cl** | **NaH** | **0 ºC** |
| **23d** | **1:2** | **$OCH_3$** | **Cl** | **NaH** | **0 ºC** |

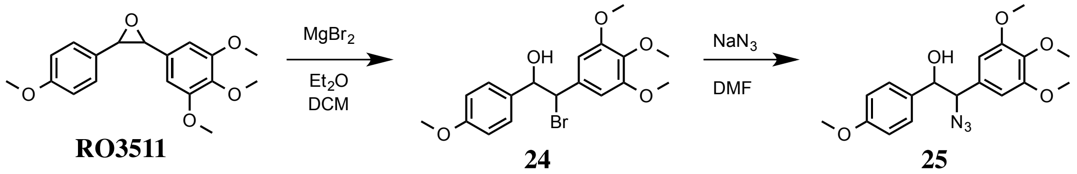
Table 2. Molar ratio of ketone (**22**) to halogen containing ester (like **15**) with appropriate R and X groups. All reactions were completed in THF.

As outlined in Murakami et al., the epoxide was opened up using sodium azide, sulfuric acid and DMSO. This reaction yielded two regioisomers, (**12a** and **12b**). The desired isomer was the one in which the azide group is alpha to the carbonyl (**12a**), which is the only theoretically 10% of the product.



Reaction 5. Opening of epoxide (**11**) in acidic conditions to create the azido-alcohol regioisomers (**12a** and **12b**) based on Murakami et al. experimental methods.

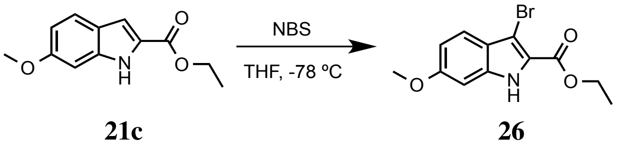
To ensure the desired isomer was obtained, an alternative method of opening the epoxide was explored. The epoxide product (**RO3511**) was obtained from a past lab member to explore the reaction conditions of an alternative method to produce the vinyl azide. When the epoxide moiety is symmetrical, the more electrophilic carbon will be the site of attack for the nucleophile. The methoxy groups are electron donating by resonance, making the carbon alpha to the trimethoxyphenyl ring be slightly more electrophilic than the beta carbon. As outlined in Oliver's procedure $MgBr_2$ was dissolved in diethyl ether.$^{20}$ This solution was then combined with the epoxide dissolved in DCM. The bromo-hydrin was not analyzed via spectroscopy due to the one-pot nature of the procedure. Once the organic solvent was removed using rotary evaporation, the azide was added to the flask and allowed to stir overnight in DMF, creating the azido alcohol. These results proved that in the case of the tentative starting material, 4,4'-dimethoxybenzophenone, the strong nucleophile $MgBr_2$ will attack the least substituted carbon of the epoxide.



Reaction 6. Nucleophilic opening of **RO3511** epoxide using the strong nucleophile, $MgBr_2$ to create the bromo-hydrin (**24**) and the azido alcohol (**25**).

## 3.4. Bromination of Indole

Allylic substitution with NBS will brominate at the 3-position of the indole, allowing for the addition of the second aryl group via a Kumada coupling reaction. This reaction must be run in a -78 ºC dry-ice acetone bath because of NBS's exothermic reactive properties.$^{19}$ Upon subjecting the methoxyindole ester (**21c**) to NBS bromination conditions, the dibrominated product (**27**) formed. This was evident due to the presence of only two doublet aromatic signals in the $H^1$-NMR spectrum. It is speculated that the indole that formed is brominated at the 3-position and at the 7-position of the indole. Future directions for this reaction include changing the molar ratios of NBS to indole, exploring different protection groups to change the resonance of the indole core, and changing the 2-position group to examine its role on carbon 3.



Reaction 7. Substitution reaction of methoxyindole ester (**21c**) with NBS to create the brominated indole (**26**).

## 4. Conclusion

The goal of this research was to create an initial indole analogue of Tamoxifen®. While this goal was not achieved, organic synthesis methodology of the Hemetsberger-Knittel and Darzen reaction on benzaldehydes, acetophenones and benzophenones was refined. Future work to achieve the target Tamoxifen® indole analogue and subsequent analogues should utilize the Hemetsberger-Knittel indole strategy with subsequent Kumada like coupling reactions. These analogues could then be evaluated for their potential as an antagonist in breast and uterine tissue, and an agonist in bone and central nervous system.

## 5. Supplemental Information

### 5.1. Instrumentation

NMR Spectra were obtained with $CDCl_3$ or deuterated DMSO as the solvents on a Varian Unity INOVA with an Oxford Instruments 400 MHz superconducting magnet at University of North Carolina Asheville and a Varian INOVA with an Oxford Instruments 500 MHz superconducting magnet at Furman University. A Thermo-Fischer Scientific Nicolet (Madison, WI, USA) iS10 FT-IR spectrometer, equipped with a germanium crystal for attenuated total reflectance was employed for infrared measurements. Spectra treatment and data manipulation were carried out on Omnic (Thermo Nicolet Corp., Madison, WI, USA) software. Note that all NMR and IR data was catalogued numerically and lab notebook correlated (ie. the spectra for all product of reaction on page 55 of the notebook may be found under MFL55).

### 5.2. Materials

All reagents were purchased from commercial sources and used as received. Prior to use in water sensitive reactions, solvents (methanol, DMF, DCM, DMSO, THF, xylenes) were dried over activated 3Å molecular sieves and for 24 hours before use.

### 5.3. Experimental Methods

### *5.3.1. ethyl azidoacetate (**18**)*

A flame-dried 2-neck 250 mL RBF cooled under nitrogen was charged with approximately 10 mL of anhydrous THF and a magnetic stir bar, heating mantle, heating adapter, stir plate, and condenser. Under an inert atmosphere of $N_2$, $NaN_3$ (5.10 g, 78.4 mmol) was added to a separate flask with 5 mL of $H_2O$ to create a slurry. Ethyl bromoacetate (7.50 mL, 67.6 mmol) was then added via syringe to the RBF with THF, and then the slurry was added, adding enough $H_2O$ to add all the slurry to the flask. The reaction was heated to 70 ºC and left to react overnight. The reaction was monitored by color change, an amber oil was observed on the bottom of a clear solution. Upon completion the reaction mixture was diluted with approximately 60 mL of $H_2O$ and transferred into a 250 mL separatory funnel and the aqueous layer was then extracted with 50 mL of diethyl ether three times. The combined organic layers were then dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to yield the product ethyl azidoacetate (7.95g, 91%) as an amber oil. $^1H$ NMR (500MHz, $CDCl_3$): δ 4.25 (q, 2H), 3.86 (s, 2H), 1.3 (t, 3H).

### *5.3.2. ethyl (Z)-2-azido-3-(3,4,5-trimethoxyphenyl)acrylate (**20a**)*

A flame-dried 2-neck 25 mL RBF cooled under nitrogen was charged with 4 mL of THF dried over 3Å molecular sieves. Under an inert atmosphere 3,4,5-trimethoxybenzaldehyde (257 mg, 1.31 mmol) and ethyl azidoacetate (500 mg, 3.87 mmol) were added to the flask via syringe. Due to the violent nature of this reaction, a bath of dry ice and acetone kept the reaction around -78 ºC. *t*-BuOK (1.26g, 11.2 mmol) was added to a separate 25 mL erlenmeyer flask with 10 mL of anhydrous THF. This base solution (5 mL) was added in drop wise over an hour at -78 ºC. The reaction was allowed to slowly reach room temperature and continued to stir overnight. Progress of reaction was monitored by TLC (80/20 hexanes(hex)/ethylacetate(EA)). Upon completion, the solution was then poured over ice and the yellow precipitated was collected via filtration. Saturated aqueous NaCl was then added to the filtrate in a separatory funnel was then extracted with 50 mL of diethyl ether three times. The combined organic layers were then dried with $K_2CO_3$ and gravity filtered. The filtrate was then condensed via rotary evaporation to yield the vinyl azide as a solid. This reaction was not quantified for yields. $^1H$ NMR (500MHz, $CDCl_3$): δ 7.92 (s, 1H), 6.91 (s, 2H), 4.37 (q, 2H), 3.90 (s, 6H), 3.89 (s, 3H), 1.40 (t, 3H).

### *5.3.3. ethyl 5,6,7-trimethoxy-1H-indole-2-carboxylate (**21a**)*

A flame-dried 100 mL 2-neck RBF cooled under nitrogen, charged with 15 mL of xylenes, was fitted with a condenser, inlet air adapter and stir bar for thermolysis. A thermometer and adapter were attached to the side arm of the RBF to monitor the temperature. The reaction was brought up to approximately 140 ºC and then ethyl 2-azido-3-(3,4,5-trimethoxyphenyl) acrylate (**21**) was dissolved in 10 mL of xylenes and extracted via 20 mL syringe and then added to the flask drop-wise with a 20 mL syringe over one hour. The formation of the indole ring was marked by the expulsion of $N_2$ gas and thus the progress of the reaction was monitored visually. The reaction was left to reflux overnight. Heat was removed and reaction flask was allowed to cool before transporting the solution into a 100 mL 1-neck RBF. The xylenes were then removed via rotary evaporation until approximately 3 mL solution remained. Hexanes were then added just until brief formations of precipitate were observed. The mixture was placed in cold storage overnight and crystallization of the product allowed. Vacuum filtration yielded the title compound as yellow crystals. This reaction was not quantified for yields. $^1$H NMR (500MHz, $CDCl_3$): δ 8.91 (s, 1H), 7.05 (s, 1H), 6.82 (s, 2H), 4.39 (q, 2H), 4.08 (s, 6H), 3.93 (s, 3H), 1.39 (t, 3H).

### *5.3.4. ethyl 6-methoxy-1H-indole-2-carboxylate (**21c**)*

A flame dried 100 mL 2-neck RBF was charged with xylenes (15 mL). Ethyl 2-azido-3-(4-methoxyphenyl)acrylate (**20a**) was dissolved in 10 mL of xylenes and extracted via 20 mL syringe and then added to the flask drop-wise over half an hour. This reaction was monitored by the presence of a fluorescing spot on TLC and the reaction was complete after 4 hours of refluxing xylenes. Heat was removed and the reaction flask was allowed to cool to room temperature before transporting the solution into a 100 mL 1-neck RBF. The xylenes were then removed via rotary evaporation until approximately 10 mL solution remained. Hexanes were then added just until brief formations of precipitate were observed. The mixture was placed in cold storage overnight to allow for crystallization of the product. Vacuum filtration yielded the title compound as yellow crystals. This reaction was not quantified for yields. $^1$H NMR (400MHz, $CDCl_3$): δ 8.78 (s, 1H), 7.55 (d, 1H), 7.17 (s, 1H), 6.83 (d, 2H), 4.39 (q, 2 H), 3.87 (s, 3H), 1.41 (t, 3H).

### *5.3.5. ethyl 3-methyl-3-phenyloxirane-2-carboxylate (**23b**)*

A flame dried 3-neck 100 mL RBF flushed with nitrogen was fitted with a inlet air adapter, thermometer, and addition funnel. An approximate amount of t-BuOK (dried, 2.5 g, 26 mmol) was added to the flask and flame dried until condensation stopped. Once cooled under nitrogen, dry THF (15 mL) was syringed in and the reaction flask was brought to 0 ºC with an ice bath. An addition funnel charged with acetophenone (2.9 mL, 24 mmol) and ethyl chloroacetate (2.6 mL, 24 mmol) diluted with THF (8 mL) was added slowly over 20 minutes via addition funnel. During addition, the reaction solution turned from clear to amber to brown. The reaction was allowed reach room temperature over time. The reaction was complete by TLC (70/30 hex/EA) after 1 hour. The reaction mixture was diluted with of brine (30 mL), washed with 30 mL diethyl ether three times. The organic layer was dried with $Na_2SO_4$, gravity filtered and condensed via rotary evaporation to give a reddish oil (4.12 g, 76.5%) $^1$H NMR (400MHz, $CDCl_3$): δ 8.0 (d, 2H), 7.6 (t, 1H), 7.5 (t, 2H), 4.21 (q, 2H), 3.69 (s, 1H), 1.61 (s, 3H), 1.35 (t, 3H).

### *5.3.6. ethyl 3-(4-methoxyphenyl)-3-methyloxirane-2-carboxylate (**23d**)*

A flame dried 3-neck 100 mL RBF flushed with nitrogen was fitted with a inlet air adapter, thermometer, and addition funnel. An approximate amount of NaH (40% NaH in mineral oil) (400 mg, 16.7 mmol) was then added to the flask and flame dried until condensation stopped. Once cooled under nitrogen, dry THF (12.0 mL) was syringed in and the reaction flask was brought to 0 ºC with an ice bath. An addition funnel was charged with commercially purchased paramethoxy acetophenone (986 mg, 9.38 mmol) and ethyl chloroacetate (2.0 mL, 19 mmol) and diluted with THF (8.0 mL) and the solution was added slowly to the NaH over 20 minutes. The reaction was complete by TLC (70/30 hex/EA) after 1 hour.[21] The reaction was allowed to reach room temperature. The reaction mixture was diluted with brine (30 mL), washed with 30 mL of diethyl ether three times. The combined organic layers were dried with $Na_2SO_4$, gravity filtered and condensed via rotary evaporation to give a reddish oil. The crude product was separated with flash chromatography. $^1$H NMR (400MHz, $CDCl_3$): δ 7.39 (d, 2H), 6.87 (d, 2H), 4.18 (q, 2H), 3.83 (s, 1H), 3.81 (s, 3H), 1.61 (s, 3H), 1.80 (t, 3H); $^{13}$C (400 MHz, $CDCl_3$) δ 172.629, 158.762, 135.724, 126.368, 113.392, 75.337, 62.071, 55.221, 25.947, 14.0.35.

### *5.3.7. 2-azido-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethan-1-ol (**25**)*

A flame dried 2-neck 50 mL RBF was obtained and cooled under nitrogen. $MgBr_{2(s)}$ (200 mg, 1.09 mmol) was dissolved in 20 mL of diethyl ether dried over sieves. A small amount of heat was added to the flask while stirring to maximize solubility. 2-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)oxirane (RO3511) (50 mg, 0.158 mmol) was dissolved in 8 mL of dry DCM. The solution of $MgBr_2$ was brought to 0 ºC using an ice bath. The DCM solution containing RO3511 was added drop wise via addition funnel. Monitoring this reaction with TLC did not provide conclusive progression of the reaction. Upon completion, the reaction was then condensed via rotary evaporation. The dehydrated bromo-hydrin product, after further during on the high vacuum pump, was observed to be have a viscous consistency and a yellow tint. $NaN_3$ (31.0 mg, 0.477 mmol) and 8 mL of DMF were added to the flask and left to stir overnight. The reaction was then diluted with 30 mL of water and washed with 30 mL of diethyl ether three times. The combined organic layers were dried with $Na_2SO_4$ and concentrated by rotary evaporation and high vacuum pump. $^1H$ NMR (400MHz, $CDCl_3$): δ 7.36 (d, 2H), 7.13 (d, 2H), 6.614 (s, 2H), 5.758 (s, 1H), 4.15 (d, 1H), 3.888 (s, 3H), 3.75 (s, 6H), 3.70 (s, 3H), 3.0 (d, 1H).

### *5.3.8. ethyl 3-azido-2-hydroxy-3-phenylbutanoate (**12a-b**)*

A flame dried 100 mL 2-neck RBF was charged with 15 mL of DMSO dried over sieves and ethyl 3-methyl-3-phenyloxirane-2-carboxylate (**11**) (626 mg, 3.04 mmol) was dissolved. $NaN_3$ (2.0 g, 30.8 mmol) and 0.15 mL of conc. $H_2SO_4$ were added to the flask. The reaction was left to react at ~90 ºC for 2 hours. The reaction solution was then poured over ice water into separatory funnel, to which it was then extracted with 30 mL diethyl ether three times. The combined organic layers were dried with $MgSO_4$, gravity filtered and condensed via rotary evaporation. (348 mg, 76%) $^1H$ NMR (400MHz, $CDCl_3$): δ 8.0 (d, 2H), 7.6 (t, 2H), 7.5 (t, 1H), 5.5 (s, 1H), 4.45 (s, 1H), 4.22 (q, 2H), 1.25 (s, 3H), 1.2 (t, 3H).

### *5.3.9. ethyl 2-azido-3-(4-methoxyphenyl)acrylate (**20c**)*

A flame dried 100 mL 2 neck RBF was acquired. Obtained NaH (750 mg, 18.7 mmol) and placed into RBF. Dry THF was added via addition funnel until dissolved (6 mL). The base solution was brought to 0 ºC using an ice bath. Ethyl azidoacetate (**16**) (2.52 g, 19.5 mmol) was added to addition funnel, along with 4-methoxybenzaldehyde (0.75 mL, 6.18 mmol) and THF (18 mL) to dilute. The solution of the addition funnel was added dropwise. The reaction was monitored via TLC and observed to be complete after 4 hours. The reaction solution was poured over $NH_4Cl$ (25 mL) and ice, and then into separatory funnel, to which it was then extracted with 40 mL diethyl ether three times. The combined organic layers were dried with $Na_2SO_4$, gravity filtered and condensed via rotary evaporation. $^1H$ NMR (400MHz, $CDCl_3$): δ 7.80 (d, 2H), 6.93 (s, 1H), 6.90 (d, 2H), 4.36 (q, 2H), 3.85 (s, 3H), 1.41 (t, 3H).

### *5.3.10. ethyl 3-bromo-6-methoxy-1H-indole-2-carboxylate (**26**)*

A flame dried 100 mL 2-neck RBF was acquired. Ethyl 6-methoxy-1*H*-indole-2-carboxylate (187 mg 0.986 mmol) (**21c**) was dissolved in the flask with 5 mL of dry THF. A bath of dry ice and acetone was to keep the reaction around -78 ºC. Dry THF (10 mL) and NBS (187 mg, 1.05 mmol) were added to an addition funnel and slowly added dropwise. TLC showed a complete reaction after 6 hours. The reaction solution was then diluted with 20 mL of brine in a separatory flask and was extracted with 20 mL of diethyl ether three times. The combined organic layers were dried with $Na_2SO_4$, gravity filtered and condensed via rotary evaporation. $^1H$ NMR (400MHz, $CDCl_3$): δ 8.90 (s, 1H), 7.57 (d, 1H), 6.97 (d, 1H), 4.46 (q, 2H), 4.00 (s, 3H), 1.45 (t, 3H).

### *5.3.11. ethyl 2-azido-3,3,-diphenylacrylate*

A flame dried 25 mL 2-neck RBF was acquired and fitted with a stir bar. The flask was then charged with 10 mL of dry MeOH, and a bath of MeOH and dry ice to keep the reaction around -10 ºC. Solid Na (209 mg, 9.09 mmol) was then added piecewise to form a white slurry. Ethyl azidoacetate (1.02 g, 7.90 mmol) and benzophenone (0.468 g, 2.57 mmol) were dissolved in 8 mL of MeOH. This solution was added dropwise via syringe over the course of an hour. The reaction flask was covered with foil to shield from the light. After 24 hours without a change in TLC, the reaction solution was poured over a saturated $NH_4Cl$ solution with ice and vacuum filtered through a Buchner funnel and P8 filter paper. Filtrate was placed in separatory funnel and extracted with 50 mL of diethyl ether three times. The combined organic layers were dried with $Na_2SO_4$, gravity filtered and condensed via rotary evaporation. Regioisomers were not separated. (0.0 g, 0%) No spectral data available.

### *5.3.12. ethyl 3,3-bis(4-methoxyphenyl)oxirane-2-carboxylate*

A flame dried 50 mL 2-neck RBF was acquired and fitted with a stir bar. Flask was then charged with NaH (195 mg, 5.28 mmol) and 10 mL of THF. An ice bath was set up to keep the reaction around 0 ºC. 2,2'-dimethoxybenzophenone (601 mg, 3.54 mmol) was added to flask, while ethyl chloroacetate (0.75 mL, 7.04 mmol) and 5 mL of THF were placed in addition funnel and added dropwise to reaction flask while stirring. After 3 hours without a change in TLC, the reaction solution was diluted with 30 mL of water into a separatory funnel and extracted with 30 mL of diethyl ether three times. The combined organic layers were dried with $Na_2SO_4$, gravity filtered and condensed via rotary evaporation. (0.0 g, 0%) No spectra data available.

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