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Synthesis of indole containing pyrimidines

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Synthesis of Indole Containing Pyrimidines

by

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Honors Thesis
in

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Advisor: Dr. John Gupton

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Acknowledgements

This research could not have been completed without the support of my advisor, Dr. John Gupton. His suggestions and guidance were truly invaluable. Also, the assistance and training from Dr. Nakul Telang, a postdoc in Dr. Gupton's research lab, helped me through the research experience. Moreover, I would like to recognize the help from the students in my research group; John Stafford, Peter Barelli, Scott Welden, Spencer Bates, Dom Gazzo, Emily Kluball and Kayleigh Hall. They truly made by undergraduate research career more enjoyable. Finally, I would like to thank NIH for funding my research during the summer of 2010.

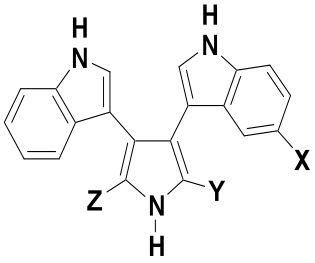
I. **Abstract**

The ability to develop compounds that are biologically active and can express anti-cancer abilities is a leading component of organic and medicinal chemistry today. In recent years, the benefits of new and interesting heterocycles and the importance of indoles has been extensively studied for their tubulin inhibition and anti-proliferative capabilities against several cancer cell lines. The synthesis of indole containing pyrimidines through an efficient three-step mechanism was developed with chemistry previously used by the Gupton group. Beginning with a commercially available reagent, 3-acetyl indole, a vinylogous amide intermediate and chloroenal intermediate were synthesized. Reaction of the indole appended chloroenal with several different amidines produced novel biheterocyclic systems in reasonable yields.

II. Introduction

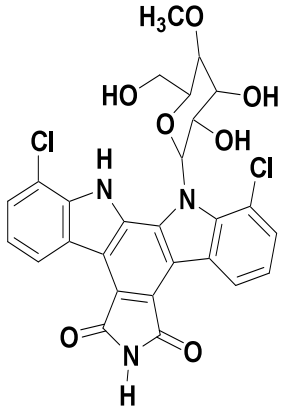
Since the early years of medicinal chemistry and pharmaceuticals, scientists have looked to natural products in efforts to cure aggressive diseases, like cancer. In past years, the Gupton group has developed new synthetic organic chemical reactions that utilize chloroenals as key building blocks for the construction of novel heterocyclic systems. Chloroenals have the ability to be reacted with binucleophilic compounds to form new and interesting heterocycles. Heterocycles are important because they often appear as part of biologically active marine natural products. These natural products remain important for the drug discovery process of new anti-cancer agents by generating new "lead compounds". The ability to prepare such molecules and to modify key structural features for the optimization of biological properties and for the minimization of undesirable side effects is invaluable.[1]

The indole functional group is a component found in many natural products, including Lycogallic acid, Lycogarubin C, Cinereapyrrole B, Lynamycin E, and Rebeccamycin, (as seen in Figure 1).[3]



X = H and Z = Y = CO_2H Lycogallic Acid
X = H and Z = Y = CO_2Me Lycogarubin C
X = OH and Z = H and Y = CO_2Me Cinereapyrrole B
X = Cl and Z = Y = CO_2Me Lynamycin E

(1)



Rebeccamycin

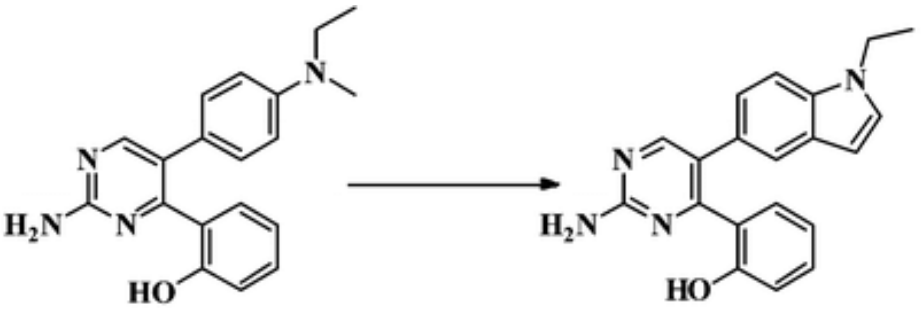
(2)

Figure 1. Natural Products containing the Indole Substituent.

For over a century, indole derivatives have been a topic of research interest due to their wide range of biological activity and potential as therapeutic agents. Indole appears in the amino acid tryptophan, a part of metabolites important in both plants and animals. It also

appears in serotonin, a neurotransmitter in animals. The indole ring also occurs in indole alkaloids, fungal metabolites and marine natural products. Indole is classified as a Pi-excessive aromatic compound, a very weak base with a high-energy HOMO and is subject to oxidative processes. Many synthetic methods look to modify the indole ring at C3, by adding on various substituents.[2]

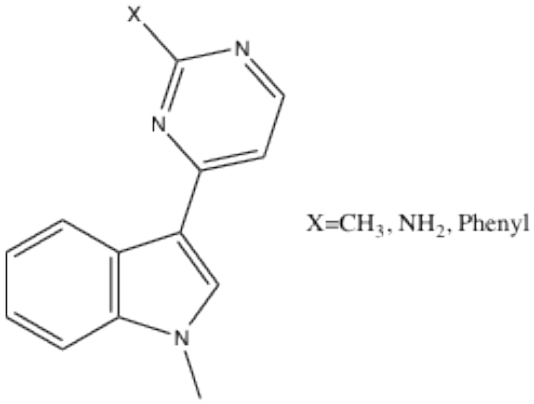
A recent article published in the *Journal of Medicinal Chemistry* reported the synthesis of 2,4,5-trisubstituted pyrimidine derivatives, and found that the indole-aryl substituted aminopyrimidine was an excellent inhibitor of tubulin polymerization (Figure 2).[4]



(3) **(4)**

Figure 2. Synthesis of indole-aryl substituted aminopyrimidine as performed in *J. Med. Chem.* in 2011 by Fuchun Xie, et. al.

The compound 4 had high antiproliferative activity against several cancer cell lines and competitively inhibited colchicine binding to tubulin. While natural products with indole and pyrroles have been studied, indole-containing pyrimidines have not been looked at yet by our research group. Using chemistry previously established in the Gupton lab, indole appended pyrimidines can be synthesized. These new compounds, containing both indole and a new heterocycle, could be a potential starting material for more complex biologically active materials.



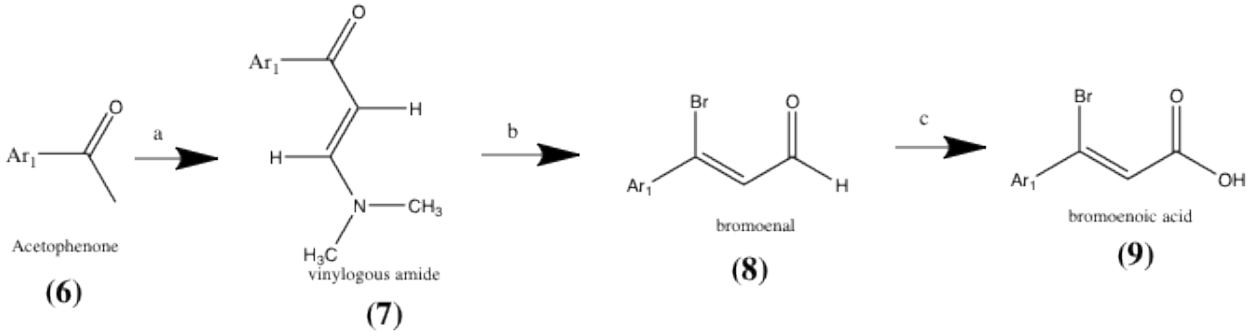
(5)

Figure 3. Desired Indole-Pyrimidine products with carrying substituents

Three different X groups were targeted throughout the project, including, amino, methyl, and phenyl, as seen in Figure 2.

III. Results and Discussion

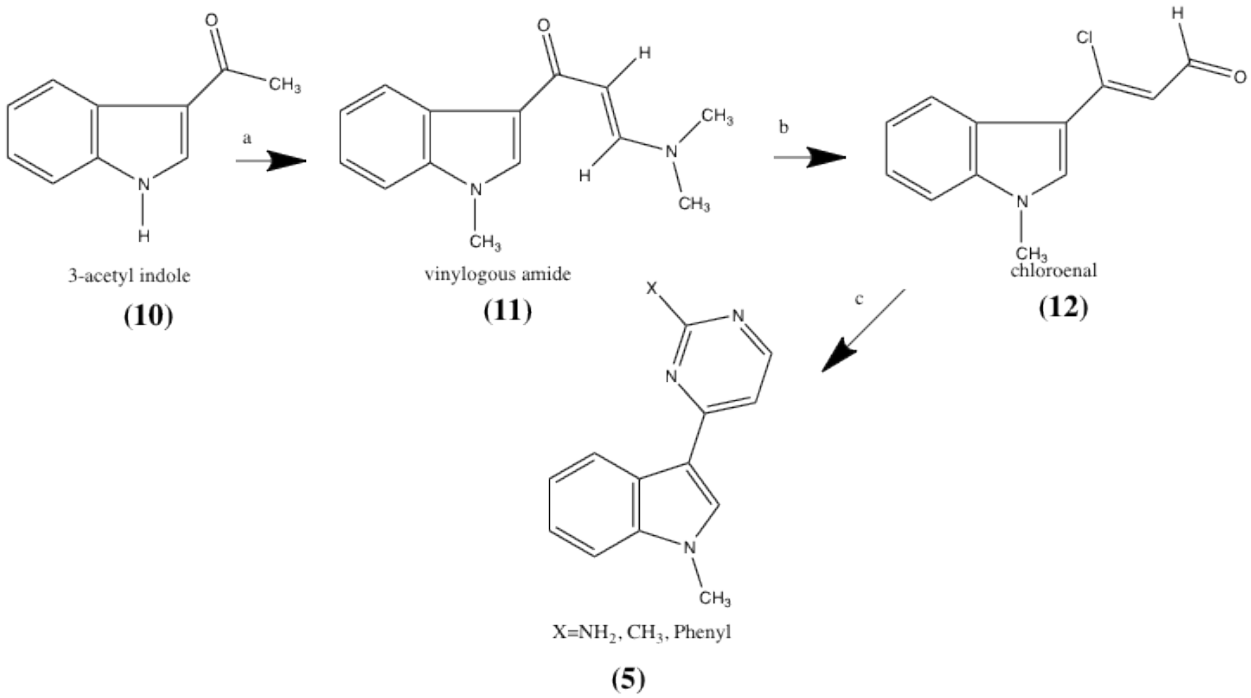
This idea for this research project was developed from previous research done in the Gupton group (Scheme 1).[5]



Scheme 1. Preparation of bromoenoic acid from acetophenone.

a. DMFA/DMF/Δ; **b.** $POCl_3/CH_2Cl_2/\Delta$ followed by H_2O/THF/rt; **c.** $NaH_2PO_4 \bullet H_2O/NaClO_2$/DMSO/rt;

Aspects of Scheme 1 were recently used to prepare possible key intermediates for the synthesis of Ningalin C. The indicated steps lead to a bromoenal, which in principle could be converted to a heterocycle by reaction with a binucleophilic substance . By using this chemistry, with indole as the aromatic group, products such as those represented in figure 2 could be synthesized. Therefore, the synthesis of the various indole appended pyrimidines with varying substituents was performed according to scheme 2.



Scheme 2. Preparation of indole pyrimidines

a. DMFA/DMF/Δ; **b.** $POCl_3/CH_2Cl_2/\Delta$ followed by H_2O/THF/rt; **c.** amidine/K_2CO_3/EtOH

When first attempting to convert 3-acetyl indole into the vinylogous amide, the crude product was not clean. An aqueous lithium chloride extraction was used to remove DMF impurities and an almost analytically pure product was obtained. This was confirmed by ^{1}HNMR with peaks for the two new vinyl hydrogens appearing at 7.60 and 5.76 ppm, as seen in Figure 3, Appendix A. A new singlet peak integrating for 6 hydrogens was also found as 3.01 ppm, representing the new N-dimethyls. The synthesized vinylogous amide was able to be converted into the chloroenal (step b) with relative ease, yielding a crude product which was analytically pure. This was confirmed by ^{1}HNMR with one of the vinyl hydrogen peaks disappearing, with only one peak appearing at 6.71 ppm. Also, the disappearance of the N-dimethyl peak and appearance of an aldehyde hydrogen at 10.20 ppm further confirmed that step b was successfully completed (as seen in Figure 7, Appendix A). However, when later rechecked by NMR to be used for step c, the compound had decomposed slightly, as a result of being left at room temperature. The reaction was therefore repeated, and the product was stored in the freezer.

Steps a and b, while never done with the indole substituent, had been done with other various aromatics, and therefore were expected to be successful. The final step,

however, was new chemistry. Theoretically, the chloroenal should react with a binucleophilic reagent. The first of the binucleophiles to be tested (as seen in Figure 4) was guanidine carbonate.

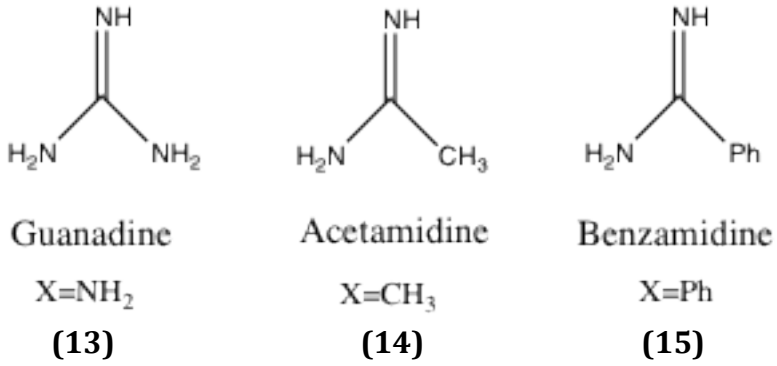


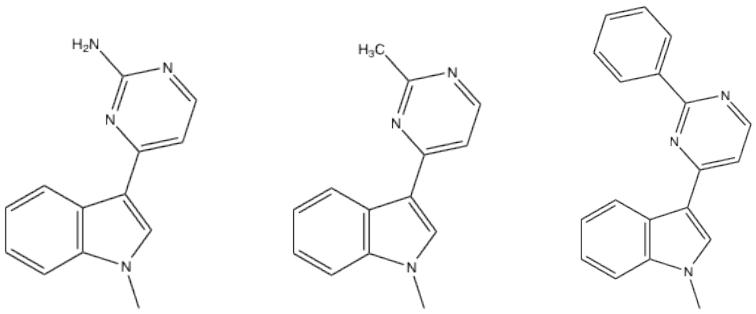
Figure 4. Nucleophilic Reagents used in step c of scheme 2 to cyclized chloroenal

Originally, the reaction mixture was refluxed and monitored by TLC for completion. The disappearance of the chloroenal TLC spot and appearance of the product appeared after 2.5 hours. However, flash chromatography of the crude material produced only a 14% yield of purified product, and the NMR still showed a product not analytically pure. Therefore, the time of the reaction was increased to overnight, and this change led to the desired product at higher yields. This was confirmed by ^{1}H NMR because all vinyl hydrogens disappeared, and the two new pyrimidine hydrogens appeared at 8.14 and 7.01 ppm (as seen in Figure 8, Appendix A).

The reaction was repeated with acetamidine and benzamidine, and crude yields were well over 100% were obtained due to leftover potassium carbonate in the mixture. Instead of purifying these products through flash chromatography, a silica/sand plug was used to remove the inorganic components. The silica plug was able to yield relatively pure products, again confirmed with the disappearance of the vinyl hydrogen and appearance of the two new pyrimidine hydrogens at 8.49 and 7.57 ppm, and 8.72 and 7.72 ppm for acetamidine and benzamadine, respectively (Figures 9 and 10, Apendix A). The methyl substituent of acetamidine group was easily identified by a peak at 2.67 ppm, integrating for 3 H. The phenyl substituent of benzamidine was more difficult to distinguish, due to the overlap of phenyl and indole peaks in the aromatic region. All expected protons were

accounted for but some overlapping absorptions were present, which made some of specific assignments very difficult.

The overall percent yields for the three synthesized products are reported in Figure 5. While the purified yields are slightly less than ideal, the procedure was optimized with each compound, with the percent yield increasing with each reaction.



(16): 49% yield **(17): 59% yield** **(18): 65% yield**

Figure 5. Reaction Products with their respective percent yields.

The development of an efficient, three step scheme to synthesize indole appended pyrimidines was established as a result of this research project. As stated earlier, substituted indole appended pyrimidines have been found to have anti-cancer abilities and antiproliferative activity. The compounds synthesized could be further elaborated and then subjected to biological testing. The difference between the indole appended pyrimidines previously reported in the literature and our new synthetic products involves the regiochemistry of indole attachment to the pyrimidine. The control of regiochemical attachment represents new chemistry that may have exciting applications. Furthermore, this reaction scheme represents a new strategy for preparing key indole building blocks, which may have many future synthetic applications.

IV. Experimental

General

All chemicals were used as received from the manufacturer (Aldrich Chemicals and Fisher Scientific) and solvents were dried over 4 angstrom molecular sieves prior to use. All synthesized compounds were characterized through H NMR, GC-MS, IR, TLC, and melting point. H-NMR spectra were obtained from a Bruker 500 MHz spectrometer in d_6-acetone

solution. Low-resolution GC-MS spectra were obtained from a Shimadzu DP 5050 instrument with a direct insertion procedure. IR spectra were obtained from a Nicolet Avatar 320 FT-IR spectrometer. Melting points were obtained from a standard apparatus. TLC analyses were performed on silica plates with either 50:50 hexane/ethyl acetate, 25:75 hexane/ethyl acetate or 50:50 ethyl acetate/acetonitrile as the eluant, depending on compound polarity.

(*E*)-3-(Dimethylamino)-1-(1-methyl-1*H*-inden-3-yl)prop-2-en-1-one

To a 100 mL round bottom flask equipped with a magnetic stir bar was added 3-acetyl indole (3.0 g, 18.9 mmol) in 25 mL DMF. DMFA (3.5 mL, 75 mmol) was added and the reaction mixture was refluxed overnight. The reaction mixture was cooled to room temperature, evaporated and dried on the kugelrohr apparatus, yielding 4.09 grams (94.9% yield). The residue was then diluted with 30 mL ethyl acetate and washed three times with 20 mL of lithium chloride solution. The organic phase was dried with anhydrous magnesium sulfate, and concentrated to yield 3.90 grams (90% yield) of a solid, which exhibited the following properties: mp: 89-93C; R_f= 0.533 in 25:75 (hex/EtoAc); ^{1}H (D_6-acetone) δ 3.01 (s, 6H), 3.89 (s, 3H), 5.76 (d, J=12.5 Hz, 1H), 7.15 (t, J=7.8 Hz, 1H), 7.22 (t, J=7.8 Hz, 1H), 7.41 (d, J=7.8 Hz, 1H), 7.60 (d, J=12.5 Hz, 1H), 8.00 (s, 1H), 8.45 (d, J=7.8 Hz, 1H); IR (neat) 3097 cm^{-1} (CH_3), 2901 cm^{-1} (CH_2), 1625 cm^{-1} (CO), 1223 (C-N); DI-GCMS *m/z* calculated for $C_{14}H_{16}N_2O$ to be 228, found BasePeak 228.

(*Z*)-3-Chloro-3-(1-methyl-1*H*-inden-3-yl)acrylaldehyde

To a 100 mL round bottom flask equipped with a magnetic stir bar was added the indole vinylogous amide (0.700 g, 3.06 mmol) and $POCl_3$ (0.575 mL, 6.29 mmol) in DCM (20 mL). The mixture was refluxed for 2 hours. DCM was removed by rotary evaporation. The residue was dissolved in 50 mL of 50:50 mixture of water:THF and stirred at room temperature for 24 hours. The mixture was extracted three times with ethyl acetate (30 mL) and washed with brine (30 mL), dried over magnesium sulfate and concentrated to yield 0.500 g (74.6% yield) of a solid, which exhibited the following properties: mp: 55-61°C; R_f=0.53 in 25:75 (hex/EtOAc); ^{1}H (D_6-acetone) δ 4.00 (s, 3H), 6.71 (d, J=7.0 Hz, 1H),

7.34 (t, J=8.0 Hz, 1H), 7.39 (t, J=8.0 Hz, 1H), 7.60 (d, J=8.0 Hz, 1H), 7.97 (d, J=8.0 Hz, 1H), 8.14 (s, 1H), 10.20 (d, J=7.0 Hz, 1H); IR (neat) 3107 (CH_2), 2828 (O=C-H), 1226 (CO): DI-GCMS *m/z* calculated for $C_{12}H_{10}NOCl$ to be 219, found BasePeak 219.

4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-amine

To a 100 mL round bottom flask equipped with a magnetic stir bar was added the chloroenal previously synthesized (0.250 g, 1.14 mmol) in 20 mL of ethanol. Guanidine carbonate (0.411 g, 2.282 mmol) and potassium carbonate (0.316 g, 2.28 mmol) were added and the reaction mixture was refluxed overnight. The reaction mixture was cooled to room temperature and the ethanol was removed using rotary evaporation and dried on the kugelrohr apparatus (0.325 g). The crude material was purified by flash chromatography on a silica column with a ethyl acetate/acetonitrile gradient in which case 0.116 g (49% yield) of purified product was obtained, which exhibited the following properties: R_f=0.29 in 50:50 (EtOAc/Acetonitrile); ^1H (D_6-Acetone) δ 3.93 (s, 3H), 7.01 (d, J=5.5 Hz, 1H), 7.18 (t, J=7.5, 1H), 7.26 (t, J=7.5 Hz, 1H), 7.47 (d, J=7.5 Hz, 1H), 8.06 (s, 1H), 8.14 (d, J=5.5 Hz, 1H), 8.60 (d, J=7.5 Hz, 1H); IR (neat) 3449 (N-H), 3145 (CH_2), 1220 (C-N); DI-GCMS *m/z* calculated for $C_{13}H_{12}N_4$ to be 224, found BasePeak 224.

1-Methyl-3-(2-methylpyrimidin-4-yl)-1*H*-indole

To a 100 mL round bottom flask equipped with a magnetic stir bar was added the chloroenal previously synthesized (0.250 g, 1.141 mmol) in 20 mL ethanol. Acetamidine hydrochloride (0.216 g, 2.28 mmol) and potassium carbonate (0.316 g, 2.28 mmol) were added and the reaction was refluxed overnight. The reaction mixture was cooled to room temperature and the ethanol was stripped off using rotary evaporation. The residue was filtered through a silica plug with 60 mL of a 50:50 mixture of hexane:ethyl acetate. The silica plug was washed twice, once with 60 mL ethyl acetate and once with 60 mL of a 50:50 mixture of ethyl acetate:acetonitrile. The solvent for each wash was removed using rotary evaporation. The residue was dried on the kugelrohr apparatus (0.15 g, 59% yield) and exhibited the following properties: mp: 64-67°C. R_f=0.42 in 50:50 EtOAc/Acetonitrile); ^1H (D_6-acetone) δ 2.67 (s, 3H), 3.60 (s, 3H), 7.25 (t, J=5.5 Hz, 1H), 7.30 (t, J=7.5 Hz, 1H), 7.51

(d, J=8.0 Hz, 1H), 7.57 (d, J=5.5 Hz, 1H), 8.19 (s, 1H), 8.49 (d, J= 5.5 Hz, 1H), 8.61 (d, J=8.0 Hz, 1H); IR (neat) 3050 (CH_2), 2929 (CH_3), 1226 (CO); DI-GCMS *m/z* calculated for $C_{14}H_{13}N_3$ to be 223, found BasePeak 223.

1-Methyl-3-(2-phenylpyrimidin-4-yl)-1*H*-indole

To a 100 mL round bottom flask equipped with a magnetic stir bar was added the chloroenal previously synthesized (0.250 g, 1.14 mmol) in 20 mL ethanol. Benzamadine acetate (0.357 g, 2.282 mmol) and potassium carbonate (0.316 g, 2.282 mmol) were added and the reaction was refluxed overnight. The reaction mixture was cooled to room temperature and the ethanol was stripped off using rotary evaporation. The residue was filtered through a silica plug with 60 mL of a 50:50 mixture of hexane:ethyl acetate. The silica plug was washed twice, once with 60 mL ethyl acetate and once with 60 mL of a 50:50 mixture of ethyl acetate:acetonitrile. The solvent for each wash was removed using rotary evaporation. The residue was dried on the kugelrohr apparatus (0.21 g, 65% yield) and exhibited the following properties:. mp: 63-68°C. R_f=0.429 in 50:50 (hex/EtOAc); ^1H (D_6-acetone) δ 4.01 (s, 3H), 7.34 (t, J=7.0 Hz, 1H), 7.47 (t, J=7.0 Hz, 2H), 7.55 (m, 3H), 7.72 (d, J=5.0 Hz, 1H), 7.95 (d, J=7.0 Hz, 2H), 8.35 (s, 1H), 8.65 (d, J=8.5 Hz, 1H), 8.72 (d, J=5.0 Hz, 1H); IR (neat) 3160 (CH_2), 1568 (Phenyl), 1255 (CO); DI-GCMS *m/z* calculated for $C_{19}H_{15}N_3$ to be 285, found BasePeak 285.

V. Appendix A

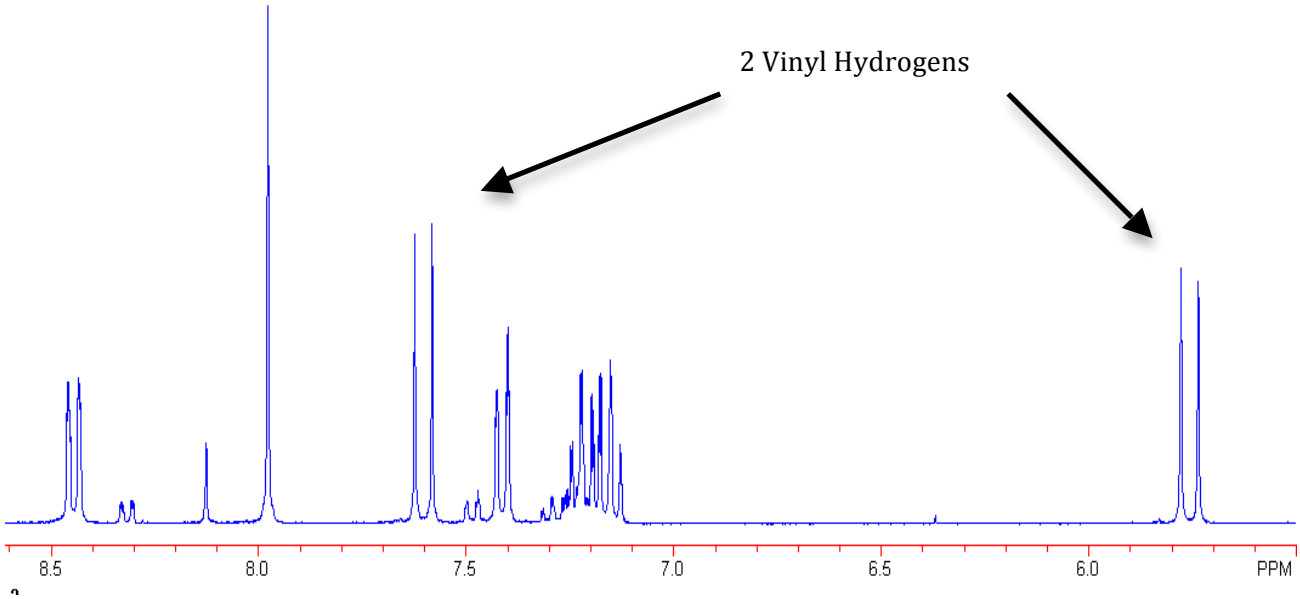


Figure 6. Magnified ^{1}HNMR spectrum of the vinylogous amide.

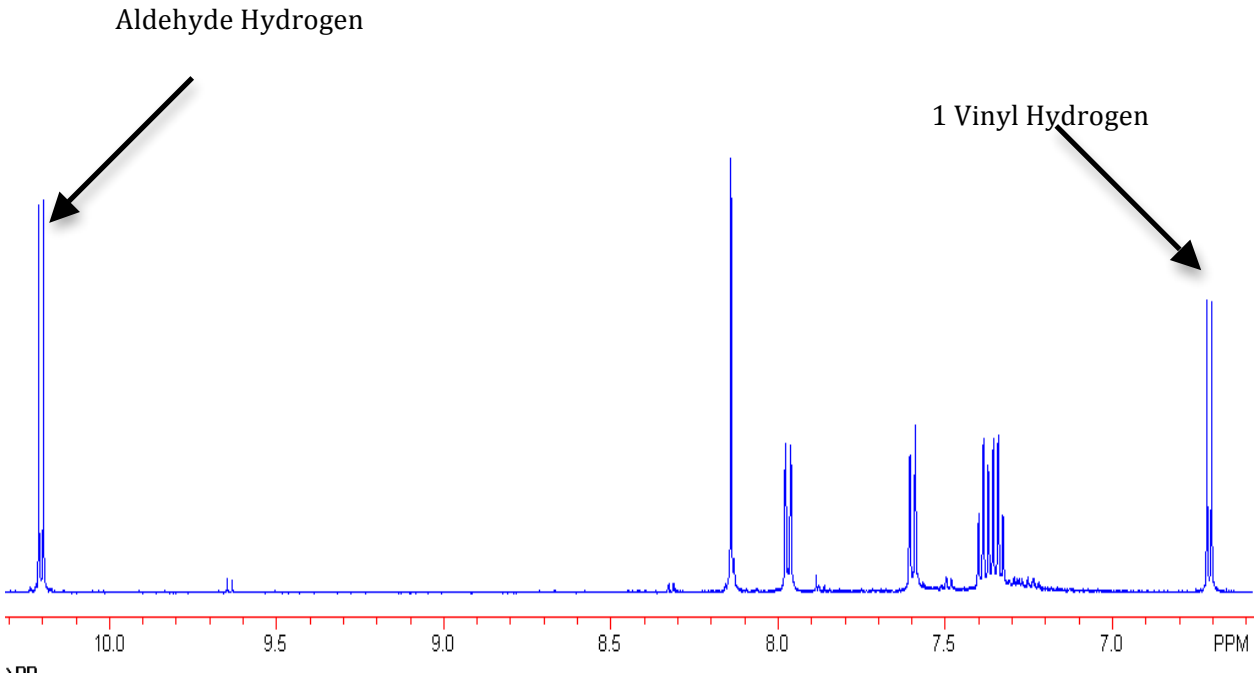


Figure 7. Magnified ^{1}HNMR spectrum of the chlorenal.

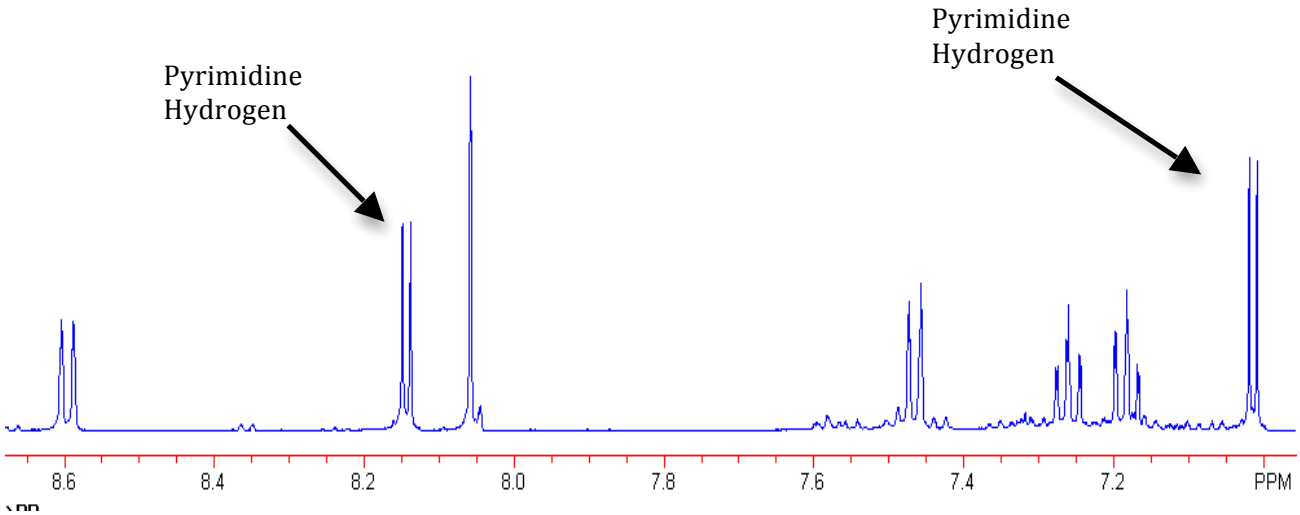


Figure 8. Magnified ^{1}HNMR of aromatic region of indole-pyrimidine with NH_2 substiuent

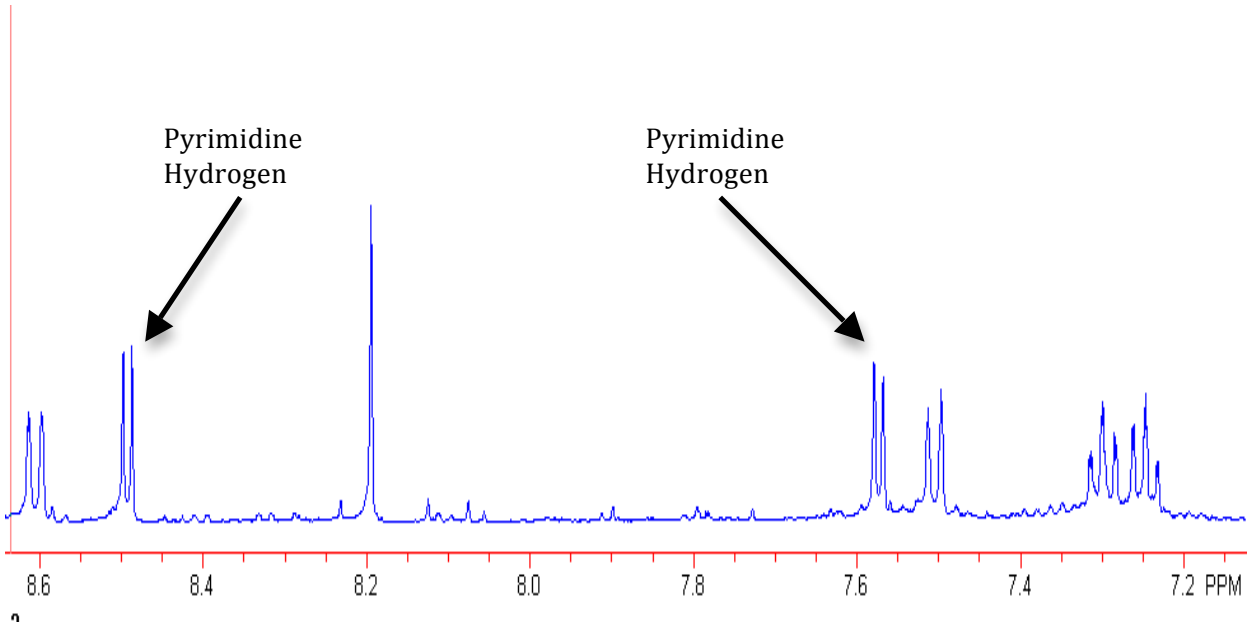


Figure 9. Magnified ^{1}HNMR of aromatic region of indole-pyrimidine with CH_3 substituent

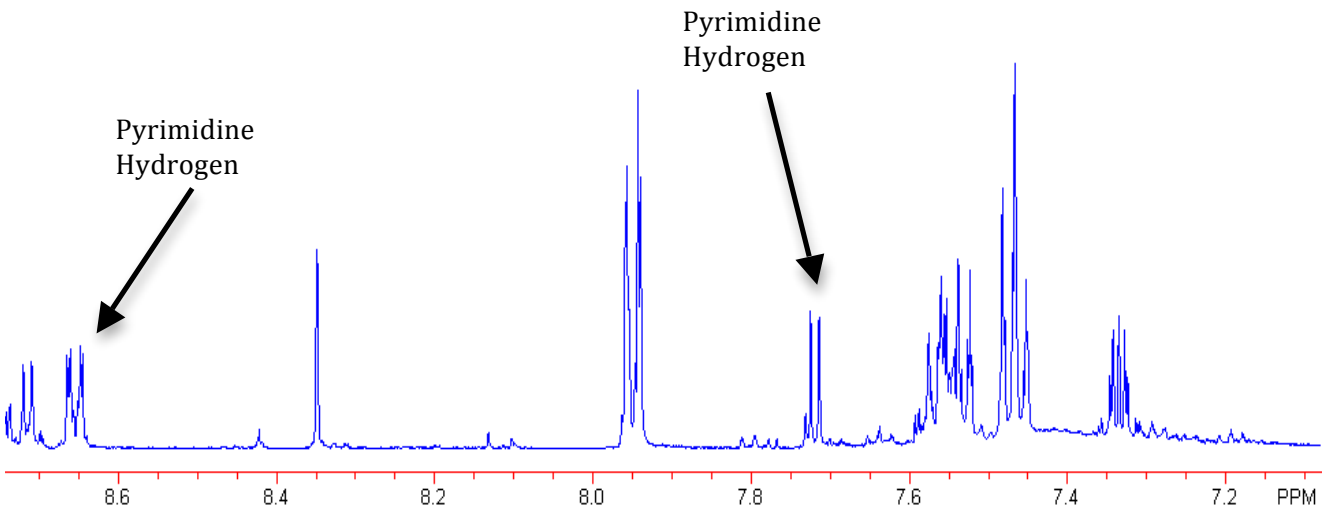


Figure 10. Magnified [1]HNMR of aromatic region of indole-pyrimidine with Phenyl substituent

VI. References

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