2013

Libraries from Libraries Approach to the Synthesis of Arylidene Oxindoles

Kyle James Knisley
Wright State University

Libraries from Libraries Approach to the Synthesis of Arylidene Oxindoles

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By
KYLE JAMES KNISLEY
B.S., Wright State University, 2011

2013
Wright State University

WRIGHT STATE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

December 5, 2013

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY KYLE JAMES KNISLEY ENTITLED Libraries from Libraries Approach to the Synthesis of Arylidene Oxindoles BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

Daniel Ketcha, Ph.D.
Thesis Director

David Grossie, Ph.D., Chair
Department of Chemistry
College of Science and Mathematics

Committee on Final Examination

Daniel Ketcha, Ph.D.

Eric Fossum, Ph.D.

Kenneth Turnbull, Ph.D.

R. William Ayres, Ph.D.

Interim Dean, Graduate School

Abstract

Knisley, Kyle James. M.S., Department of Chemistry, Wright State University, 2013.
Libraries from Libraries Approach to the Synthesis of Arylidene Oxindoles.

A libraries from libraries combinatorial chemistry approach was employed to synthesize fluorinated derivatives of both oxindoles and isatins as potential pharmaceuticals or targeting agents for imaging purposes related to cancer or Alzheimer's disease. Synthesis for these fluorinated derivatives are described by routes involving, either: a) *N*-alkylation of 5-substituted isatins followed by Wolff-Kishner reduction to the corresponding oxindoles and final Knoevenagel condensation with aryl aldehydes, or; b) Wolff-Kishner reduction of the isatins followed by condensation and finishing with the *N*-alkylation of the aldol products. In specific cases, a "click" reaction followed the *N*-alkylation of the aldol products to form the isatin 1,2,3-triazole which could be utilized to perform radiochemistry with a [^{18}F]-radiolabel for the imaging of cancer. The strategy for the synthesis of such potential inhibitors was guided by SAR studies of peptide based inhibitors, as well as small-molecule inhibitors based upon the isatins scaffold. Previously, it was shown increasing functionality by adding 3 points of variability with the incorporation of an electron-withdrawing group such as a chlorine atom at the C-5 position allowed for increased potency of the oxindole derived inhibitors. Herein, a library of arylidene oxindoles was synthesized utilizing 3 points of variability with the incorporation of the electron-withdrawing group fluorine. Furthermore, a novel

alternative synthesis was established for the creation of arylidene oxindoles which allowed for increased functionality through the incorporation of *N*-propargyl inhibitors. Finally, the ability to create *N*-propargyl compounds lead to the synthesis of isatin 1,2,3-triazoles was also explored for the possibility as potential imaging agents for cancer.

Table of Contents

List of Figures/Schemes

Page

List of Tables

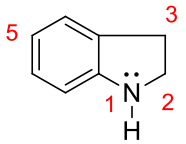
Page

Acknowledgements

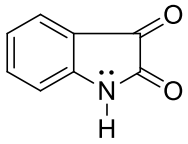
I would like to give special thanks to Dr. Ketcha for supporting me throughout my time at Wright State University. I appreciate all the support and opportunities you have provided for me and I couldn't have asked for a better advisor or person to work with over the past two years. I would also like to give thanks to all the Chemistry faculty members at Wright State as well as supporting staff, friends, and visitors.

Introduction

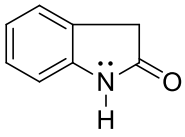
In recent years the *drug discovery process* has relied to some extent on the precepts of *combinatorial chemistry*[1] as a means of rapidly synthesizing and evaluating diverse *libraries* of compounds for biological activity. Such compound libraries are centered about *scaffolds* which can be defined as the "core portion of a molecule common to all members of a combinatorial library."[2] Certain chemical structures (often polycyclic heterocycles) have been found to be particularly attractive scaffolds for drug discovery libraries as they are often capable of binding as ligands to multiple, unrelated classes of protein receptors and have been defined as *privileged structures*.[3] One such family of privileged structures includes the benzo-fused nitrogen heterocycles, including indole (**1**)[4] and its oxidized congeners isatin (**2**) and oxindole (**3**).



1



2



3

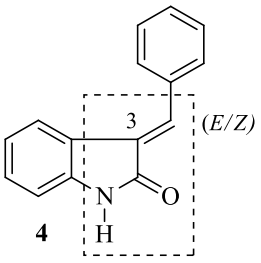
It is generally appreciated that libraries developed around such privileged structures should "yield medicinally active compounds with high hit rates at significantly reduced library size compared to large classical libraries obtained from combinatorial chemistry efforts based on non-privileged templates."[5] As this thesis relates to the construction of fluorinated derivatives of both oxindoles and isatins as potential

pharmaceuticals or targeting agents for imaging purposes related to cancer or Alzheimer's disease, a brief overview of the diverse biological activities of these heterocycles is presented herewith.

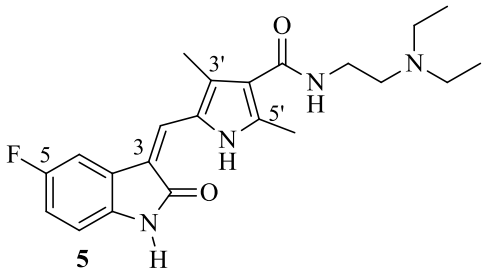
Oxindole

Oxindole (indolin-2-one, **3**) is a reduced derivative of the isatin family and was first synthesized by Baeyer at the end of the 19th century through the reduction of isatin.[6] The chemistry and synthesis of oxindole was last reviewed by Sumpter in 1945.[7] Given the biological activity of this heterocyclic scaffold, much recent work has been devoted to rapidly generating diversity by taking advantage of the reactive C-3 ketone carbonyl group of isatin precursors in multicomponent reaction processes so as to afford the corresponding spirocyclic oxindoles.[8] Although the broad range of biological activities exhibited by oxindoles warrant their inclusion into the classification of privileged scaffolds, perhaps their most significant role is that of protein kinase inhibitors.[9] Arylidene oxindoles were among the first structures identified as receptor tyrosine kinase (RTK) inhibitors by SUGEN in 1998, wherein it was found that: (1) 3-[(pyrrole)methylidenyl]indolin-2-ones are highly specific against the VEGF (*Flk-1*) RTK; (2) 3-(substituted benzylidenyl)indolin-2-ones (e.g., **4**) containing bulky groups in the phenyl ring at the C-3 position showed high selectivity toward the EGF and Her-2 RTKs; and, (3) pyrrolic compounds containing an extended side chain exhibited high potency and selectivity when tested against the PDGF and VEGF (*Flk-1*) RTKs.[10] Crystallographic evidence demonstrated that both the proton at the *N*-1 position and the oxygen atom at the C-2 position of the indolin-2-one were found to be coordinated to the

peptide backbone within the ATP binding pocket of these RTKs, and it was reasoned that such bidentate hydrogen bonding between the indolin-2-one flat core in the adenine binding site might serve to block entry of ATP in the site. Additionally, it was found that alkylation at the *N*-1 position of the indolin-2-ones greatly decreases the inhibitor potency of these oxindoles, and that the substitution around the indolin-2-ones may be key determinants for the potency and especially specificity of the inhibitors. This recurring (*vide infa*) "nitrogen-linked Michael acceptor"[11] structural motif of general structure **4** is found in many biologically active oxindoles, and has led to development of **SU11248** (5-(5-fluoro-2-oxo-1,2-dihydroindol-(3Z)-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (**5**) or Sunitinib.[11]



4

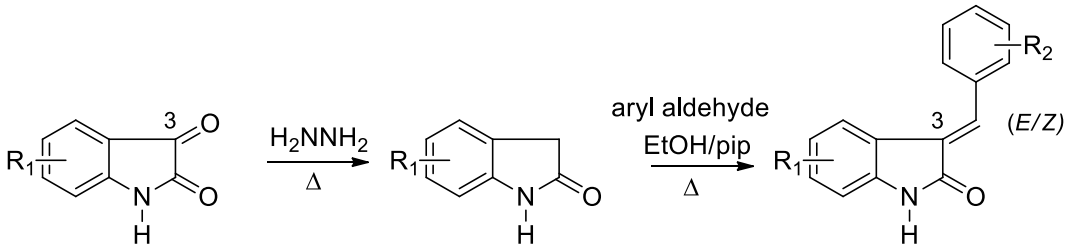


5
Sunitinib

Sunitinib was developed as a tyrosine kinase inhibitor that would target both vascular endothelial (VEGF) and platelet-derived growth factor (PDGF) RTKs because of their critical roles in tumor growth and the fact that both RTKs have been found to be expressed on tumor cells and affect tumor cell production.[11] It was found to possess the best inhibitory potency against the VEGF-R2 and PDGF-Rβ targets in biochemical and cellular assays, displays solubility under both neutral and acidic conditions, and protein binding properties in the presence or absence of serum or blood proteins.[12] Sunitinib also had very good oral bioavailability, was highly efficient in a number of preclinical

tumor models and was so effective that it was placed into clinical phase I trials for the treatment of cancer.[12]

It should also be mentioned that the synthetic sequence developed by SUGEN remains the most commonly employed approach to arylidene oxindoles and involves an initial Wolff-Kishner reduction of the C-3 carbonyl group of substituted isatins **6** (which in general are more readily available) to the corresponding oxindoles **7** in refluxing hydrazine hydrate, followed by an aldol type condensation with an appropriate aldehyde in refluxing ethanol with a catalytic amount of piperidene.

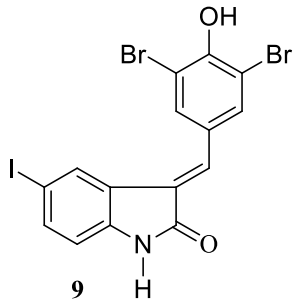


6 **7** **8**

Such arylidene oxindoles **8** (which can exist as a mixture of *E/Z* isomers) can serve as potent protein kinase inhibitors[9-22], exhibit moderate antitumor [23,24] and antiviral activity (HIV),[25] and can serve as caspase-3 inhibitors.[26] As described earlier, oxindoles are most often recognized as protein kinase inhibitors. Protein kinases are involved in a number of diseases such as cancer, diabetes, and inflammation in which the protein kinase-mediated cell signaling pathways are activated by these disease states. The human genome encodes over 518 protein kinases that share a catalytic domain conserved in sequence and structure but are vastly different in the way catalysis is regulated.[9] Protein kinase inhibitors are designed around the ATP binding pocket between two lobes within

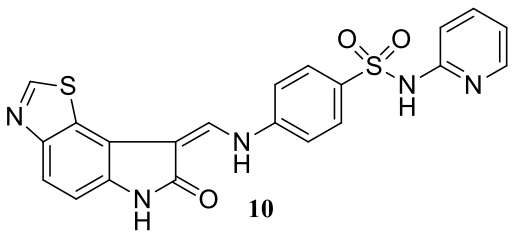
the kinase fold which when combined with less conserved surrounding pockets has allowed for differences in kinase structure and flexibility in order to achieve selectivity.[9]

Benzylidene oxindoles with a phenolic group at C-3 represent another important class of kinase inhibitors. Workers at Glaxo Wellcome first demonstrated the efficacy of such analogues for the inhibition of cRaf1, a kinase required for Ras signal transduction and the first enzyme in the mitogen-activated protein (MAP) kinase cascade consisting of the three kinases cRaf1, MEK and ERK.[13] The c-Raf protein kinase is one of three Raf proteins possessed by mammals, the other two Raf proteins are known as A-Raf and B-Raf. B-Raf is expressed primarily in the nervous system and is the most potent activator of MEK, (A-Raf is the weakest), and c-Raf is expressed throughout the body. The Raf proteins are involved in a signaling pathway that has been implicated in the promotion of neuronal survival which exists as the Raf-MEK-ERK pathway. Researchers have discovered that disruption of either c-Raf or B-Raf results in embryonic lethality.[14] Workers at the University of Texas at Dallas later discovered that one of the Glaxo c-Raf inhibitor compounds, **GW5074** (5-iodo-3-[(3,5-dibromo-4-hydroxyphenyl) methylene]-2-indolinone, **9**)[14] was a potent inhibitor of neurodegeneration both in cell culture and in an animal model. It inhibits the death of cultured cerebellar granule and cortical neurons induced by a variety of different apoptotic stimuli and also prevents striatal degeneration and improves behavioral performance in mice treated with 3-nitropropionic acid which is commonly used as an *in vivo* paradigm of Huntington's disease.[14] Although compound **9** represented the first demonstration of neuroprotection by pharmacological inhibition of c-Raf, the major drawback to **GW5074** lies in the fact that it is neurotoxic at concentrations that are not much higher than those at which it is protective.



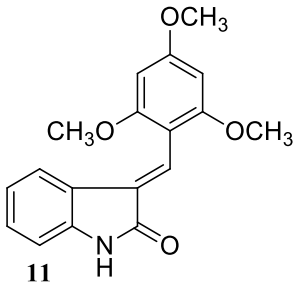
9

Benzylidene oxindole derivatives possessing the (C=O)NH and the C-3 pyrrolyl N-H moieties capable of making three hydrogen bonds with the backbone motif of the kinase hinge region have also been demonstrated as inhibitors of cyclin-dependent kinases (CDKs).[15] CDKs play an important role in cell-cycle proliferation and are attractive targets in cancer chemotherapy. An enamino analog **GW8510** (**10**) was discovered by Davis *et al*.[17] The cyclin-dependent protein kinase has been linked to neuronal apoptosis in which an abortive re-entry of neurons into the cell cycle occurs. **GW8510** was developed as an inhibitor of cyclin-dependent kinase 2 (CDK2) and inhibits cerebellar granule neurons caused by switching them from high potassium (HK) medium to low (LK) potassium medium. Johnson *et al*. showed that **GW8510** was one of seven 3-substituted benzylidene oxindoles that prevents LK-induced death of cerebellar granule neurons.[18] These findings demonstrate that the oxindole scaffold is very effective for the development of neuroprotective compounds that could be utilized in the treatment of neurodegenerative conditions.



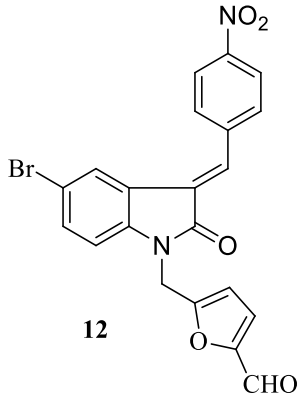
10

The casein kinase CK1 inhibitor **IC261** (**11**) developed by Flajolet *et al*. is another example of an oxindole derivative being utilized as a protein kinase inhibitor.[19] The CK1 kinase inhibitor and γ-secretase are targets for therapeutic intervention in the treatment of cancer and Alzheimer's disease. Alzheimer's disease is an illness that prevents patients from managing their own lives and is accompanied by protein aggregates in the brain largely composed of amyloid-β-peptide (Aβ) which are better known as amyloid plaques. Aβ is generated by the degradation of the type I transmembrane amyloid precursor protein (APP) by two aspartyl proteases, β- and γ-secretase. The γ-secretase is a target for therapeutics because it liberates various Aβ-peptides with the lengths of 38, 40, and 42 amino acids. The toxicity of such amyloid monomers is dependent upon the length of the peptides, in which case $A\beta_{42}$ is the most toxic and $A\beta_{38}$ is non-toxic.[20] **IC261** was shown by Flajolet to be an ATP-competitive casein kinase 1ε (CK1ε) inhibitor and causes a significant reduction of $A\beta_{40}$ and $A\beta_{42}$. Hottecke *et al*. attempted to analyze the relationship between CK1 kinase and γ secretase inhibitory activity.[20] It was observed that cellular γ-secretase inhibition occurred at high concentration of **IC261** or through a metabolic activation of **IC261**. Variations of **IC261**were synthesized and it was observed that subtle variations to the substitution pattern caused a loss in the CK1 inhibition.



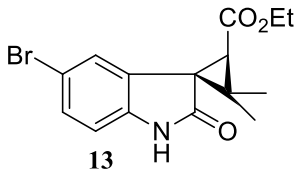
11

Zhou *et al*. examined the antitumor activities of 3-substituted 1-(5-formyl furfuryl) indolin-2-ones such as (*Z*)-5-bromo-1-(5-formylfurfuryl)-3-(4-nitro benzyli denyl)indolin-2-one (**12**).[23] In the development of **12**, forty-two analogues were synthesized and screened of which thirty one compounds were found to be highly potent against human intestinal Caco-2 cell line. It was observed that EWG substitutions in the molecules were favorable for their antitumor activities. Compound **12** was found to be the most active with an IC_{50} value of 0.19 μM.[23]



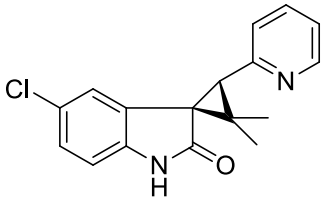
12

Oxindole derivatives have also been shown to exhibit antiviral activity in the form of non-nucleoside reverse transcriptase inhibitors (NNRTI). The main disease associated with NNRTI is HIV/AIDS, the leading cause of death due to infectious disease in the world. Treatment failures for this disease remain high due to the evolution of drug-resistant viruses. The most commonly prescribed drugs for HIV are the NNRTI. Jiang *et al*. synthesized **13** as a potential NNRTI, and although **13** was a very potent inhibitor for HIV replication, it exhibited high clearance, low exposure, and low bioavailability[25].

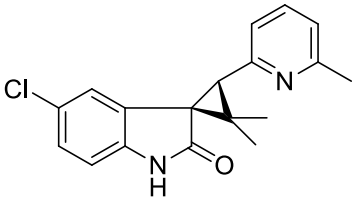


13

The inhibitor was optimized by replacing the ester moiety with a tetrazole since esters are known to be metabolically unstable, and using a 5-chloro- rather than a 5-bromo-substituent. It was discovered that one form of the tetrazole was active while another form was inactive depending on the position of the methyl group on the tetrazole ring, and the incorporation of a 2-pyridinyl moiety in place of the tetrazole (*e.g.*, **14)**, exhibited EC_{50} values in the single digit nanomolar range (0.008 μM) suggesting that the pyridine nitrogen is important in the interaction with the enzyme. Additionally, incorporation of a methyl group at the *meta*-position of the 2-pyridyl ring of **14** increased the inhibitory potency of the HIV inhibitor **15** to 0.005 μM. The study also demonstrated that compounds **14** and **15** exhibited significantly improved exposure and improved water solubility compared to that of compound **13** indicating they might be better HIV replication inhibitors.[25]



14



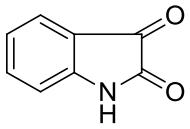
15

As can be seen from the examples above, oxindole derivatives have been employed in the treatment of a variety of pathological diseases ranging from cancer to neurological disorders. There are numerous possibilities for a potential drug lead with

the oxindole scaffold which makes it an interesting and popular starting point for many researchers when searching for the next big discovery in the pharmaceutical world. Isatin is also considered a privileged structure that presents similar features to those of oxindole which is the reason it too is a very popular starting point as a drug lead.

Isatin

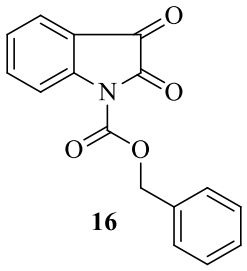
Isatin (**2**, 1-H-indole-2,3-dione)[27] is a naturally occurring product found in a variety of plants such as the genus *Isatis* and found as a metabolic derivative of adrenaline in humans.[28] Isatins are another member of a family of benzo-fused nitrogen heterocycles which exhibit a range of pharmacological properties including both antiprotozoal activities and anticancer activities.[29,30] Previously, isatin derivatives were associated with dye synthesis but recent reviews on isatin have shown that these heterocycles exhibit a variety of beneficial effects towards many pathological and physiological diseases.[27] Due to the fact that various isatin derivatives are known to possess extensive biological activity, the isatin scaffold is also considered a "*privileged scaffold*",[31] and as such can serve as a convenient starting point in the search for new receptor agonists and antagonists.



2

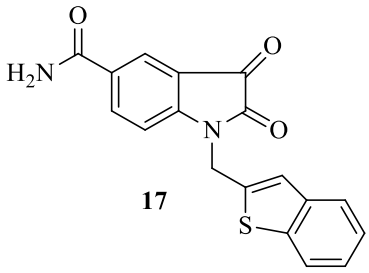
Iyer and co-workers demonstrated that simple N-Boc and N-Cbz-isatin analogues (e.g., **16**) acted as reversible, slow binding inhibitors of serine proteases and exhibited selectivity for α-chymotrypsin over porcine pancreatic elastase.[32] This is important

because serine proteases have been associated with a number of pathological conditions such as neurodegenerative diseases and arthritis and these findings demonstrated that minor structural modifications of the isatin scaffold can lead to potentially useful protease-specific inhibitors.



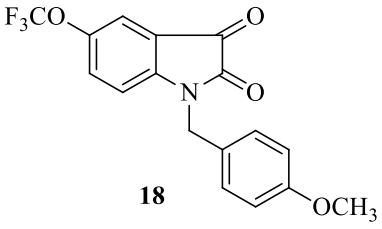
16

Webber and coworkers described an extensive study of isatin derived inhibitors of HRV 3C protease, [33] and developed a new class of active, reversible, non-peptidic inhibitors of this enzyme. The HRV 3C protease is responsible for the common cold, therefore an inhibitor of this protease would allow for treatment of the common cold. The structure activity relationship (SAR) studies showed that molecular recognition of the carboxamide in the P_1 subsite was an important aspect when considering the design of the inhibitor. Also, it was determined that a carbonyl or a carbonyl isostere was required at the C-5 position on the isatin scaffold for the most active HRV 3C protease inhibitors. These findings allowed researchers to develop 1-benzo[b]thiophen-2-ylmethyl-2,3-dioxo-2,3-dihydro-1H-indole-5-carboxylic acid amide (**17**) which was shown to be the most potent inhibitor for this protease.

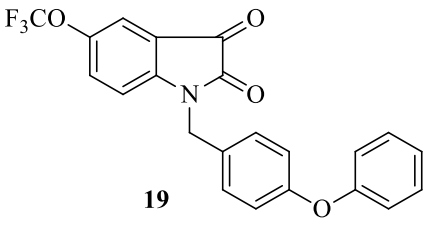


17

Lindsley and coworkers discovered the first positive allosteric modulator (PAM) of muscarinic acetylcholine receptor subtype 5 (M-5).[34] These muscarinic acetylcholine receptor subtypes participate in a variety of functions within many physiological processes. Through the *N*-alkylation of the isatin scaffold, Lindsley was able to create the first M-5 muscarinic acetylcholine receptor ligand in the form of 1-(4-methoxy-benzyl)-5-trifluoromethoxy-1H-indole-2, 3-dione (**18**).[34] SAR studies indicated that isatin derivatives with a methoxybenzyl group at the *N*-position favored M-5 activity and the trifluoromethoxy at the C-5 position favored a dual M-1/M-5 activity. Through optimization of **18**, it was discovered that replacement of the methoxy phenyl moiety with a biphenyl ether moiety (e.g., **19**) increased activity towards M-5 producing an EC_{50} value of 1.9 μM and exhibited decreased activity meaning it was selective against M1-M4. Compound **19** was the most active and selective M-5 PAM to date.[35]



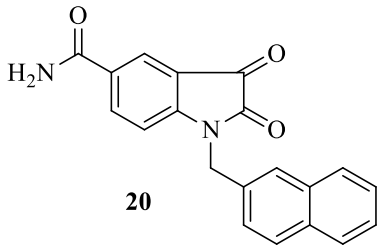
18



19

Through the work of Luhua and coworkers it was shown that isatin derivatives could also be utilized as noncovalent (SARS) coronavirus 3C-like protease inhibitors.

SARS coronavirus (severe acute respiratory syndrome) is a life threatening form of atypical pneumonia.[36] From the SAR studies it was concluded that a large hydrophobic group placed at the *N*-1 position could fit in to a putative hydrophobic pocket leading to high activity. It was also concluded that placing a carboxamide at the C-5 position of the isatin scaffold made the molecule 3-4 times more active compared to the iodic substitution in which iodine was utilized as a hydrophobic, electron affinitive group as opposed to the carboxamide which can form multiple hydrogen bonds. The final conclusion made by the group was that the C-3 oxygen formed a hydrogen bond to the protein which was important for inhibition activity.[36] These conclusions led to the creation of 1-naphthalen-2-ylmethyl-2,3-dioxo-2,3-dihydro-1H-indole-5-carboxylic acid amide (**20**) which was the most potent inhibitor of the SARS Coronavirus.



20

Biological Background

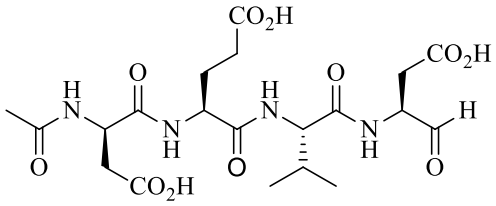
Recently, a new application for the isatin scaffold was discovered in the role of caspase inhibitors. Caspases are *cys*teine *asp*artyl-specific prote*ases*[37] and regulate the highly conserved mechanism of cell removal known as apoptosis (programmed cell death).[38,39] Apoptosis is essential in cell disposal as it serves to maintain homeostasis in multi-cellular organisms. However, dysregulated apoptosis is believed to be involved in

pathological conditions in humans such as cancer, autoimmune disorders and some neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases.[37] There are three groups of caspases; the first group is involved in inflammation and includes caspases-1, -4, -5, and -13; the second group is the initiator caspases which include caspases -6, -8, and -10; and the final group is the excutioner caspases which include caspases 2,-3, and -7.[40]

Caspases are among the most specific of proteases.[37] The nomenclature for protease substrate cleavage is P_n, P_1, P_2, P_3, P_4, P_4', P_3', P_2', P_1', P_n' which assigns amino acid side chains of the peptide substrate and the nomenclature for the corresponding bonding sites in the protease active site is S_n, S_1, S_2, S_3, S_3', S_2', S_1', S_n' subsites.[41] Cleavage occurs at the amide bond between the P_1 and P_1' residues known as the scissile bond.[40] Caspases have an absolute requirement for aspartic acid to be P_1 in the S_1 subsite which is believed to be caused by hydrogen-bonding interactions between aspartic acid and three residues of the caspase (Arg179, Gln283, and Arg341).[40] Substitution of aspartic acid-P_1 with a different amino acid from the S_1 subsite results in >100-fold reduction in catalytic efficiency.[40] Caspases have an equally stringent specificity for at least four amino acids P_4-P_3-P_2-P_1 to the left of the cleavage site and this primary sequence recognition is a necessary requirement for catalysis.[40] Thornberry established that caspases catalyze the hydrolysis of amide bonds through nucleophilic attack by the activated cysteine thiol from the enzyme onto the amide carbonyl bond of the P_1 amino acid to form a tetrahedral intermediate which causes the cleavage of the peptide bond when the double bound is reformed.[40]

Peptide Based Cell Death Inhibitors

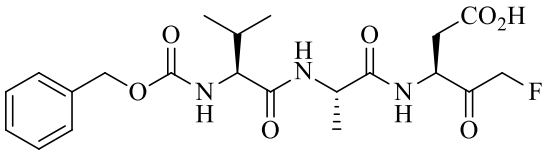
The first class of caspase inhibitors studied was peptide based. A typical peptide based caspase inhibitor is comprised of a tetrapeptide sequence with an electrophilic functionality known as a "warhead". The "warhead" is typically located at the C-terminus of the peptide which may act as a reversible or irreversible moiety. Reversible warheads are groups that can bind to the caspase temporarily through an intermediate and then released, such as aldehydes, ketones or nitriles. Irreversible warheads possess good leaving groups that are nontoxic, such as α-substituted ketones, so as to allow the leaving group to be released once the nucleophilic caspase attacks and becomes attached to the inhibitor.[40] Also, the P_4 amino acid is often capped with a neutral group like a acyl moiety. Numerous peptide based caspase inhibitors have been synthesized and studied for the inhibition of apoptosis. A potent peptide inhibitor that was one of the first to be studied was acetyl-aspartyl-glutamyl-N-(2-carboxy-1-formylethyl)-valinamide (Ac-DEVD-CHO, **21**) because DEVD is the cleavage sequence of poly(ADP-ribose) polymerase (PARP) which is the DNA repair enzyme cleaved at the onset of apoptosis.[42]



21

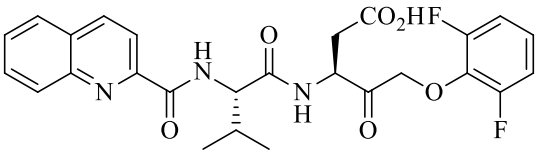
Ac-DEVD-CHO is a potent and effective caspase inhibitor for *in vitro* studies however it was less effective or useful for *in vivo* studies due to the three CO_2H groups that reduced the inhibitor's ability to penetrate cells. Since the effectiveness of Ac-DEVD-CHO as a caspase inhibitor was limited due to the three CO_2H groups, *N*-

benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk, **22**) was designed as a peptide inhibitor likely to be more effective for *in vivo* studies. [43]



22

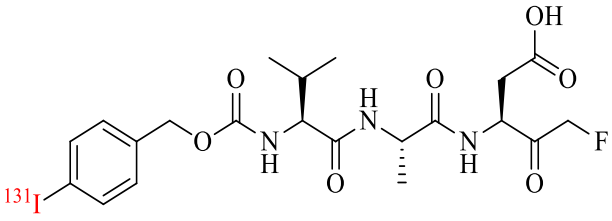
Whereas the caspase inhibitor zVAD-fmk was shown to prevent apoptosis, however it required high doses leading to toxicity because of the conversion of fluoromethylketone into toxic fluoroacetate.[44,45] Also, zVAD-fmk showed poor tissue penetration indicating it too would not be suitable for diseases of the central nervous system due the inability to penetrate the blood brain barrier.[45,46] Brown *et al.* discovered that the caspase inhibitor quinolyl-valyl-O-methylaspartyl-[2,6-difluorophenoxy]-methyl ketone (Q-VD-OPh, **23**) was significantly more effective in preventing apoptosis than any other peptide-based caspase inhibitor.[47]



23

Q-VD-OPh exhibited increased potency, stability, and cell permeability compared to other peptide caspase inhibitors and was non-toxic at high concentrations.[47] Although Q-VD-OPh exhibited significantly more attractive features than previous peptide based caspase inhibitors, it has the disadvantage of being extremely difficult to produce in large quantities.

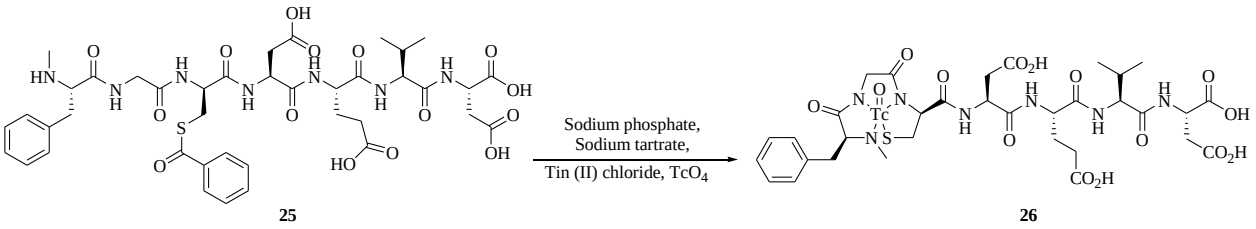
Haberkorn *et al.* introduced the concept that peptide based caspase inhibitors, more specifically z-VAD-fmk, could be useful as an imaging agent of caspases.[48] An electrophilic aromatic substitution reaction was utilized to incorporate a radioactive iodide at the benzyloxycarbonyl protecting group of the z-VAD-fmk creating [^{131}I]-zVAD-fmk (**24**). This compound was screened for its ability to measure apoptosis in induced Morris hepatoma cells in which [^{131}I]z-VAD-fmk was shown to have low cellular uptake and high lipophilicity which caused unspecific binding.[48] This poor cell permeability hindered the intracellular targeting of activated caspases thus preventing *in vivo* application.[48]



24

More recently, Bohn *et al.* aimed to label a peptide recognized by active caspase-3 with a phenylalanine-glycine-cysteine (FGC) moiety that could complex technetium.[49] The DEVD moiety was chosen as the peptide to target caspase-3. Stability and *in vitro* studies were conducted to evaluate the usefulness of the peptide for monitoring the effectiveness of cancer treatment.[49] To perform such a procedure, the (Me)FGC(Bz)DEVD peptide was purchased and effectively labeled using a phosphate buffer, sodium tartrate and tin(II) chloride to yield ^{99m}Tc-(Me)FGCDEVD **26**.[49] Tin(II) reduces the pertechnetate to technetium while the tartrate and the phosphate buffer stabilize the technetium core prior to trans-chelating to the FGC motif. The benzoyl

group in the precursor **25** has to be removed by heating (100 °C, 30 min). During the labeling process, the pH must remain neutral to enable the detection of the labeled compound.



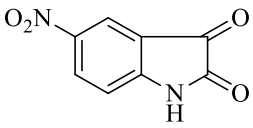
25 **26**

The 99mTc-(Me)FGCDEVD was tested in tumor bearing mice treated with cisplatin which induces apoptosis in tumors. Test subjects were re-evaluated three days after initial treatment in which it was observed that uptake of 99mTc-(Me)FGCDEVD was greater in subjects treated with cisplatin compared to control tumors.[49] Therefore, it is believed 99mTc-(Me)FGCDEVD has the potential to be a new apoptotic tracer for *in vivo* studies in *Single Photon Emission Computed Tomography (SPECT)* but further studies like toxicity and cell permeability need to be conducted.

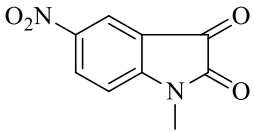
It is unrealistic to pursue the use of peptides as effective caspase inhibitors or imaging agents because of the numerous disadvantages they present such as the fact that they can be metabolized *in vivo.* Also peptides have difficulties crossing the Blood Brain Barrier (BBB) because of their size and the fact that they are mostly polar molecules which is unfortunate since the treatment of neurological disorders require the ability to cross the BBB. Due to the disadvantages presented by peptide based caspase inhibitors, the use of small molecule caspase inhibitors was explored to greater success.

Small Molecule Approach

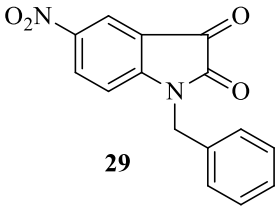
Due to the limitations presented by peptide-based caspase inhibitors, researchers looked for alternative small molecule inhibitors. SmithKline Beecham used a high-throughput screen of their compound library for inhibitors of caspase-3, which resulted in the identification of 5-nitro-isatins **27**, **28**, and **29** which possessed IC_{50} values of 3 μM, 1 μM, and 0.25 μM, respectively.[50]



27

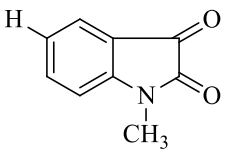


28



29

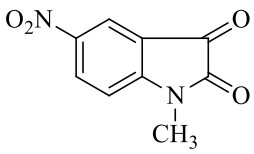
The role of C-5 substituents was then examined to assess the importance of the 5-nitro functionality. A relationship was observed between the electron-withdrawing ability of the 5-substituent (σ_m) and the potency of inhibition of caspase-3. The nitro (**28**), cyano (**31**), methoxycarbonyl (**32**), and iodo (**33**) derivatives led to low micromolar inhibition potencies but the protio (**30**) and carboxylate (**34**) compounds exhibited no activity at 50 μM.[50]



IC_{50} >50 μM

σ_m 0

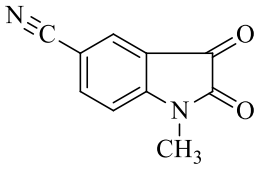
30



IC_{50} 1 μM

σ_m 0.71

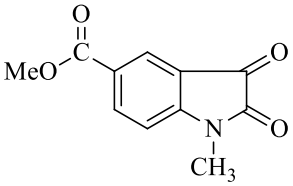
28



IC_{50} 0.6 μM

σ_m 0.56

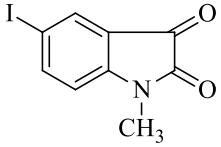
31



IC_{50} 15 μM

σ_m 0.36

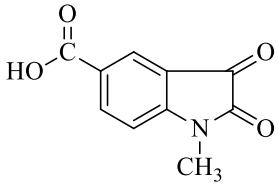
32



IC_{50} 7 μM

σ_m 0.35

33

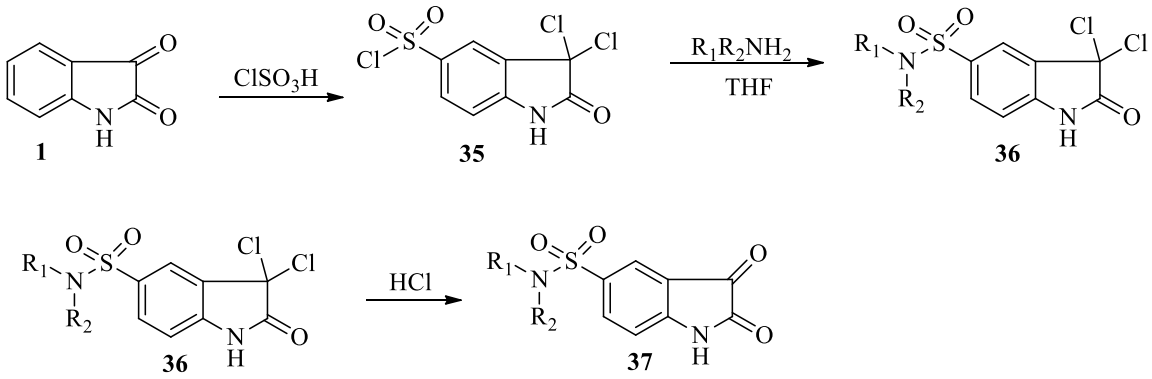


IC_{50} >50 μM

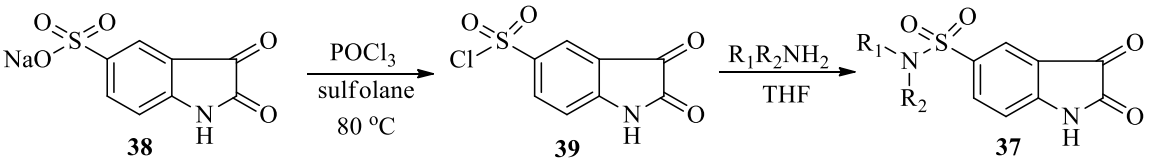
σ_m - 0.1

34

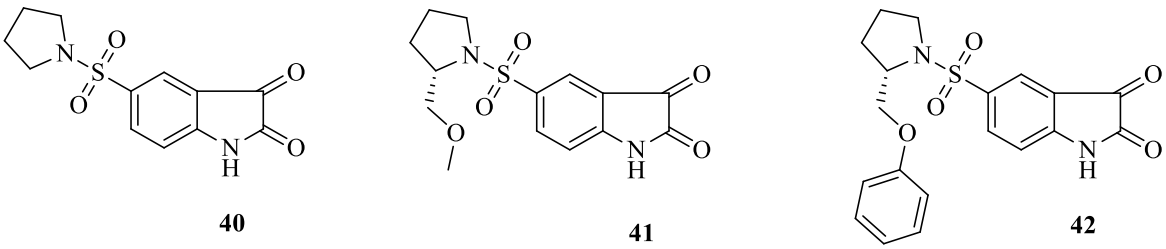
As can be inferred from the values shown above, the electrophilicy of the isatin C-3 carbonyl was deemed critical for activity and this indicated that the mechanism of action involved addition of the catalytic cysteine residue of the enzyme to this functionality.[50] Binding models were developed based on X-ray structures which allowed for the identification of the formation of a tetrahedral intermediate between the catalytic cysteine thiol of caspase-3 and the isatin carbonyl group.[51,52] It was deemed necessary to find a replacement for the 5-nitro group since nitro groups can be subject to metabolic reduction, and also in the interests of identifying a replacement functionality that would allow for the facile incorportaion of molecular diversity.[50] To this end, the class of 5-*N*,*N*-dialkylisatin sulfonamides was developed and the new para 5-*N*,*N*-dialkylisatin sulfonamides were ultimately prepared by condensing secondary amines with 5-chlorosulfonylisatin. However, in contrast to the original report in which isatin **2** was heated at 70°C in chlorosulfonic acid,[53] the Glaxo team only obtained the gem-dichloro derivative **35**. To then attain the desired 5-isatin sulfonamide, the gem-dichloro derivative **35** was reacted with an amine to give **36**, and subsequent hydrolysis of the gem-dichloro species with 3 N HCl afforded the 5-isatin sulfonamides **37**.[50]



An alternative synthesis was later developed by Martinez *et al.* in which 5-chlorosulfonylisatin was prepared by treating sodium 5-isatin sulfonate (**38**) with phosphorus oxychloride in sulfolane at 80 ^{o}C. The resulting 5-chlorosulfonylisatin **39** was then reacted with 1 equiv. of amine in the presence of 1 equiv. diisopropylethylamine in tetrahydrofuran to yield the 5-isatin sulfonamide **37.**[54]

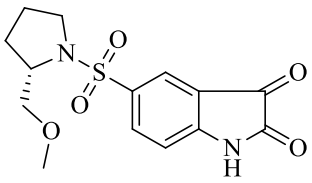


A series of isatin sulfonamides were then synthesized and evaluated for inhibition of caspases which resulted in the identification of isatin sulfonamide **40** which exhibited substantial activity against caspase-3 (K_i = 1.4 μM) and much more selectivity for caspases-1, -3, and -7 relative to the active 5-nitro group of **28**. This led to the preparation of an extensive series of compounds with variation of groups about the sulfonamide functionality and isatin nitrogen ultimately leading to the development of isatin sulfonamides **41** and **42** which exhibited K_i(app) values of 60 and 15 nm inhibition respectively against caspase-3.[50]

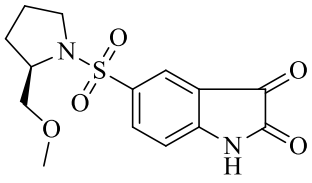


Lee *et al.* examined SAR utilizing pyrrolidine derivatives in which compound **40** was modified by extending groups from the C-2 position of the pyrrolidine ring. This led

to the finding that chirality of the extended functionalities was significant. For example, both *R* and *S* configurations of the methoxymethyl groups for **41** and **43** were examined, wherein it was found that the (*S*)-configuration was found to be significantly more active than that of the *R*-stereochemistry.[50]



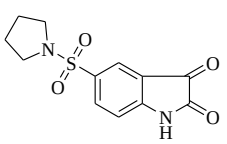
(S)-configuration
IC_{50} 120 nM
41



(R)-configuration
IC_{50} 18,000 nM
43

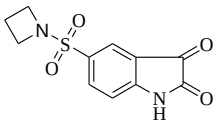
These isatin sulfonamides inhibitors exhibited high selectivity towards caspases-3 and -7 which was possibly caused by the three hydrophobic residues, Tyr^{204}, Trp^{206}, and Phe^{256} that are located in the S_2 pocket which are unique to caspase-3 and -7.[50] The x-ray co-crystal structure also revealed that a tetrahedral intermediate formed between the catalytic cysteine thiolate and the isatin ketone carbonyl group indicating that the S_2 pocket was involved in extensive hydrophobic contacts with the pyrrolidine ring of the inhibitor. This supported the Lee *et al.* theory that the hydrophobic pocket allows for the observed specificity of the isatin sulfonamides, which may be the reason **28** (e.g., N-CH_3) only exhibited moderate selectivity.[55] Previously, it was believed that the S_1 subsite of caspases required a P_1 aspartic acid residue in peptide-based inhibitors for recognition, however, the co-crystal structure revealed that the S_1 subsite in the case of isatins is occupied only by a water molecule.[50] This sulfonamide co-crystal structure model revealed that caspase-3 exhibits minimal interactions with the S_3 and S_4 subsites and selectivity is obtained by way of hydrophobic contacts between the pyrrolidine ring of the inhibitor and residues of the S_2 hydrophobic pocket.[50]

The SmithKline Beecham group had previously evaluated the importance of ring size of the isatin sulfonamides by assessing compounds **40**, **44, 45** and **46**. From this study it was discovered that there was no great effect on activity based on the ring size from five to seven carbons, however the azetidine ring of **44** caused a significant increase in potency compared to that of the analogous pyrrolidine moiety **40.**[50]



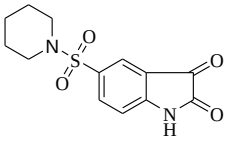
IC_{50} 2,800 nM

40



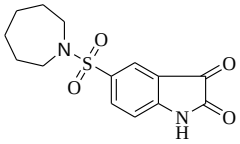
IC_{50} 170 nM

44



IC_{50} 2,200 nM

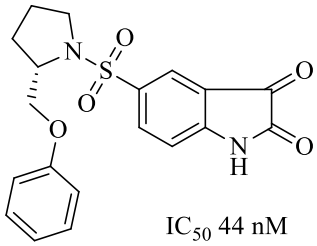
45



IC_{50} 1,900 nM

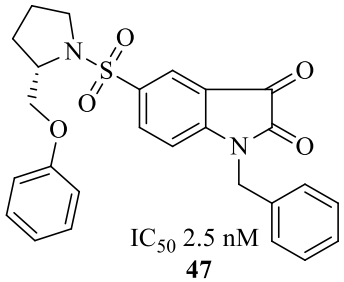
46

However, the four member ring was an impractical choice for a lead since the chiral five membered rings could be derived from either isomer of proline which would allow for the facile incorporation of methyl or benzyl ether substituents with optimal chirality (*vide infra*).[50] For example, the potency of the SmithKline Beecham compounds was further increased by adding a hydrophobic moiety such as a phenoxy group (e.g., **42**) or a methoxymethyl group attached to the pyrrolidine ring of **41** compared to those compounds without a substituent such as **40** and **44.** The potency was further increased by *N*-alkylating the isatin sulfonamide compounds wherein it was observed that *N*-alkylation caused an increased activity for **47** over the N-H version **42.**[50]



IC_{50} 44 nM

42

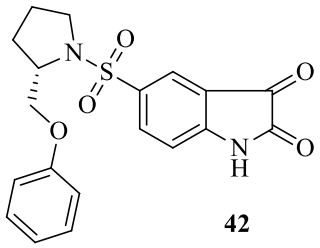


IC_{50} 2.5 nM

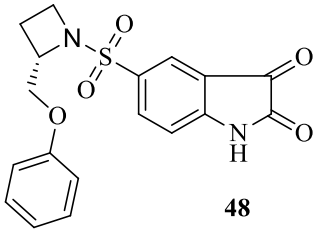
47

Isatin Sulfonamides: Developments of the Washington University Group

Upon the initial discovery of the casapase inhibitory properties of isatin sulfonamides by SmithKline Beecham, the Mach group from Washington University School of Medicine further expanded the SAR of this scaffold **47**.[56] Salient structural changes included substituting the *para*-position of the *N*-benzyl group, replacing the pyrrolidine ring with a chiral C-2 substituted azetidine ring, and replacing the benzene ring of the pendant phenoxymethyl moiety with a pyridine ring. As previously discovered by SmithKline Beecham, *N*-alkylation of the isatin nitrogen of **42** with a benzyl group **47** resulted in a 10 to 20 fold increase in potency for inhibiting caspase-3, which led Mach to look at various *N*-alkylated derivatives.[50] It was found that incorporation of the azetidine ring on the isatin sulfonamide **44** caused an increase in activity over the corresponding 5-membered congener. Mach found also that replacement of pyrrolidine analogue with an analogous C-2 substituted azetidine analogue (e.g., **48**) unexpectedly resulted in little difference in potency for caspase-3, a finding which was later explained by molecular modeling studies which revealed a high degree of overlap in binding of the azetidine and pyrrolidine analogues to activated caspase-3.[56]

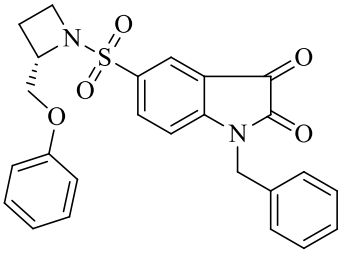


42

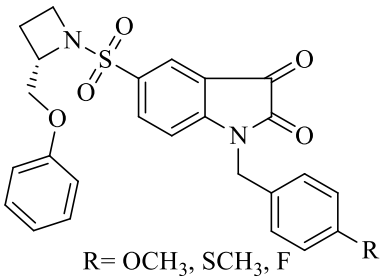


48

However, *N*-alkylation of the azetidine compounds possessing an O-benzyl/ substituted benzyl, or a pyridylmethyl group (e.g., **49-51**, respectively) resulted in a 10 to 50 fold increase in potency against caspase-3.

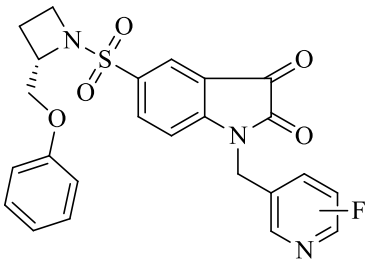


49



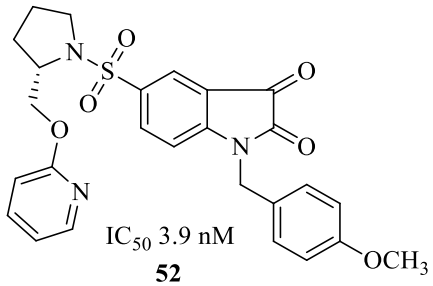
R= OCH_3, SCH_3, F

50



51

Optimal caspase-3 inhibition (IC_{50} value of 3.9 nM) was achieved by replacement of the benzene ring of the 2-(phenoxymethyl)pyrrolidine moiety with a pyridine ring along with introducing a *para*-methoxy substituent on the *N*-benzyl substituent as in **52**.[56]



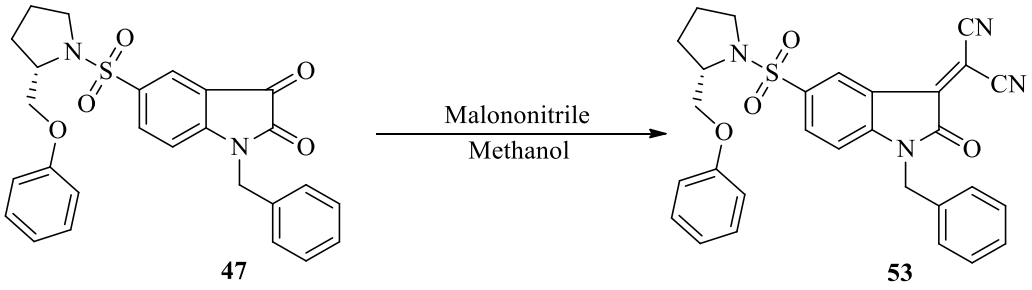
IC_{50} 3.9 nM

52

The increase in potency of the pyridine analogues seems to be associated with a possible hydrophilic interaction between the pyridinylmethyl moiety and the S_3 binding domain of caspase-3. All isatin sulfonamide caspase-3 inhibitors were found to be involved in a $\pi - \pi$ interaction with Phe381, however, the pyridines appeared to be perfectly oriented to involve a hydrogen bond between the pyridine nitrogen and the hydroxyl group of Ser381.[56] Overall, the substitution of the pyridine ring for the benzene

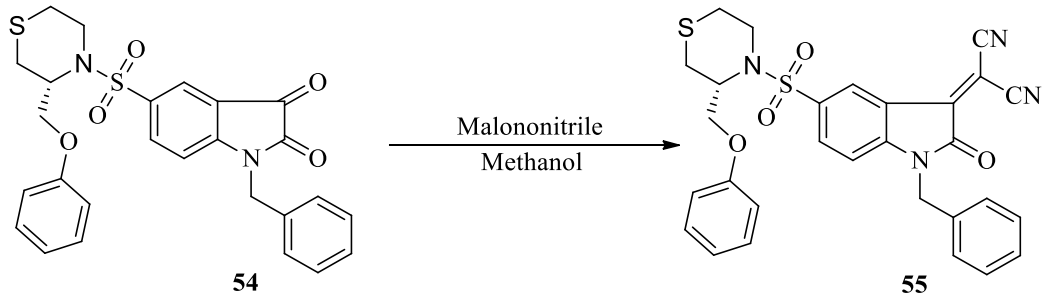
ring in the phenoxymethyl moiety resulted in a dramatic reduction in the overall lipophilicity of the isatin analogues further increasing the effectiveness.[56]

Mach *et al.* also developed an alternate series of isatin sulfonamide substrates called "isatin Michael acceptors" (IMA)[57] that incorporate a Michael acceptor group at the C-3 position of this scaffold. Such Michael acceptor inhibitors would allow for an alternate mechanistic mode of attack by the cysteine thiol nucleophile upon the α,β-unsaturated carbonyl compound in an irreversible manner. One such representative of this alternate class of inhibitor was prepared by reaction of the isatin sulfonamide **47** with malononitrile in methanol to give **53.**



47 **53**

This IMA analog **53** showed an increased potency of roughly 10-fold for caspase-6 when compared to similar isatin precursors but still retained high selectivity for both caspases-3 and -7 as well.[57,58] Replacement of the phenoxymethylpyrrolidine ring in **47** with a thiomorpholine ring followed by reaction of isatin sulfonamide **54** with malononitrile led to the creation of the IMA analogue **55** which exhibited significantly increased caspase-6 selectivity and reduced selectivity for caspase-3.[58] Although such isatin-based IMA derivatives represent the most potent (high nano) nonpeptidic caspase-6 inhibitors, such molecules also display only moderate selectivity for this caspase.



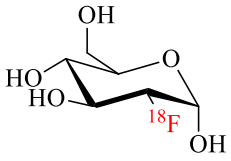
54
Caspase-3: 1028 + 259 nM
Caspase 6: 12833 + 1258 nM

55
Caspase-3: 1454 + 170 nM
Caspase-6: 151 + 2 nM

Small Molecule Imaging Agents for Cancer

To date, imaging of cancer by Positron Emission Tomography (PET) has been mainly limited to the use of [^{18}F]-fluorodeoxyglucose (FDG)[59-61]or ^{99m}Tc-annexin-V,[62-64] although both methods are beset with significant limitations (*vide infra*). Since 2006, several groups have been developing radiotracers based on the *isatin* sulfonamide class of caspase inhibitors and designed to assess the induction of apoptosis in tumors as a response to chemotherapy.

The mainstay of PET imaging of cancer remains [^{18}F]-FDG **56** which is based on a change in the uptake of glucose in many tumor types.[59]



56

[^{18}F]-FDG has many limitations including: an inability to detect small tumor volumes; the fact that it is not useful with less glycolytic tumors, and; the need to perform baseline scans. Moreover, glycolysis is also associated with inflammation and tissue repair in response to damage which could lead to misinterpreted diagnosis. Recent strategies have begun to move away from monitoring the differences in metabolism

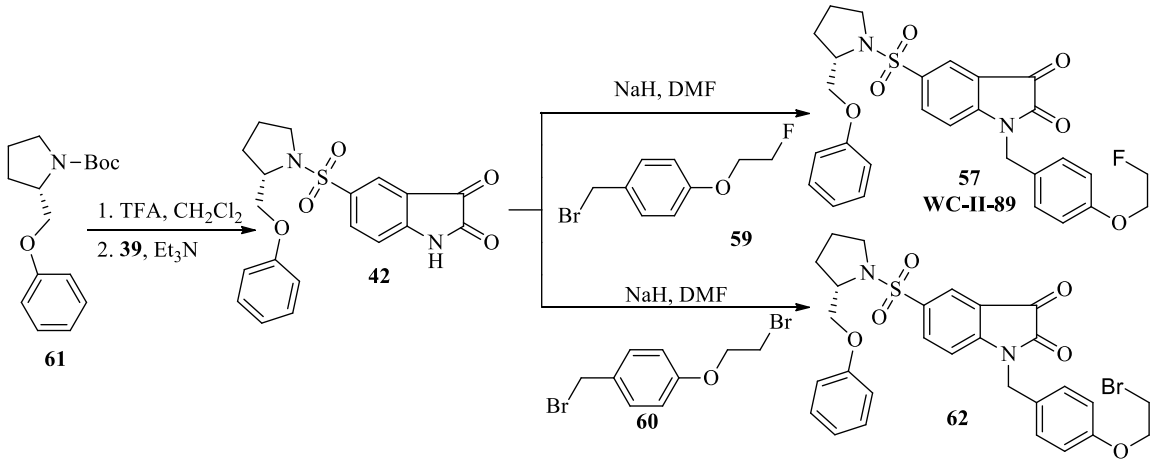
between cancerous and normal cells and have begun to look for specific biomarkers associated with cell death or the lack thereof.[65] Cell death exhibits two types of biomarkers, intracellular and extracellular. Intracellular biomarkers include effector caspase activation, mitochondrial membrane potential, cytosolic proteins, and exposed DNA, while extracellular biomarkers are associated with plasma membrane phospholipids, histones, and plasma membrane depolarization.[65] Since the ability to evade apoptosis is recognized as one of the hallmarks of cancer, the presence of caspases in tumor cells after treatment can be indicative of a positive response to therapy.[66] The capability to noninvasively monitor the ability of a drug to induce or halt apoptosis immediately after treatment would be of tremendous value to the research and clinical community.

Initial strategies for the PET imaging of biomarkers associated with apoptosis focused on ^{99m}Tc-Annexin-V, a 36-kDa protein which binds selectively and with high affinity to externalized phosphatidylserine (PS) residues. Phosphatidylserine is normally situated at the interior of the cell membrane but translocates to the exterior in the early stages of apoptosis after activation of caspase-3.[66] As opposed to FDG which takes days to weeks for confirmation of an effective treatment, ^{99m}Tc-Annexin-V bound to externalized PS gives immediate confirmation. However, since the externalization of PS also occurs in necrosis, this method is incapable of distinguishing between these two modes of cell death. Additionally, the slow clearance of radiolabeled Annexin-V from non-targeted tissues requires imaging studies be conducted 4-6 h after administration which is not compatible with the short-lived lifetimes of the radionuclides used in PET.[66]

Recently, Wang *et al*. were able to develop a ^{18}F- labeled Annexin B1 specifically designed for *in vivo* PET imaging. Like ^{99m}Tc-Annexin-V, [^{18}F]-Annexin B1 is a 38 kDa peptide which also binds selectively and with high affinity to externalized PS.[67] Interestingly, ^{18}F-AnxB1 had great stability in vitro and *in vivo* without significant degradation which prompted *in vivo* biodistribution and apoptosis-targeting studies with PET/CT imaging. To study biodistribution, normal rats were injected with ^{18}F-AnxB1 and imaged by PET/CT at different time points after probe administration to visualize the whole-body biodistribution, organ uptake and tissue clearance.[67] A series of PET/CT images demonstrated that ^{18}F-AnxB1 concentration was the highest in the liver and kidney, cleared through renal excretion system, and accumulated finally into the bladder. As opposed to ^{99m}Tc-Annexin-V which required 4-6 h to clear main organs, ^{18}F-AnxB1 exhibited sufficiently rapid clearance from main organs so that only modest activity remained after 2 h. Finally, ^{18}F-AnxB1 was then used to detect cyclophosphamide (CTX) induced-apoptosis in tumor bearing rats using PET/CT. It was discovered that tumors treated with CTX could be observed by PET imaging with the best contrast at 2 h after treatment with ^{18}F-AnxB1 and tumors without CTX treatment presented low contrast due to lack of apoptotic regions indicating a noninvasive apoptosis detection imaging agent was created.[67]

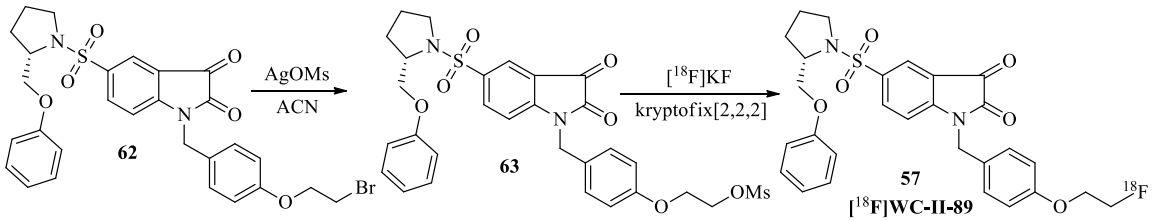
The recognition that isatin sulfonamides are capable of acting as potent and selective inhibitors of caspase-3 has led to the development of radiolabeled analogs based upon this scaffold for imaging cancer. Mach *et al*. from Washington University Medical School developed the isatin sulfonamide analog **WC-II-89** (**57**) which was suitable for radiolabeling with fluorine-18.[68] **WC-II-89** was synthesized from 5-chloro

isatinsulfonamide through a procedure in which methyl 4-hydroxybenzoate underwent *O*-alkylation with sodium hydride in THF at 0^{o}C followed by the addition of 1-bromo-2-fluoroethane to produce (4-(2-fluoro-ethoxy)benzoic acid methyl ester.[68] This ester was then reduced with $LiAlH_4$ in ether to create the alcohol [4-(2-fluoro-ethoxy)-phenyl]-methanol, which was then converted to a bromo analog **59** through treatment with tetrabromomethane and triphenylphosphine. 1-(2-Bromoethoxy)-4-(bromomethyl)benzene (**60**) was obtained by benzylic bromination of 1-bromoethoxy-4-methyl-benzene with NBS in CCl_4. With these benzylic bromides in hand, the *N*-Boc pyrrolidine **61** was with TFA and the resulting secondary amine was coupled with 5-chlorosulfonylisatin (**39**) in THF using triethylamine as an acid scavenger to produce 5-(2-phenoxymethyl-pyrrolidine-sulfonyl)-1*H*-2,3-dione, **42**. Compound **42** was then *N*-alkylated at the isatin nitrogen through treatment with sodium hydride in DMF at 0 ^{o}C followed by addition of **59** or **60** to give compound **WC-II-89** or **62** which is the precursor utilized to generate the [^{18}F]-radiolabeled compound **58**.[68]



To afford the radiolabeled version of W**C-II-89**, compound **62** was heated to reflux with silver methanesulfonate in acetonitrile to generate the precursor **63**.

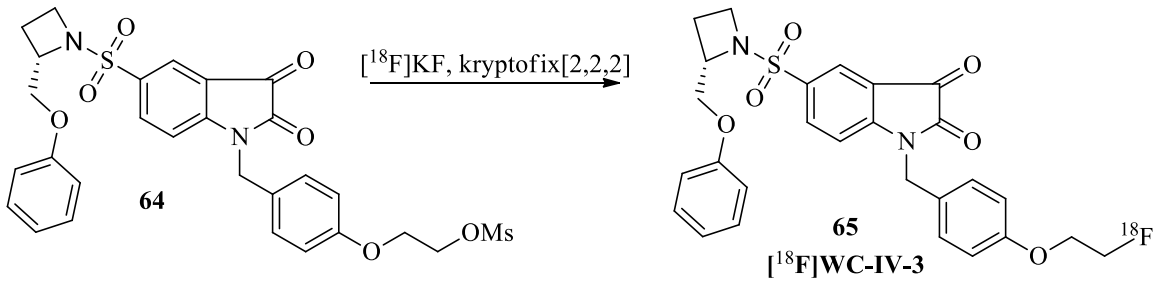
[^{18}F]WC-II-89 was then generated starting from **63** using a nucleophilic displacement of the mesylate group with [^{18}F]-fluoride ion using the radiochemical method developed by Yoo *et al.*[69]

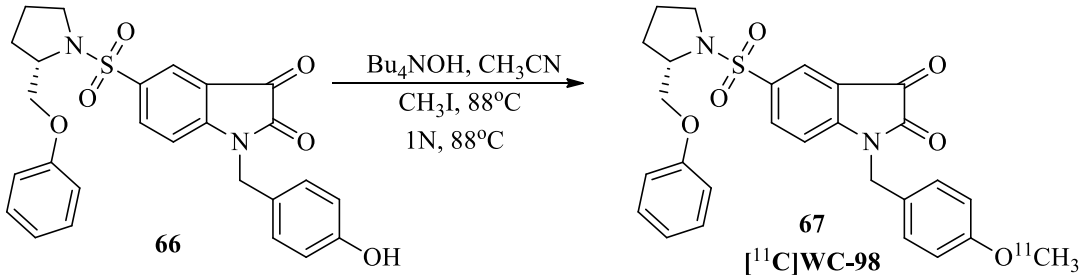


[^{18}F]WC-II-89 was evaluated as a radiotracer for imaging caspase-3 activation through the use of an animal model that utilizes the protein synthesis inhibitor cycloheximide (CHX) which induces apoptosis in rat liver in both a dose- and time-dependent manner.[68] Biodistribution studies of **[^{18}F]WC-II-89** were conducted in normal and CHX treated male rats wherein it was discovered that initial uptake of **[^{18}F]WC-II-89** was higher for CHX-treated rats than control rats. However, this difference between control and treated rats was reduced with time except for in the liver and spleen.[68] Isatin sulfonamides are competitive inhibitors of caspase-3, meaning **[^{18}F]WC-II-89** binds to the activated form of caspase-3 in tissues undergoing apoptosis, which is why the liver and spleen of CHX treated rats exhibited slower washout of radioactivity. Another promising observation from the biodistribution studies was that there was a very low uptake of radioactivity in the bones, indicating that defluorination was not a concern.[68] Researchers then performed Western Blot testing to correlate caspase-3 activity to the biodistribution results. This revealed that the level of cleaved caspase-3 in the spleen and liver of the treated rats was much higher than in the control animals indicating the imaging agent could indeed be used for imaging caspase-cleaved apoptosis. Following the promising results from the biodistribution studies and Western blot testing, Mach *et al*

performed microPET images of a rat liver at 10-60 min post-iv injection of **[^{18}F]WC-II-89**. The microPET images revealed that a rat receiving 3 h pre-treatment of CHX displayed a higher uptake of **[^{18}F]WC-II-89** in the liver versus the control rat. The increased accumulation of **[^{18}F]WC-II-89** in the treated rat liver versus the control rat is consistent with drug-induced caspase-3 activation. A two-fold increase in uptake of **[^{18}F]WC-II-89** was observed in the treated rats compared to control rats and the normal rat liver displayed a faster washout of radioactivity. Through these results, Mach *et al* were able to demonstrate that apoptosis could be measured and imaged by PET using ^{18}F-labeled caspase-3 inhibitors in the form of **[^{18}F]WC-II-89**.

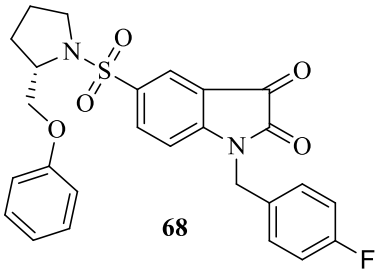
Given the success of **[^{18}F]WC-II-89** as an ^{18}F-labeled caspase-3 targeting agent for PET imaging, the Washington University group set out to compare the effectiveness of two additional radiolabeled isatin analogs. An azetidine analog (**[^{18}F]WC-IV-3**, **65**) and a pyrrolidine analog (**[^{11}C]WC-98, 67)** were synthesized and compared to the successful **[^{18}F]WC-II-89** analog. The **[^{18}F]WC-IV-3** was synthesized from a mesylate precursor **64** through a nucleophilic substitution using [^{18}F]fluoride/Kryptofix 2.2.2 complex. Alternatively, the **[^{11}C]WC-98** was synthesized through an *O*-methylation of a precursor **66** using [^{11}C] CH_3I.[70]



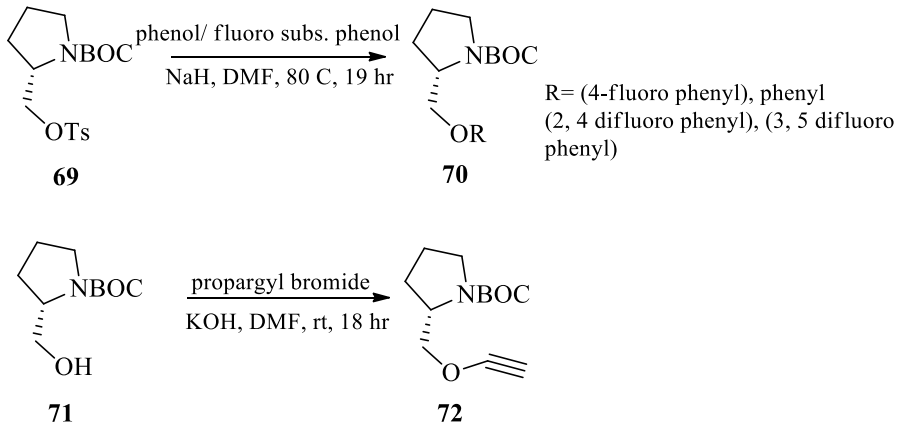


After completing the synthesis of both **[^{11}C]WC-98** and **[^{18}F]WC-IV-3**, biodistribution studies were conducted to determine the similarity to the successful **[^{18}F]WC-II-89** analog. These studies revealed that in the control animals both **[^{11}C]WC-98** and **[^{18}F]WC-IV-3** analogs behaved similarly to the **[^{18}F]WC-II-89** analog, with rapid clearance from blood and normal excretion. Both **[^{11}C]WC-98** and **[^{18}F]WC-IV-3** uptake levels in the spleen of CHX treated rats versus control was not as high as those seen in the **[^{18}F]WC-II-89** analog indicating that **[^{11}C]WC-98** and **[^{18}F]WC-IV-3** may not discriminate between varying caspase-3 levels *in vivo* as well as **[^{18}F]WC-II-89**.[70]

Concurrent with these studies, Aboagye *et al.* from Imperial College further expanded on the idea of employing isatin sulfonamides as ^{18}F-labeled caspase-3 targeting agents for PET imaging of apoptosis by developing a [^{18}F]-labeled isatin with improved metabolic profile, reduced lipophilicity, and subnanomolar affinity for caspase-3.[71] In this case, the previously synthesized 1-(4-fluoro-benzyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole-2,3-dione (**68**) was chosen as the lead compound and modifications were made to the left side ether moiety and at the *N*-1 position.

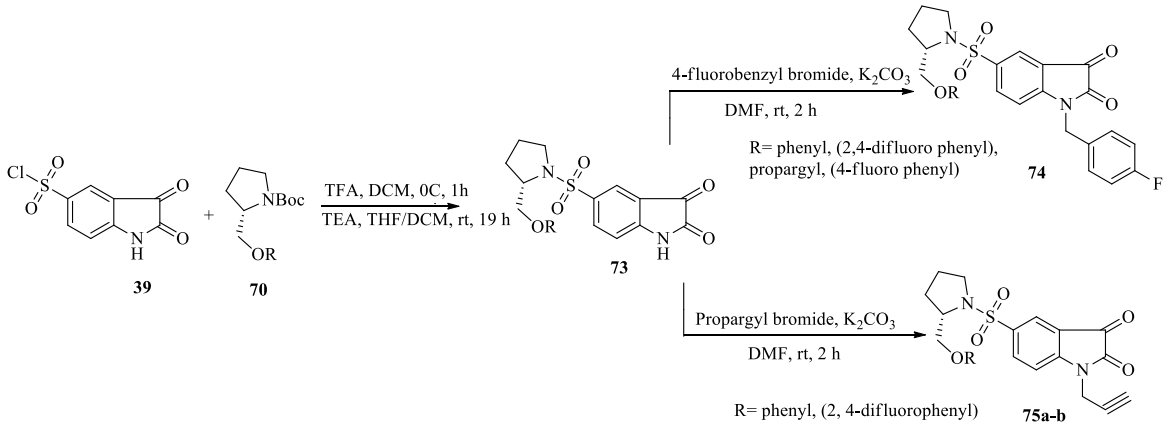


The goal was to improve the biological stability of **68** while retaining selectivity and affinity for caspase-3.[71] This was achieved by incorporating fluorine groups into the left side phenyl ether group which was thought important because it was believed that the major metabolic pathway for degradation of the isatins in the body involves aromatic hydroxylation. Since the introduction of fluorine to aromatic groups is known to block P450-catalyzed ring hydroxylation of the substituted carbon and also reduces metabolic attack on neighboring nonsubstituted carbons by exerting a strong electron withdrawing effect, it was believed that the 2,4-difluorophenyl ether would be significantly more stable *in vivo* compared to unsubstituted phenyl ethers.[71] Furthermore, the tolerance of different heterocycles on the left side ether moiety was investigated in which the main focus was the tolerance to 1,2,3-triazoles since these groups are inert to metabolic degradation[72] and can be easily labeled with fluorine-18.[73] The target compounds were synthesized by condensation of functionalized pyrrolidines with 5-chlorosulfonylisatin and subsequent alkylation of the isatin nitrogen using potassium carbonate and DMF. Reaction of commercially available phenols as well as 4-hydroxytetrahydropyran with tosylate **69** provided the pyrrolidines **70**, while *O*-alkylation of **71** with propargyl bromide afforded 1-methyl-2-prop-2-ynyloxymethyl-pyrrolidine (72).[71]

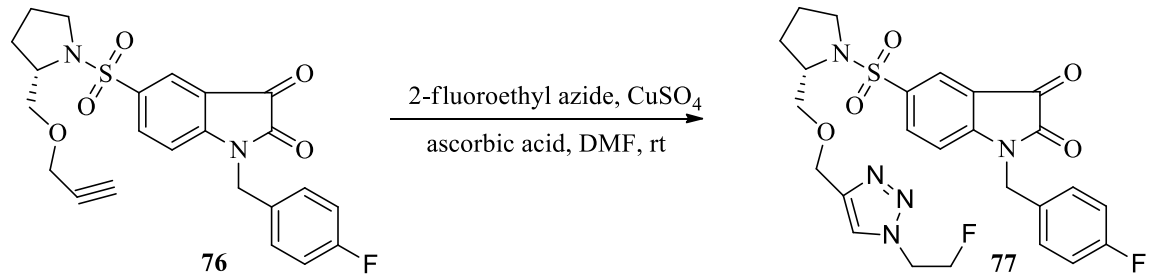


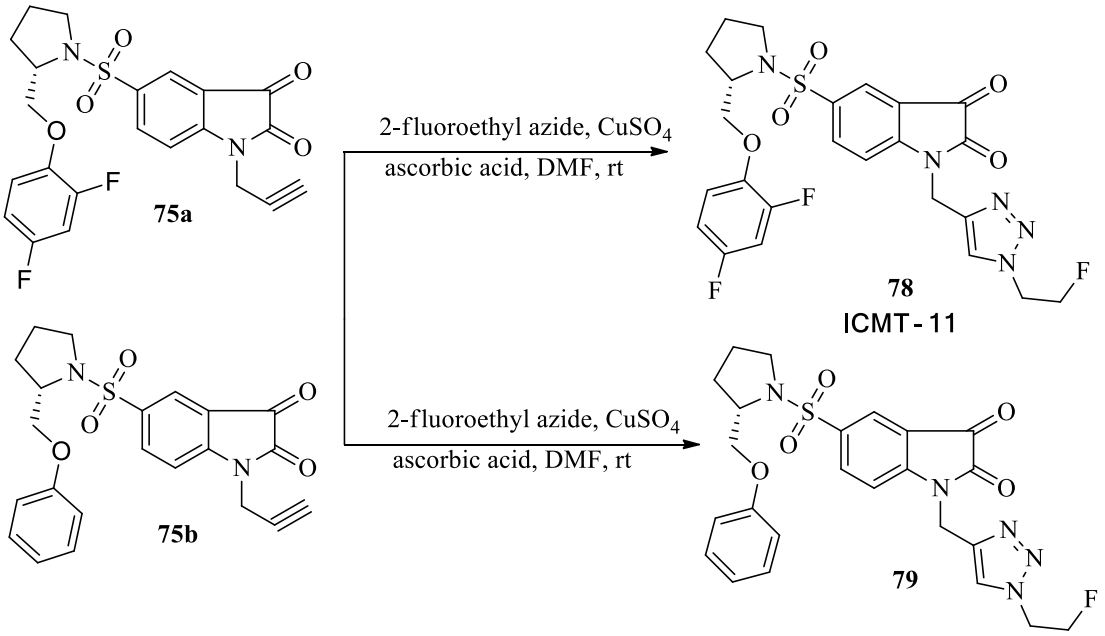
69 **70** **71** **72**

Deprotection of the *N*-Boc protected pyrrolidines with trifluoroacetic acid followed by conjugation with 5-chlorosulfonylisatin (**39**) provided the sulfonamides **73**. Further *N*-alkylation of 4-fluorobenzyl bromide or propargyl bromide with potassium carbonate in DMF yielded the target compounds **74** and **75.**[71]

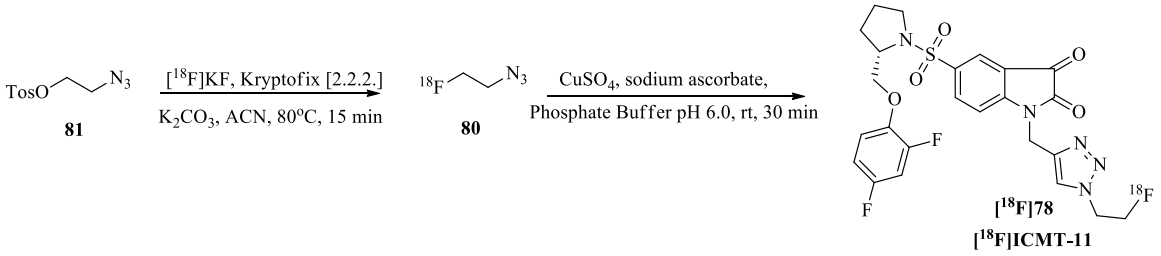


The three desired triazoles (e.g., **77**, **78**, **79**) were prepared by the copper catalyzed cycloaddition of 2-fluoroethylazide with the alkyne precursors **75a-b** and **76**. However, the isatin scaffold was found to decompose on heating at 90 ^{o}C in the presence of the copper sulfate and ascorbic acid resulting in poor yields of the three target triazoles. This problem was partially resolved by increasing the copper sulfate concentration, utilizing ambient temperatures, and reducing the reaction time from 2 h to 1 h producing the triazoles in higher yields.[71]



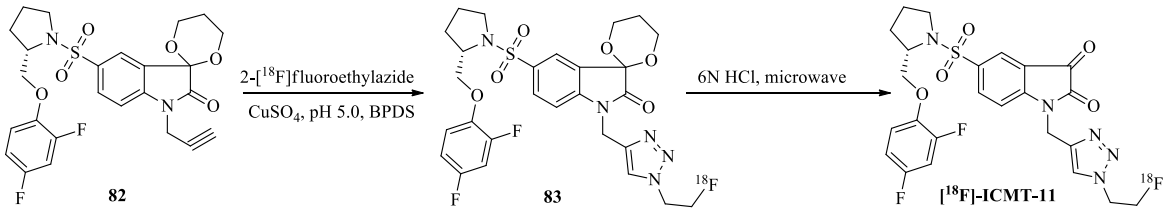


Likewise, the ^{18}F version of the triazole **78** was prepared by copper catalyzed cycloaddition of 2-[^{18}F]fluoroethylazide (**80**) with the alkyne precursor **75a**. In turn, 2-[^{18}F]fluoroethylazide (**80**) was prepared by reaction of [^{18}F]fluoride with the corresponding tosylate precursor **81.**

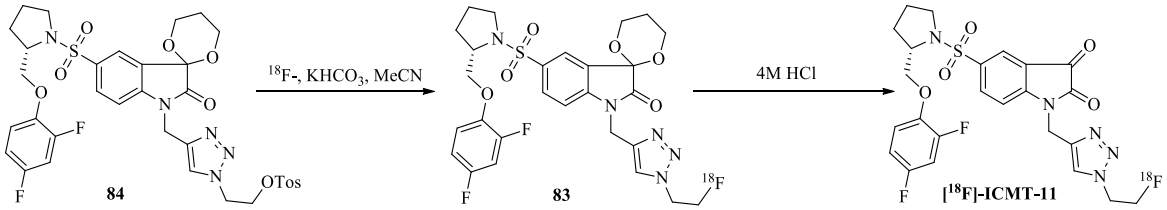


This initial click chemistry strategy to synthesize **[^{18}F]ICMT-11** avoided the prior protection of the reactive dicarbonyl function, however, this process gave rise to a stable by-product which could not be removed from the ^{18}F tracer. Due to the potential complications with caspase-3 binding, an optimization of the radiochemistry protocol was explored to remedy the issue. Aboagye *et al.* improved the existing protocol by introducing bathophenanthroline disulfonic acid disodium ligand (BPDS) as an additive

to stabilize the Cu(I) catalyst.[74] It was discovered that BPDS reduced the amount of required alkyne precursor and shortened the reaction time. In this new synthesis, an acetal protected isatin alkyne precursor **82** was reacted with 2-[^{18}F]fluoroethylazide, $CuSO_4$/Na-ascorbate, and BPDS for 30 min at room temperature. The resulting intermediate **83** was then deprotected by microwave heating with HCl or conventional heating with sulfuric acid to produce **[^{18}F]ICMT-11.**[74]



In the automated radiosynthesis protocol, Aboagye *et al.* used a pre-formed triazole ring containing tosylate precursor **84** which underwent nucleophilic displacement with ^{18}fluoride to yield **83**. Ketal hydrolysis then afforded **[^{18}F]ICMT-11** that displayed high yield in a shorter time with very high specific activity.[75]



To determine if the modifications made to the left side ether moiety were effective against caspase inhibition, Aboagye *et al.* utilized fluorimetic *in vitro* caspase inhibition assays which revealed that the fluorine substituents on the phenyl ether were well tolerated and the affinity for caspase-3 increased 2-fold as compared to the nonfluorinated phenyl ether. The high potency of the fluorinated analogs was

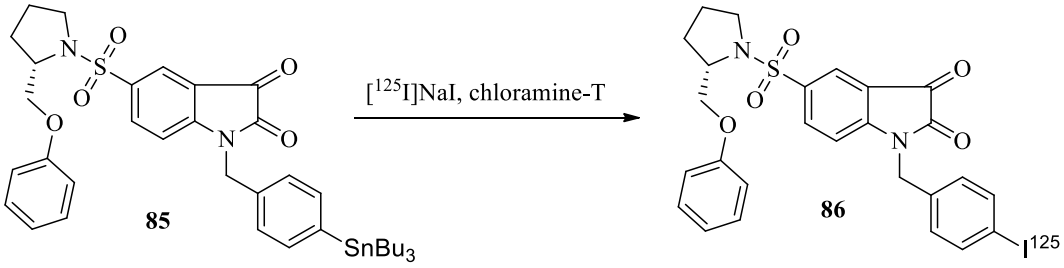
hypothesized to be attributed to π-stacking or hydrogen bonding with other groups in the S_3 domain such as Ser381. The most important observation collected from the enzyme assays was the knowledge that a 2'-fluoroethyl-1,2,3-triazole on either side of the molecule led to a sharp increase in potency towards caspase-3. Furthermore, the triazole **ICMT-11** was by far the most potent inhibitor of caspase-3 and -7 with affinities of 0.5 nM and 2.5 nM, respectively which was greatly improved compared to **68** which possessed affinities for caspase-3 and -7 of 50.5 nM and 19.8 nM, respectively. Given the high potency towards caspase-3 and -7 inhibition of the difluorinated phenyl ether analogues, the next logical step was to perform biodistribution studies on the analogues to ensure metabolic stability and toxicity The biodistribution studies were performed using the radiotracer **[^{18}F]ICMT-11** which was shown to rapidly distribute to tissues and was rapidly eliminated with high localization of radioactivity in the kidney, urine and liver indicating the importance of the renal and hepatic routes for elimination. A key observation obtained from the biodistribution study was the lack of uptake in the bones which suggested an absence of *in vivo* defluorination and hence metabolic stability of the 2'-fluoroethyl-1,2,3-triazole moiety. Tumor uptake studies were conducted with **[^{18}F]ICMT-11** in control and cisplatin (CDDP) treated RIF-1 tumor bearing mice in which the CDDP treated tumor exhibited 2.9 fold increase in **[^{18}F] ICMT-11** derived radioactivity compared to that of the control tumor.[75,76]

After enzyme and distribution studies demonstrated that **[^{18}F]ICMT-11** would have potential utility for imaging caspase-dependent cell death, Aboagye *et al.* performed a study to validate the ability of **[^{18}F]ICMT-11** to non-invasively image drug induced tumor apoptotic processes *in vivo* and its potential for the early detection and monitoring

of a response to anticancer therapy in an experimental apoptosis tumor model. The **[^{18}F]ICMT-11** PET imaging was performed 24 h after the treatment of 38C13 xenograft-bearing mice with cyclophosphamide (CPA) and the tumor was then excised for tracer biodistribution. Overall, the distribution was consistent with the normal tissue biodistribution of **[^{18}F]ICMT-11** in which there was rapid distribution of the radiotracer to tissues together with rapid elimination through hepatic and renal routes. PET images of the CPA-treated mice showed an increase tumor uptake of **[^{18}F]ICMT-11** in which a 1.5-fold increase of tumor uptake was observed in CPA treated mice compared to control mice. Thus, Aboagye *et al.* was able to develop the first caspase-3/7 specific PET tracer for tumor apoptosis imaging. This [^{18}F]-labeled isatin sulfonamide, has desirable attributes for PET imaging of apoptosis which include high affinity for active caspase-3, high metabolic stability, reduced lipophilicity and ease of radiosynthesis which is why it has been selected for clinical development.[75,76]

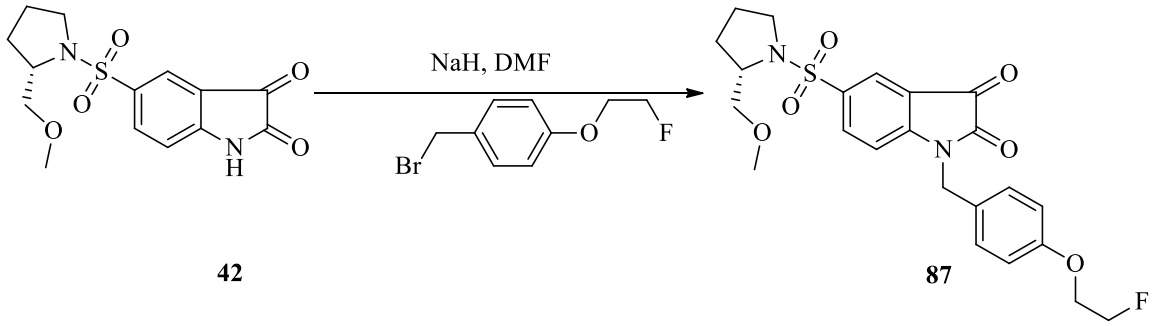
During the time Mach *et al.* was developing **WC-II-89**, Kopka *et al*.[77] from Germany were developing a series of 5-pyrrolidinylsufonyl isatins as caspase binding radioligands (CBRs) potentially capable of directly targeting apoptosis *in vivo.* The caspase inhibitor (*S)*-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**41**) was chosen as the lead structure for the development of CBRs. A series of 2-methoxymethyl-pyrrolidinyl analogues were *N*-alkylated with NaH in DMF to yield *N*-1-substituted isatins which were evaluated to determine caspase inhibition potency for caspase-3. A radioiodinated CBR **86** was synthesized by *N*-alkylation of 5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl) isatin with *p*-tributlylstannylbenzyl mesylate to obtain the

tributylstannylated precursor **85** which was then placed through a iododemetalation reaction using [^{125}I]NaI in the presence of chloroamine-T hydrate used as an oxidant.[77]



Kopka *et al*. first evaluated the binding potencies of a nonradioactive reference compound of **86** as well as various modified 5-pyrrolidinylsulfonyl isatin analogs for the ability to inhibit caspase-3/7. In accordance with the Washington University group, it was found that all *N*-alkylated analogs display similar or even better caspase-3 binding potencies compared with **38**, thus confirming that modification of the isatins at the *N*-position increases binding potency. However, Kopka *et al*. discovered that attaching bulky *N*-benzyl as well as *p*-substituted *N*-benzyl residues does not influence the binding potency of the isatin analogs in vitro.[77] Since the groups *para* on the *N*-benzyl moiety do not affect binding, he chose to attach the radiolabel at that site. The 1-(4-iodo-benzyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)isatin reference compound was found to be less effective at inhibiting caspase-3, thus indicating that the radioactive version **86** would also likely be less effective. The (*S*)-1-(4-(2-fluoroethoxy)benzyl)-5[1-(2-methoxy-methylpyrrolidinyl)sulfonyl]isatin (**87**) which was synthesized through the *N*-alkylation of 5-pyrrolidinylsulfonyl isatin **42** with 1-bromomethyl-4-(2-fluoro-ethoxy)-benzene using sodium hydride (NaH) and DMF, exhibited moderate lipophilicity and was selected for kinetic studies to determine the mechanism of inhibition for caspase-3 activity. This

study revealed **87** displays competitive inhibition which was consistent with previous studies which demonstrated that the isatins bind to the cysteinyl active site of the activated caspase.[55]

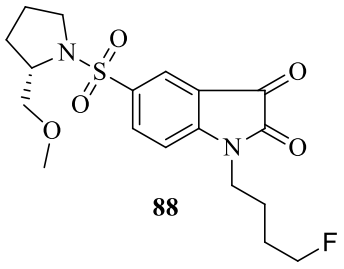


42 **87**

The modified 5-pyrrolidinylsulfonyl isatin analogs were then evaluated in cellular apoptosis assays in which the potency of inhibiting apoptosis was measured. These studies were performed on human umbilical vein endothelial cells (HUVEC) undergoing apoptosis which were incubated with different concentrations of isatin analogs for 8 h. Western blot analysis revealed that caspase processing was inhibited by the 2-methoxymethyl-substituted 5-pyrrolidinylsulfonyl isatins at concentration of 10 μM while the 2-phenoxyphenyl analogs were less effective in inhibiting caspase-3 processing. This indicated that future work with the 5-pyrrolidinylsulfonyl isatins should focus on the use of the 2-methoxymethyl analogs.[77]

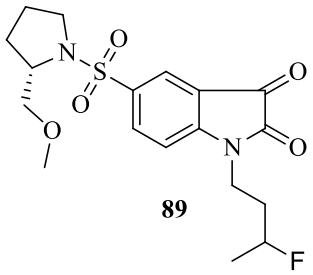
Kopka *et al*. then focused on the synthesis of new fluorinated and non-fluorinated 5-pyrrolidinylsulfonyl isatins as caspase inhibitors because it was believed that introduction of a fluorine substituent would enhance the potency of **42**.[78] The fluorine analogs were incorporated through *N*-alkyl chains on the isatin nitrogen of the 2-methoxymethyl-5-pyrrolidinylsulfonyl isatins. These new pyrrolidinylsulfonyl isatin analogs were evaluated for their ability to inhibit caspase-3 and -7. This study revealed

that the fluoroethyl and fluoropropyl isatins exhibited very weak inhibitory activity toward caspase-3/-7 while butyl derivatives were the most potent caspase -3 and -7 inhibitors. For example, the 4-fluorobut-1-yl derivative **88** was a very potent inhibitor and 3-fluorobut-1-yl derivative **89** was the most potent caspase inhibitor. The study also showed that terminal difluroalkyl and trifluoralkyl derivatives exhibited moderate activity.[78]



88

Caspase 3: 41 ±17 nM
Caspase 7: 28 ±6 nM



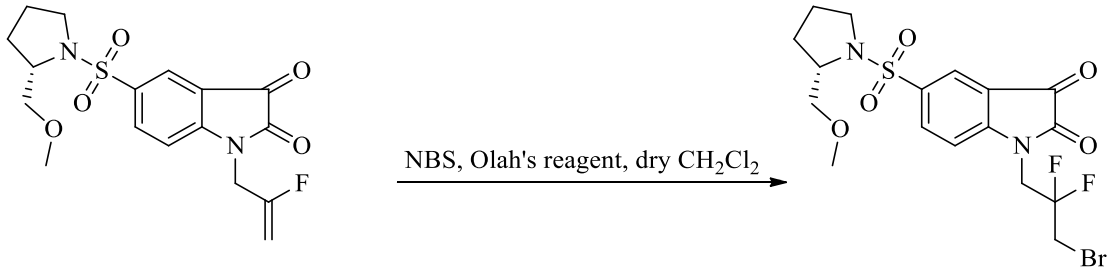
89

Caspase 3: 25 ±3 nM
Caspase 7: 6.8 ±1.9 nM

Given the promising results obtained from the inhibition studies of the fluorinated 5-pyrrolidinylsulfonyl isatins caspase inhibitors above, Podichetty *et al*. turned their focus to other fluorinated derivatives that could be radiolabeled to monitor apoptosis by PET. The focus was to develop a radiofluorination methodology that would introduce a [^{18}F]fluorine radionuclide which might lead to isatin variants based on fluorohydrins.[79] The chemistry utilized to perform such reactions was halofluorination techniques like bromofluorination which is a versatile method to introduce fluorine to unsaturated compounds along with a second reactive function into organic compounds. One of the most common procedures for bromofluorination uses *N*–bromosuccinimide (NBS) as the source of the bromonium ion and different amine-HFcomplexes.[80,81] Katzenellenbogen *et al.* demonstrated that bromofluorination could serve as a radiochemical tool for introducing fluorine-18 into medicinally relevant compounds and facilitate their use as

imaging agents for PET.[69] In this case, bromofluorination of olefins as precursors and epoxide ring opening reactions were used to obtain vicinal fluorohydrins.[82-84]

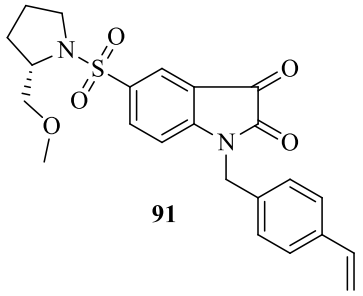
A series of 5-pyrrolidinylsulfonyl isatins were created with varying carbon units through the *N*-alkylation of the isatin nitrogen to create the olefin **89, 91, 92** and terminal epoxide precursors **93**.[79] The fluorinated isatin analogues were then prepared from these precursors by bromofluorination and epoxide ring-opening with different amine/HF reagents.



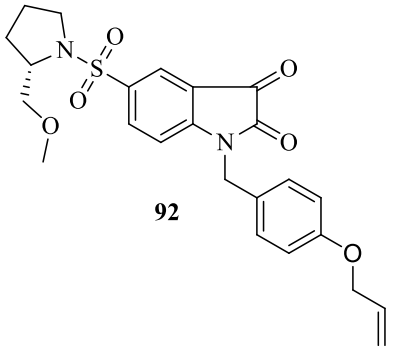
89 **90**

The potencies for caspase inhibition of all the synthesized analogues were measured using fluorogenic *in vitro* caspase inhibition assays.[79] From these inhibition assays, it was found that all fluorinated 5-pyrrolidinylsulfonyl isatin analogues with the exception of the *gem*-difluoride derivative **90** exhibited moderate to high inhibition potencies for caspase-3 and -7. The most active terminal olefin against caspase-3 was **91** which had an IC_{50} value of 9.3 nM while **92** was the most active toward caspase-7 with an IC_{50} value of 0.6 nM while the most active terminal epoxide **93** against both caspase-3 and caspase-7 was the styrene oxide derivative which exhibited IC_{50} values 6.6 nM and 1.9 nM, respectively.[79] Although the olefin and epoxides would not be present in the desired products, it was important to examine their potencies against caspases-3 and -7 to

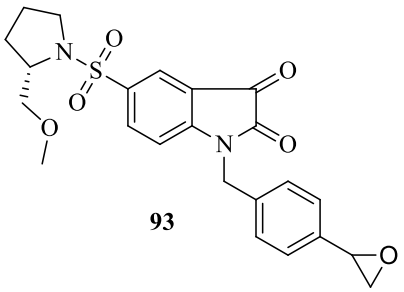
determine the best potential radiolabeling precursors to facilitate quicker development of an active compound.



91

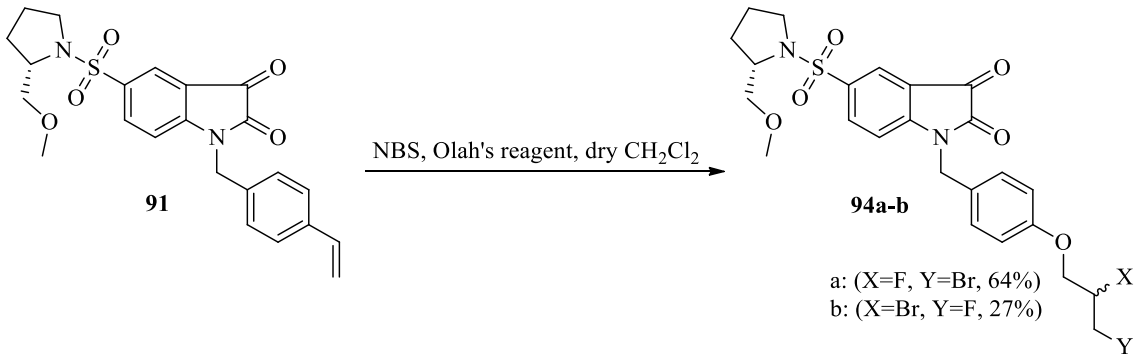


92

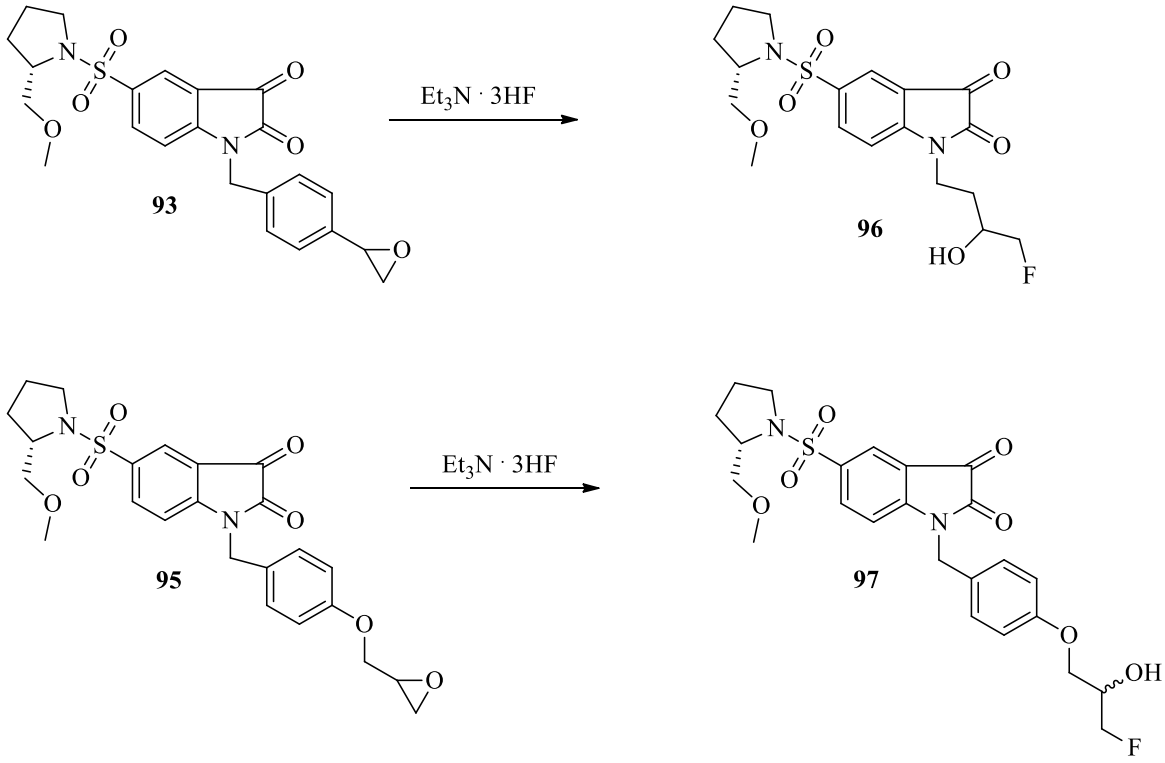


93

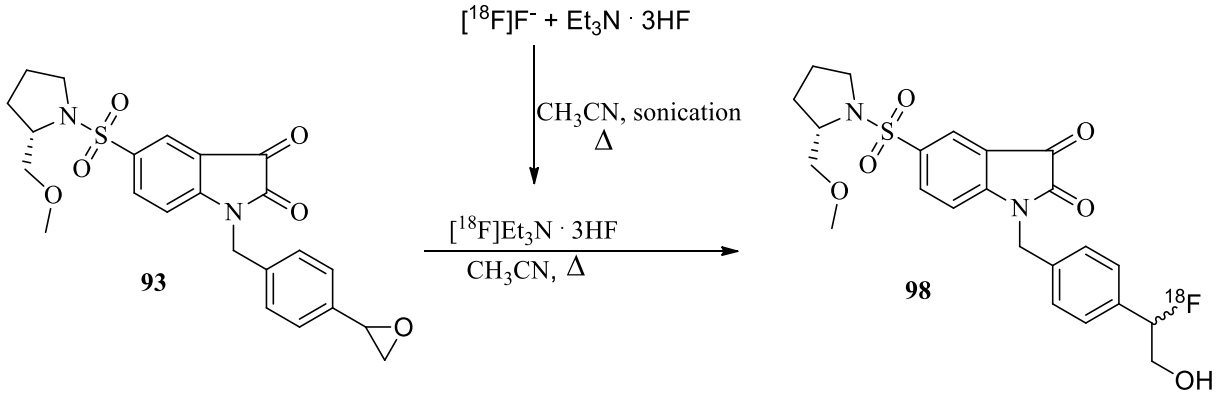
The most potent bromofluoride obtained was the mixture of regioisomers (7:3) **94a-b** which exhibited high inhibition potency towards caspase-3 (IC_{50}: 26 nM) and caspase-7 (IC_{50}: 1.9 nM). To synthesize compound **94a-b**, a bromofluorination occurred in which **91** was reacted with (NBS), and Olah's reagent which is a nucleophilic fluorinating agent that consists of a mixture of 70% hydrogen fluoride and 30% pyridine and convert alcohols to alkyl fluorides.[79]



Through the analysis of the fluorohydrins it was discovered that **96** was the most active inhibitor of caspase-3 and the fluorohydrin **97** was the most potent against caspase-7. Both compounds were synthesized through the reaction of an epoxide derivative (**93, 95)** with triethylamine trihydrofluoride.[79]



A proof of concept synthesis of a fluorine-18 labeled model compound was performed on compound **93** to produce the [^{18}F]**98.** Through optimization of the synthesis, the radiochemical yield of [^{18}F]**98** was optimized to 7% when [^{18}F] triethyl-amine trihydrofluoride ([^{18}F]$Et_3N \cdot 3HF$) was utilized for radiofluorination. This was the first time a nucleophilic fluorinating reagent generated from $Et_3N \cdot 3HF$ and [^{18}F]F^- via isotopic exchange was successfully applied in the synthesis of the [^{18}F]labeled target.[79]



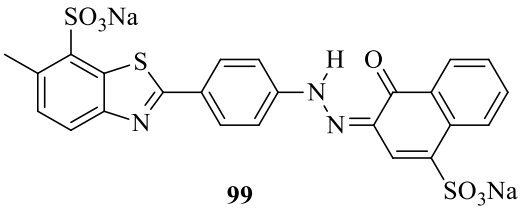
Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly. It is a progressive neurodegenerative disorder characterized by dementia, cognitive impairment, and memory loss. The early symptoms of AD are often overlooked since they are very similar to the signs of natural aging, however, the symptoms gradually worsen until patients lose the ability to perform basic functions like speak, recognize people, and memory becomes non-existent.[85] AD is characterized by significant losses of neurons and synapses and diagnosed by the appearance of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary lesions in the brain.[85,86] As AD progresses, the density and spatial distribution of lesions yield information on the disease, however, total plaque density correlates poorly with cognitive decline and neurodegeneration.[86] The neurofibrillary lesion formation has been shown to correlate more closely with disease progression of AD.[86,87] These lesion formations consist of neurofibrillary tangles (NFTs) which contain paired helical filaments (PHF) resulting from the hyper-phosphorylation of the microtubule-binding protein tau, which plays an essential role in maintaining microtubule stability similar to ties along railroad tracks.[85] In AD, neuronal tau is phosphorylated and proteolyzed resulting in an impairment of the normal functions of tau and even more importantly, tau appears decades before neocortical Aβ deposition and signs of dementia can be detected which makes tau a possible diagnostic tool for AD.[85,86] Both tau and Aβ filaments consist of parallel β-sheets aligned perpendicular to the fibril axis, resulting in a cross-β-sheet structure.[88] This parallel orientation of the β-sheets generates channels extending along the length of the filament to which aromatic molecules can bind via $\pi - \pi$

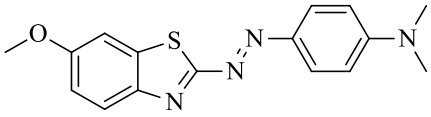
interactions.[89,90] Presently, there are a few small molecules such as Pittsburgh Compound B developed for use in various modalities of whole brain imaging to capture the spatial distribution of amyloid lesions *in situ.*[91] However, most of these ligands bind cross-β-sheet structures common to both tau and β-amyloid bearing lesions which indicates that the information associated with neuritic lesions will be contaminated by cross reactivity with amyloid plaques making it non ideal AD diagnostic tool.[86] A new diagnostic tool was proposed in which a tau selective binding agent would interact with aggregates composed of full-length tau protein which appear much earlier in lesion formation and even come before the formation of insoluble proteolytic products.[86,92] The use of full length tau proteins incubated in the presence of anionic surfactant inducers yield products that react with commonly used fluorescent probes for cross-β-sheet conformation such as Thioflavin S (ThS) and T (ThT) which support aggregation at low micromolar bulk tau concentrations, facilitating screening for high affinity ligands.[85,93] Since the presence of anionic surfactants speeds aggregation rate and lowers bulk protein levels required for fibrillization, it is possible to compare ligand binding to all major AD disease associated protein targets.[85,94]

Kuret *et al.* used this approach in the pursuit of selective binding molecules for tau. In this work, a chemical library of over 72,000 compounds was attained and screened in order to identify ligands capable of interfering with the ThS fluorescence signal which was used to monitor tau conformation. The ThS-reactive tau was prepared with octadecyl sulfate (ODS) which is an alkyl sulfate inducer of tau conformational change and aggregation. A full length tau was used as substrate because it aggregates in early stage AD therefore representing an early marker for AD progression.[86] The ODS

was utilized as an inducer because it efficiently drives conformational changes at low concentrations of full-length tau over short time periods under reducing conditions.[95] Following the assay of the 72,000 compounds, 45 compounds representing 35 active and 10 structurally related inactive analogs were chosen for additional studies. In this follow up study, the inducer arachidonic acid replaced ODS to exclude nonspecific alkyl-sulfate-mediated effects on ThS fluorescence. The 35 active compounds were screened once again and all but eight showed similar dose response curves and half maximal activity concentration (AC_{50}) values in the presence of arachidonic acid. The eight inactives were then eliminated from the study while Thiazine red **99** and **100** were added to the library because of their ability to bind to protein aggregates.[86] Thiazine red **99** is known to selectively bind to neurofibrillary lesions in AD tissue while **100** binds Aβ aggregates *in vitro*.[96, 97]

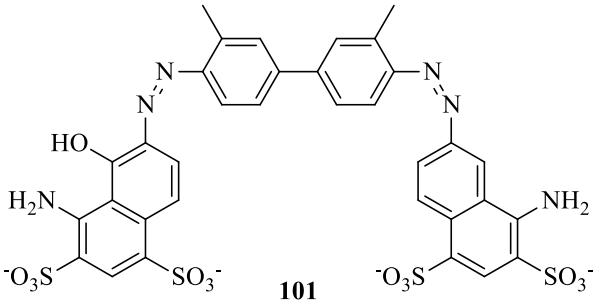


99

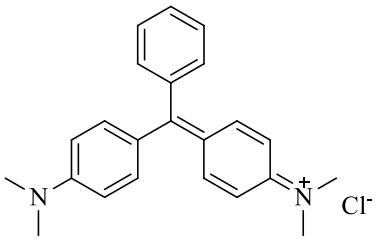


100

The compounds were grouped into six classes which included benzothiazoles, phenylazenes, quinoxaline nitriles, anilines, anthraquinones, and indolinones. These compounds were then measured for their relative affinity for tau, α-synuclein, and $A\beta_{1-42}$. Several of the compounds measured exhibited significant fold selectivity for tau relative to at least one other substrate. For example, Evans blue **101** and aniline crystal violet **102** which were exclusively selective for tau against both α-synuclein, and $A\beta_{1-42}$.

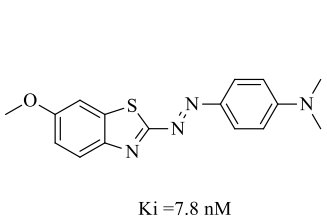


101



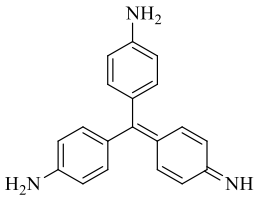
102

As opposed to the benzothiazole **100**, the aniline **103**, the anthraquinone **104**, and the indolinone **99** were all selective for both tau and $A\beta_{1-42}$ relative to the α-synuclein with the benzothiazole being the most potent with a K_i of 7.8 nM.



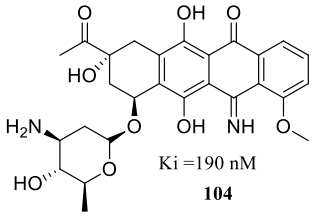
Ki =7.8 nM

100



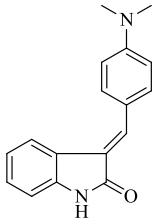
Ki =150 nM

103



Ki =190 nM

104



Ki = 95 nM

105

Kuret *et al*. found that aggregates composed of tau, α-synuclein, and $A\beta_{1-42}$ display an overlapping variety of small molecule binding affinities. Through the study of six different scaffold classes it was discovered that tau aggregates were the most discriminating substrate tested with the strongest binding affinities, while α-synuclein fibrils were the least discriminating and exhibited weaker overall affinity for the test compounds. A structural feature shared by the two most potent compounds towards tau (e.g., **99** and **101**) was the presence of at least three aromatic or rigid moieties connected by two rotatable bonds. For efficient tau binding the requisite geometry has been proposed to be completely planar. Also another common feature for tau binding is the

utilization of a hydroxyl group *ortho* to an azo linker which is predicted to form a hydrazone over the azo tautomer, thereby creating a six-membered ring through hydrogen bonding which would result in a third ring possibly being important for selective interaction with tau filaments. The indolinone **105** was an effective binder of all filamentous substrates tested and other studies have shown hydroxyindoles as $A\beta_{1-40}$ and $A\beta_{1-42}$ fibrillization inhibitors so they may have applications as tau binders.[98]

The indolinone, phenothiazine, and triarylmethines scaffolds, which were very potent as ThS displacement agents, shared the commonality of having a planar, rigid structure that could substitute highly delocalized aromatic pi-electrons when appropriately substituted with electron donating and accepting groups.[97] Due to this, certain derivatives of these scaffolds were highly polarizable and capable of supporting strong van der Waals interactions between the ligand and binding sites exposed on the fibril surfaces therefore introducing the idea of compound polarizability as an important determinant of tau fibril binding affinity.[99,100] To test this theory, an SAR was conducted on phenothiazine, triarylmethine, and indolinone scaffold derivatives while interacting with both authentic and synthetic tau filaments. It was found that polarizability is an important descriptor of tau filament binding affinity for these ligands. This was accomplished by examining closely related analogs of phenothiazine, triarylmethine, and indolinone in which net charge, surface area, sterics, charge, hydrophobicity and number of rotatable bonds was held constant or controlled. However, the binding affinity within scaffold families was lost when compounds from multiple classes were combined indicating that additional factors beyond polarizability contributed to tau filament binding affinity.[99]

The eventual goal of Kuret's work is to develop a tau imaging[101] agent for the diagnosis of AD. Currently, the methods for whole-brain imaging of dementia patients utilize positron emission tomography (PET) to image the binding of radiolabeled compounds to lesions containing filamentous amyloid-β (Aβ) peptide. The current radiolabeled PET compounds consist of Aβ-directed agents that have high sensitivity for detection of AD but have limited specificity. Another drawback to the Aβ-directed agents lies in the fact that Aβ levels plateau as the disease progresses which limits the utility of pre-mortem Aβ detection for longitudinal assessment.[101] In addition, Aβ-directed agents cannot distinguish different forms of frontotemporal lobar degeneration which does not accumulate Aβ aggregates from AD.[101] Thus, Kuret anticipates that selective radiotracers for tau-bearing neurofibrillary lesions could complement the established Aβ imaging in many ways. For example, neurofibrillary lesions appear in large numbers at certain sites years before the onset of dementia in AD providing a potential biomarker for the detection of the disease in early stages.[101] Furthermore, due to the relationship between disease progression and spatial distribution of neurofibrillary pathology, tau-based imaging could help monitor the effectiveness of drug treatments over time.[101] A tau directed imaging agent must fulfill four principal criteria which include: 1) the ability to cross the BBB after intravenous injection while simultaneously having the capability of rapid elimination; 2) the tau imaging agent should be capable of engaging their target within cells undergoing neurofibrillary degeneration and also must bind to a target that varies in composition and post-translational modification, and; 3) tau imaging agents must bind tau aggregates with sufficient selectivity so that neuritic lesion spatial distribution is not disrupted by other lesions that appear in the disease.[101] As

mentioned earlier, the first tau aggregate binding agents were identified on the basis of direct fluorescence in tissue and include Thioflavin T and its neutral benzothiazole derivatives[102], BF-168[103], and X-34.[104,105] The tau directed imaging agents have potential for diagnosing and staging of AD. However, despite the numerous advantages which they afford, there are still disadvantages which include the complexity of the target. The target for tau directed imaging agents presents up to six tau isoforms in varying states of post translational modification and its low concentration relative to Aβ aggregates which provide a source of off target binding sites. Due to this, tau directed imaging agents must deliver substantial binding selectivity if their diagnostic potential is to be realized.[101]

Aims and Background to Research

WSU Approach

Given the proven efficacy of benzylidene oxindoles as cell-death inhibitors (*Ketcha/Apoptrol*) as well as the potential of this scaffold to serve as ligands for various biological targets, the goals of this project were to design and synthesize a library of benzylidene oxindoles (especially fluorinated analogues) as potential cell death inhibitors by strategies which maximize the creation of sub-libraries, and if possible prepare molecules capable of serving as PET targeting agents for cancer or AD. Since (as will be detailed) the incorporation of a strongly Electron Withdrawing Group (EWG) such as chlorine at the C-5 position was determined to be of importance for cell death inhibitors, it was determined that the use of a fluorine atom at the C-5 position would be the next logical step. Not only is fluorine a strong EWG but it is also serves critical functions in many active drug leads.

The incorporation of fluorine atoms into the core structures of pharmaceutical agents has been a recurring and sometimes uncommented upon strategy in drug design.[106] In terms of caspase inhibitors, Brown in 2003 made the *ex post facto* suggestion that the effectiveness of Q-VD-OPh (**23**) to inhibit apoptosis was attributable to the ability of the carboxy terminal 2,6-difluorophenoxy warhead to increase cell permeability, stability and efficacy.[47] Additionally, it has also been postulated that the mechanism of action of Q-VD-OPh involves the formation of an irreversible thioether bond between the aspartyl warhead in the inhibitor and the active site cysteine of the caspase, with displacement of the non-toxic 2,6-difluorophenol leaving group.[107] Alternatively, in designing the isatin sulfonamide PET imaging agent **[^{18}F]ICMT-11** (**72**), introduction of fluorine atoms onto the left side ether moiety was originally intended to enhance metabolic activity by retarding P450-catalyzed ring hydroxylation of the phenolic ring, and in the event, the difluorinated isatin was found even more potent against caspase-3 than the non-fluorinated analog.[71]

Some of the design considerations for benzylidene oxindole cell-death inhibitors in the Ketcha group were based on a consideration of the structural features of Q-VD-OPh as well as the isatin sulfonamide class of inhibitors. In terms of the peptide inhibitor Q-VD-OPh, it was deemed important to incorporate a quinoline or possibly a pyridine ring into the core structure of the WSU scaffold, as well as some type of fluorinated aromatic ring by analogy to the difluorophenoxy ring at the C-terminus. The main structural features obtained from the isatin sulfonamides included the use of a strong electron withdrawing group at the C-5 position and the incorporation of a group (such as a substituted benzyl substituent) at the *N*-position on the isatin or oxindole ring.[50] Such

structural design considerations were therefore incorporated into the benzylidene oxindole cell-death inhibitors with 3 potential points of variability. **Figure 1**

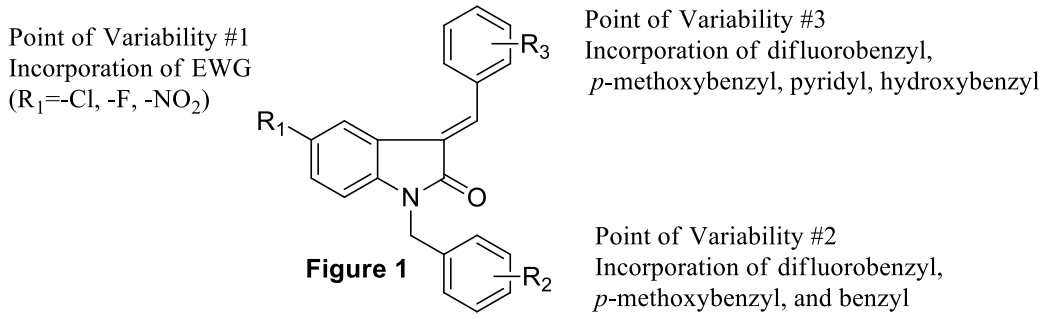
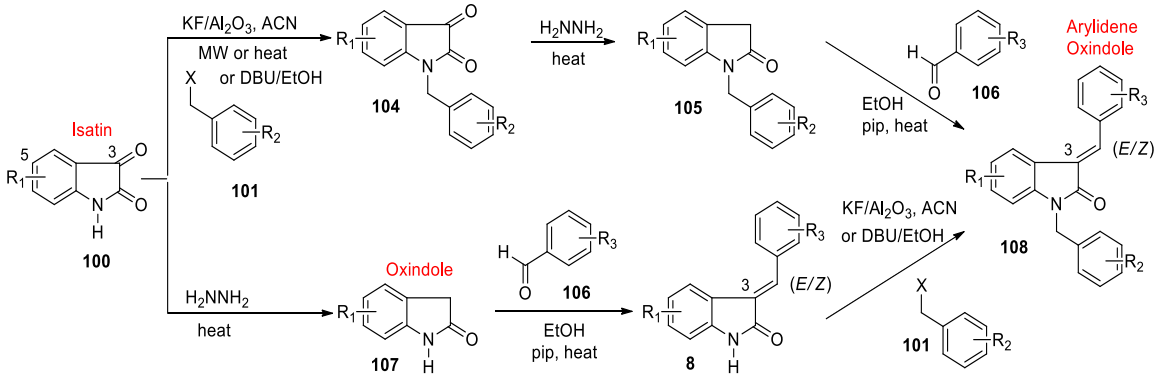


Figure 1

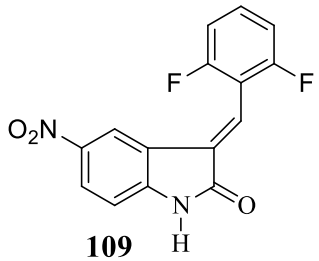
Another overarching stratagem of this design concept was to achieve these goals through a '*libraries from libraries*' approach, wherein sub-libraries of precursor compounds could be prepared and screened for alternative applications before subjecting these compounds to further elaboration for subsequent screens in terms of cell-death inhibition or Alzheimer's. Since both isatin and oxindoles are "*privileged scaffolds*"[4] creating sub-libraries of each class would allow for the benefits of screening each subset for biological targets (e.g., kinase inhibitors, tau binding agents) normally associated with those scaffolds. Ultimately, this would lead to the goal of creating a benzylidene oxindole library with 3 points of variability. The creation of each sub-library can be demonstrated through the multistep reaction manifold in **Scheme 1**



Scheme 1

In the upper manifold of this scheme, *N*-alkylation of isatins **100** with benzylic halides **101** would initially give a sublibrary of *N*-alkylated (mainly benzylic) derivatives **104**; Wolff-Kishner reduction of these would then afford an *N*-alkylated oxindole sublibrary **105**. Finally, along this top branch, aldolization with various aromatic aldehydes **106** would then provide the desired *N*-alkylated benzylidene oxindoles with three points of variability **108**. Following the lower pathway, after reduction of the isatins to the corresponding oxindoles **107**, aldolization gives an *N*-H benzylidene oxindole (potential kinase inhibitors) sub-library **8**, wherein the ultimate target can be achieved by final *N*-alkylation.

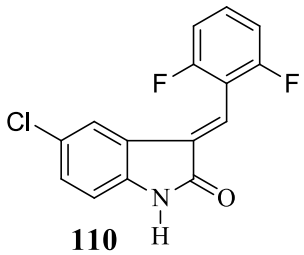
An overview of previous results from the Ketcha group regarding small-molecule cell-death inhibitors is provided herewith. The first successful small-molecule cell-death inhibitor created at WSU was synthesized by Abdullah in which the 2,6-difluorobenzyl-moiety characteristic of the C-terminal warhead of Q-VD-OPh was utilized in the creation of 5-nitro-3-(2,6-difluorobenzylidene)indolin-2-one (**109**). Compound **109** exhibited the same level of activity as the peptide inhibitor Q-VD-OPh matching the 5μM inhibition against Jurkat T-cells (*Ketcha/Apoptrol*).[47,107]



109

However, since the nitro group of **109** was viewed as metabolically unstable, it was deemed impracticable as a cell death inhibitor. Due to this issue, a subsequent study

was performed in an attempt to find an alternative EWG at the C-5 position that would allow for enhanced or equivalent activity. Originally, fluorine was thought of as a suitable replacement for the nitro group but it was deemed more cost effective to use the less electronegative chlorine because the cost of 5-fluoroisatin starting material ($12.84/g) was far greater than that of the 5-chloroisatin ($1.23/g). Additionally, it was also found more difficult (less efficient) to reduce 5-fluoroisatin to 5-fluorooxindole compared to converting 5-chloroisatin to 5-chlorooxindole. In light of those considerations, Repasky then undertook the task of creating analogues possessing a 5-chloro substituent and was able to synthesize the variant comparable to **109**, namely 5-chloro-3-(2,6-difluorobenzylidene)indolin-2-one (**110**).



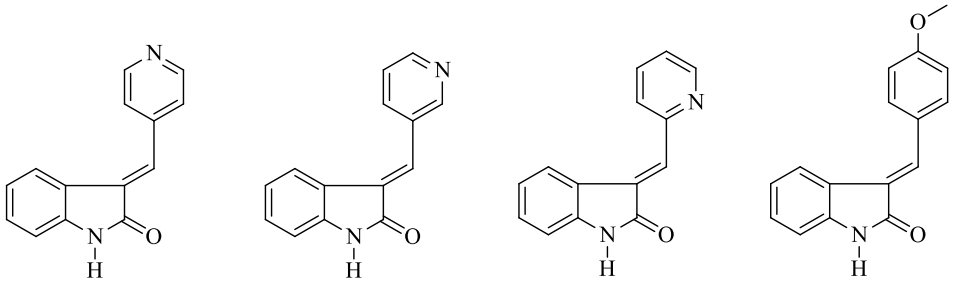
110

No inhib at 100 μM

Surprisingly, **110** showed no inhibition of Jurkat T-cell death at 100 μM, which was quite unexpected given the fact that the 2,6-difluorophenyl- moiety was thought to be partially responsible for the initially observed activity. It was therefore reasonable to suspect that while the incorporation of a chlorine might not be as effective as the metabolically unstable nitro group, the 5-chloro derivative might still have been thought to show inhibition at the 20-50 μM level.

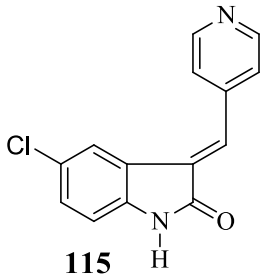
Since the presence of a 2,6-difluorophenyl- group at the C-3 position was apparently not an absolute requirement, it was then decided to incorporate the alternate

structural feature of Q-VD-OPh, namely a quinoline type group (*e.g.*, pyridyl) or at least one that contained lone pair electrons at the C-3 site. For simplicity, mimics of the quinoline type group examined were the pyridyl (2-, 3-, and 4-) moiety and also the *p*-methoxyphenyl group. To that end, Abdullah and Clay synthesized 3-(pyridine-4-ylmethylene)indolin-2-one (**111**), 3-(pyridine-3-ylmethylene)indolin-2-one (**112**), 3-(pyridine-2-ylmethylene)indoline-2-one (**113**), and 3-(4-methoxybenzylidene)indolin-2-one (**114)**.



111 50 μM **112** 100 μM **113** 100 μM **114** 50 μM

It was determined that all pyridine derivatives exhibited some cell-death inhibitory activity against Jurkat T-cells, wherein the 4-pyridyl-moiety was the most active at 50 μM. It was also determined that the *p*-methoxyphenyl- moiety would be a good candidate since it also showed inhibitory activity at 50 μM. From these findings involving an oxindole lacking a C-5 substituent, it was deemed valuable to create a 4-pyridyl- bearing compound with an electron withdrawing group *(i.e.*, chlorine) at the C-5 position. This was accomplished by Repasky in synthesizing 5-chloro-3-(pyridine-4-ylmethylene)indolin-2-one (**115**).



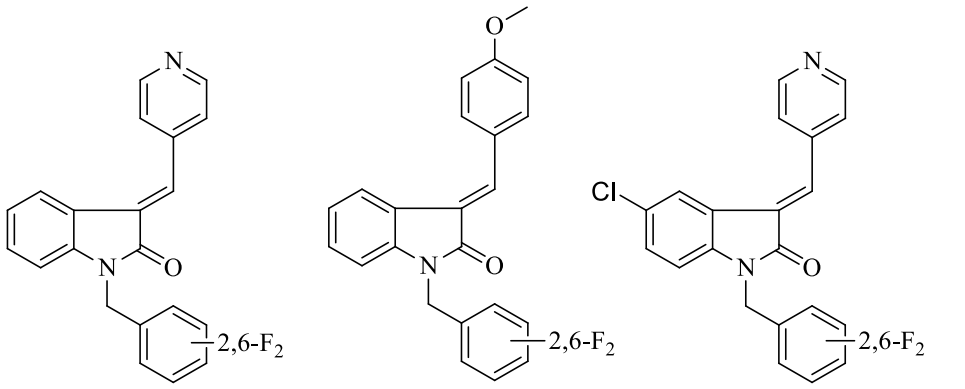
115

50 μM

Surprisingly, compound **115** showed the same inhibitory activity of 50 μM against Jurkat T-cells as compound **111** which did not contain an EWG at the C-5 position. In contrast to the isatin sulfonamide inhibitors, it was now questionable if incorporating an EWG at the C-5 position of a benzylidene oxindole was in fact crucial for activity. Thus, while a strong EWG might be expected to increase the electrophilicity of a C-3 ketone in the case of isatins, such a group may play little role in enhancing the presumptive nucleophilic attack of the caspase thiol upon the beta-carbon of the α,β-unsaturated amide moiety of the oxindole class. Moreover, it was beginning to appear that hydrogen bond acceptor properties of a C-3 substituent might play an important role as suggested by Loeser et al.[107] who postulates that hydrogen binding between caspase and inhibitor influences the effectiveness of the inhibitor. This led to the idea that substituent groups with available lone pair electrons like hydroxyl, methoxy, and pyridyl groups could be utilized as necessary structural features in the design of potential inhibitors of caspases.

Although there is no established correlation of the ability of isatin sulfonamides to inhibit caspases by nucleophilic attack of the thiol at the C-3 ketone carbonyl and the presumed ability of benzylidene oxindoles to serve as Michael acceptors for this enzyme, the value of incorporating an *N*-benzyl group seemed appealing as it typically enhanced

activity by roughly an order of magnitude for the isatins.[37] Inspection of the Q-VD-OPh molecule suggested that the addition of a 2,6-difluorobenzyl moiety at the *N*-position might be a good choice because of the EWG effects and the expected increased cell permeability as a consequence of the fluorines. Therefore, the 2,6-difluorobenzyl moiety was incorporated onto the already active lead compounds **111**, **114**, and **115** at the *N*-position of the oxindole scaffold so as to afford the corresponding *N*-substituted analogues, *N*-(2,6-difluorobenzyl)-3-(pyrid-4-yl-methylene)indolin-2-one (**116**), *N*-(2,6-difluorobenzyl)-3-(4-methoxy-benzylidene)indolin-2-one (**117**), and *N*-(2,6-difluorobenzyl)-5-chloro-3-(pyrid-4-yl-methylene)indolin-2-one (**118**).



116
15-20 μM

117
Inactive

118
90% inhibition at 10 μM

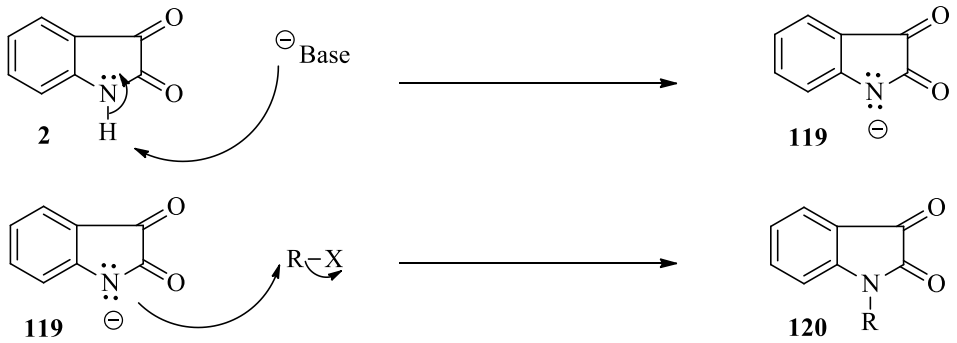
In general, a trend towards increased activities against Jurkat T-cells was found to be associated with introducing the 2,6-difluorobenzyl group versus the *N*-unsubstituted counterparts. For instance, compound **116** exhibited activity at 15-20 μM (versus 50 μM for **111)**, and the most active compound **118** showed activity at 10 μM compared to 50 μM for **115**. Mysteriously, in the case of the 4-methoxy derivative **117**, a decrease in cell-death activity was observed wherein the N-substituted derivative was found to be inactive at 100 μM in contrast to the corresponding N-H derivative **114** which exhibited 50 μM

activity. While at present the lack of a synergistic effect for a molecule possessing two favorable fragments (e.g.,4-methoxy/*N*- 2,6-difluorobenzyl) is obscure, the main conclusion developed through these SAR studies was that the 3 points of variability can possibly work in a synergistic way to increase the inhibitory activity of these benzylidene oxindole cell death inhibitors. With that overview of previous work relating to benzylidene oxindoles as cell-death inhibitors in the Ketcha Group completed, a more detailed examination of the relevant chemistry towards the target sub-libraries will ensue.

Results and Discussion

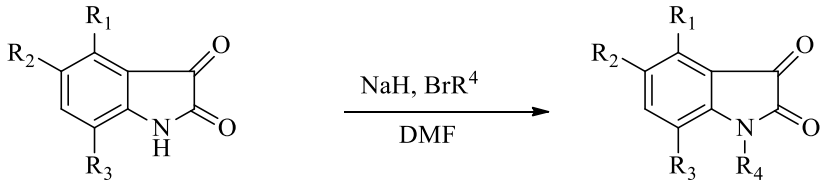
N-Alkylation of Isatins

The preparation of *N*-alkylated isatins has been reported utilizing numerous alkyl-, allyl-, benzyl-, and propargyl halides under a variety of basic conditions. In most examples, the use of conventional heating methods under reflux were utilized, however more recent efforts have focused upon conducting the *N*-alkylation of isatins utilizing microwave irradiation. Regardless of the heating source, the *N*-alkylation of isatin occurs through the reaction of isatin **2** with some base-solvent combination. Herein the base abstracts a proton from the nitrogen forming the highly conjugated (and often highly colored) isatin anion **119**. The alkyl halide then undergoes nucleophilic attack by the nitrogen anion forming the *N*-alkylated isatin **120**.



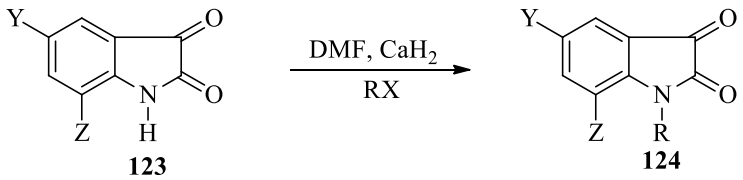
The most popular base-solvent combination utilized in the *N*-alkylation of isatin with alkyl halides is sodium hydride in N,N-dimethylformamide (DMF).[37,50,109] For example, Chen *et al*. alkylated numerous 4,5,7-substituted isatin derivatives **121** with

sodium hydride and various alkyl bromides in DMF to provide the corresponding *N*-alkylisatin derivatives **122** in moderate yields depending on the substituent groups.



121 **122**

Due to the flammibility issues associated with the use of NaH, it is often replaced with calcium hydride (CaH_2). Garden *et al.* utilized calcium hydride in DMF to synthesize a set of 7-substituted-*N*-alkylisatin derivatives, wherein a 7-substituted isatin **123** was reacted with an alkyl halide in DMF at temperatures ranging from 25-50^{o}C to produce the isatin derivatives **124**. It was observed that isatin derivatives bearing an electron-withdrawing substituent at the C-5 and C-7 position reacted more readily with CaH_2 at room temperature reflecting the increased acidity at the amide proton.[110]

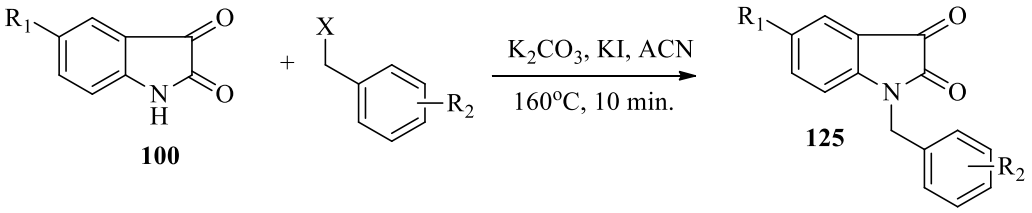


123 **124**

Due to the advances in technology, the use of microwave irradiation as an alternative heat source has gained popularity in recent years. The use of microwave irradiation is particularly attractive because it allows for rapid and highly efficient synthesis while minimizing the thermal decomposition of products that is sometimes associated with conventional heating due to longer reaction times. The advantages of utilizing microwave irradiation in organic synthesis have been reviewed on a number of

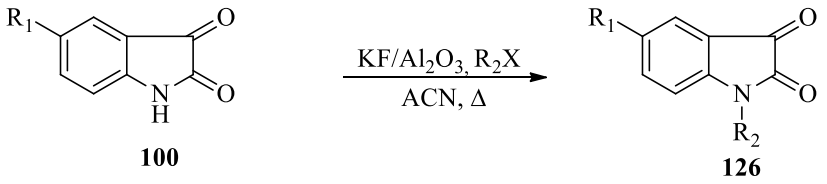
topics including classic organic reactions,[111] green organic synthesis,[112] and multi-component reactions.[113]

The most successful approach for the *N*-alkylation of isatins using microwave irradiation is with K_2CO_3 and alkyl halides in a minimal amount of DMF.[114] Additionally, several groups have devised parallel methods minimizing purification and which are amenable to automation under thermal or microwave conditions. Noteworthy examples include a parallel microwave procedure by Lindsley which employed K_2CO_3/KI in acetonitrile (160 ^oC, 10 min),[34] or an analogous process in DMF (150 ^oC, 5-15 min) developed by Wee *et al.*[115] In Lindsley's case, a variety of *N*-benzyl isatins **125** were synthesized by reacting 5-substituted isatins **100** with a variety of benzylic halides (2.5 equiv) and K_2CO_3 (2.0 equiv) with KI (0.1 equiv) as the catalyst in acetonitrile (ACN).[34]



Alternatively, in regards to devising highly efficient and rapid solution-phase methodologies for the parallel synthesis of diverse isatins, Shuttleworth utilized polymer-supported 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) on polystyrene for the preparation of a library of serine protease inhibitors.[116] Another fairly new approach to the *N*-alkylation of isatin is through the use of potassium fluoride on alumina (KF/Al_2O_3), a solid-supported base introduced by Ando *et al.*[117] in 1979 for *N*-alkylation. The main advantage of the use of the solid base KF/Al_2O_3 in solution phase chemistry involves avoiding difficult workups, because

the base can be removed through vacuum filtration. Chibale employed this less expensive solid-supported base to perform parallel solution phase synthesis of *N*-alkyl isatins **114** in ACN starting from a substituted isatin **115**.[118] More recently, the Ketcha group reported the use of KF/Al_2O_3 in ACN for the *N*-alkylation of isatins under thermal conditions or microwave-irradiation (180 ^oC).[115,116]



100 **126**

The use of the solid base KF alumina by the Ketcha group was envisioned as an expedient protocol since the catalyst might be removed by simple filtration and this methodology might then be utilized in an automated synthesizer in multiparallel fashion using an instrument such as the Quest 210. However, upon filtration, evaporation of the ACN led to a product containing all organic impurities and purification by recrystallization or chromatography was then necessary. Thus, the first objective of this work entailed examining the use of a soluble organic base in a solvent capable of executing a crystallization of the product upon cooling. Therefore, initial studies involved a comparison of the *N*-alkylation of isatin derivatives with the solid base of KF/alumina utilizing conventional heating to those obtained with the liquid base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in ethanol (EtOH) utilizing microwave irradiation. Although the *N*-alkylation of isatins with DBU as a base had yet to be explored, if this base were sufficiently strong to effect the amide *N*-H abstraction, then this approach would allow for the expedited synthesis of substrates wherein the products

might precipitate from the reaction mixture in high yields and high purity and be isolable by simple filtration without column chromatography.

N-Alkylation of Isatin Utilizing DBU

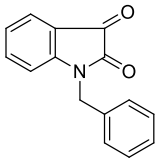
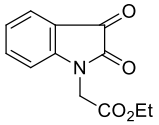
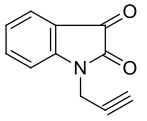
To explore the possibility of employing DBU as a potential base for the *N*-alkylation of isatins, a study was conducted employing isatin, 5-chloroisatin, and 5-fluoroisatin and a series of alkylating agents including benzyl chloride, 2,6-difluorobenzyl bromide, 2,6-dichlorobenzyl bromide, propargyl bromide, and ethyl bromoacetate. The parameters set forth for the reaction involved the use of the isatin (2.00 mmol), DBU (1.1 equiv), and the alkyl- or benzylic halide (1.1 equiv) in EtOH (3 mL) in a microwave vial (10 mL). Reactions were conducted at temperatures from 120°C to 140°C and at time intervals of 10 to 25 min after which reactions were monitored by TLC and GC/MS to ascertain completeness of reaction; the results are presented below in **Table 1**. In the case of isatin itself, the *N*-alkylations required a time of 10 min at 140 °C, while effective alkylations of 5-chloroisatin and 5-fluoroisatin could be effected at a temperature of 120 °C for 20 min and 25 min, respectively. The lower temperatures and longer reaction times are attributed to the electron withdrawing effect of the chlorine and fluorine atoms, which are thought to weaken the nucleophilicity of the isatin salt thus making it easier to abstract the proton but reducing the attack on the alkyl halides. Generally, it was also found that increasing the amount of DBU to 1.5 equiv did not result in substantially enhanced conversion. In all cases, after heating for the appropriate time, the reaction vessel was then allowed to stand in a freezer followed by a vacuum filtration to afford the pure (TLC, GC/MS) products as light orange (or red) solids. The

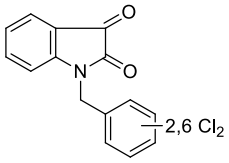
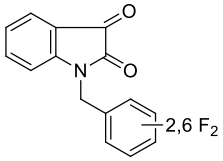
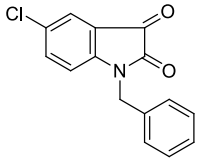
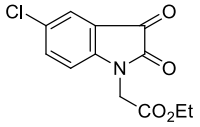
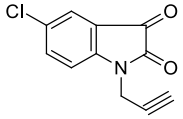
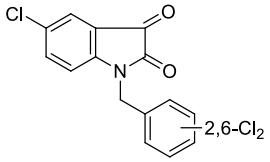
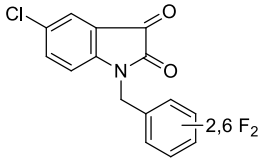
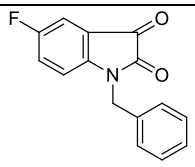
filtrate could then be evaporated under reduced pressure to afford an oil and subsequently purified by silica gel chromatography (70:30 hexanes/EtOAc) to provide additional product if desired. The only deviation from the above procedure involved the case of 1-(prop-2-yn-1-yl)indoline-2,3-dione wherein a ramping technique was required to obtain higher yields.

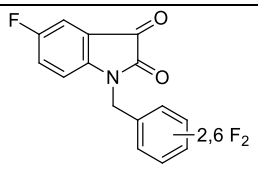
100 **127**

The percent yield and melting points for all cases are reported in **Table 1** below along with the structure of the product of interest.

Table 1. *N*-alkylation of 5-substituted isatin derivatives via DBU

Compound Number	Compound	Melting Point	Lit. Melting Point	Percent Yield
128		129-134°C	128-133°C[119]	61%
129		105-110°C	124-129°C[119]	47%
130		147-149°C	157-158°C[110]	81%

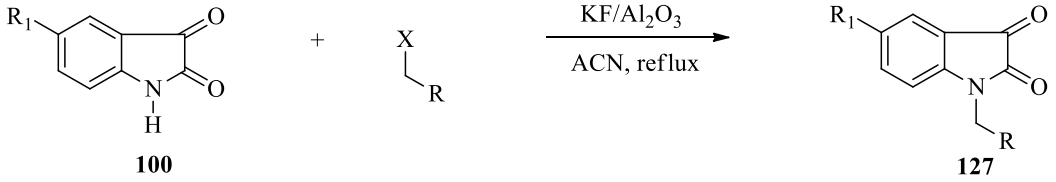
131		184-186°C	184-189°C[119]	93%
132		155-158°C	154-156°C[119]	84%
133		130-133°C	134°C[119]	71%
134		126-128°C	130-135°C[119]	68%
135		157-159°C	N/R[150]	64%
136		232-235°C	233-235°C[119]	84%
137		171-174°C	172-177°C[119]	72%
138		130-132°C	130-132°C[137]	88%

139		129-132°C	N/R[34]	82%

The above success using the soluble base DBU and a solvent from which the product might spontaneously crystallize, ensured that a substantial savings in time-consuming steps would be achieved as opposed to previous methods in which a workup or purification was required after filtration. The yields listed are a summation of the pure solid obtained upon the first crystallization and that obtained by chromatographic purification of the filtrate. In all instances, the material obtained by crystallization from the reaction medium was pure as determined by GC/MS and in most cases provides sufficient amounts of material to be carried on in subsequent steps if desired. In general, electron-withdrawing substituents at the C-5 position of the isatin allowed for alkylations at lower temperatures albeit with longer reaction times. Additionally, electron withdrawing groups on the benzylic halides served to enhance observed reactivity in comparison to those rings not so substituted or the propargyl halide. It is also worthy to note that it was found that ethyl 2-(5-chloro-2,3-dioxoindolin-1-yl)acetate **124** reacted at room temperature while waiting to be placed in the microwave reactor. Thus, the use of the soluble base DBU in conjunction with a crystallizing solvent under microwave conditions allows for the expedited construction of a small library of *N*-alkylated isatins which could be isolated in a pure state and in good yields by simple filtration.

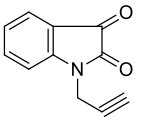
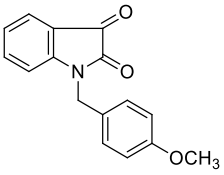
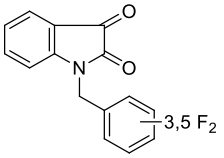
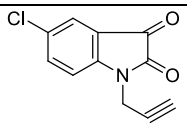
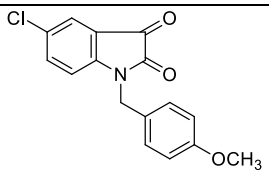
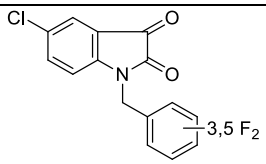
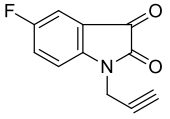
N-Alkylation of Isatins utilizing KF/Al_2O_3

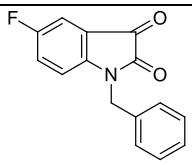
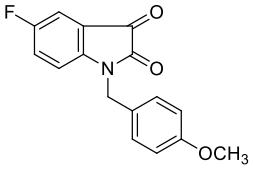
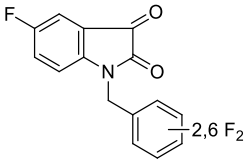
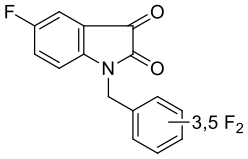
Concurrent with the investigations to develop an expedited microwave synthesis of *N*-alkyl isatins amenable to automation, wherein product isolation could be achieved by simple filtration, efforts were also devoted to extending the scope of the KF/Al_2O_3[115,116] protocol previously devised in our group. Despite the more extensive workup required for this thermal process, it is in general a more scalable procedure allowing for the larger quantities of *N*-alkyl isatins **131** required for our intended multi-step sequences.



Following the procedure originally developed by Clay,[119,120] the isatin starting materials **130** (R_1 = H, F, Cl) were refluxed in ACN for approximately 24 h utilizing 6 equiv of KF/Al_2O_3 and a slight excess of the appropriate alkyl halide (X = Cl, Br, 1.5 equiv). The crude products were separated from the solid base by vacuum filtration and the solid catalyst was washed with additional cold solvent. The resulting filtrate was then evaporated under reduced pressure to afford an orange (or red) solid. The solids could then be recrystallized from dichloromethane and hexanes to afford the pure (TLC, GC/MS) products as bright orange or red crystals. The percent yield results and melting points for these reactions are reported in **Table 2** below along with the structure of the product of interest.

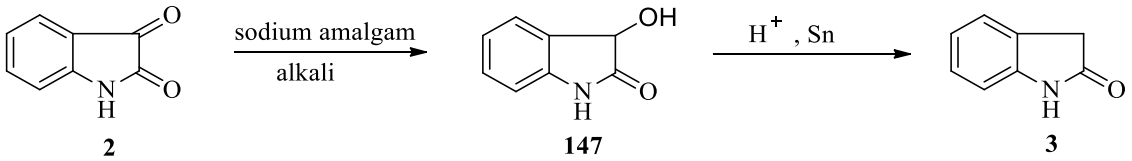
Table 2: Alkylation of 5-substituted isatin derivatives via KF/Al_2O_3

Compound Number	Compound	Melting Point	Lit. Melting Point	Percent Yield
130		153-156 °C	157-158 °C[110]	69%
140		150-153 °C	N/R[146]	55%
141		161-164°C	N/R	50%
135		164-168°C	N/R[150]	52%
142		148-150°C	152-153°C[145]	75%
143		173-175°C	N/R	95%
144		124-126°C	N/R	36%

138		128-129°C	130-133°C[137]	82%
145		132-135°C	138-139 °C[146]	71%
139		143-145°C	N/R[34]	85%
146		160-161°C	N/R	96%

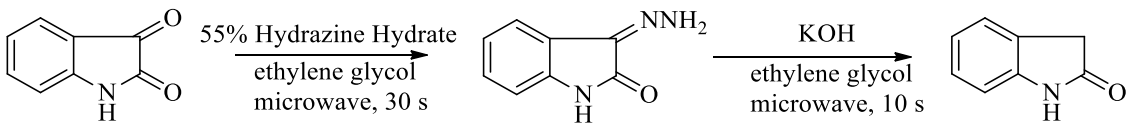
Reduction of Isatin

Having in hand new or expanded protocols for the effective *N*-alkylation of isatins (thereby creating a new sub-library of diversified isatins), it was then deemed valuable to conduct the reductive-deoxygenation of such derivatives to produce a library of *N*-alkylated oxindoles in anticipation of final conversion to benzylidene oxindoles. The reduction of isatins to oxindoles is a well-established procedure first reported by Baeyer in 1866,[6] wherein isatin **2** was reduced with sodium amalgam in alkaline medium to create 3-hydroxyoxindole **147**. Further reduction of the dioxindole with tin and mineral acids or by sodium amalgam in acid medium gave oxindole **3**.[6]



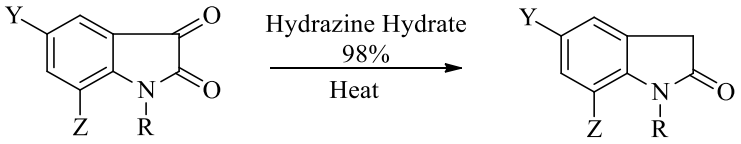
2 **147** **3**

One well-known method for the reduction of a carbonyl group in an aldehyde or ketone to a methyl or methylene group is the Wolff-Kishner reduction in which the corresponding hydrazones of aldehydes and ketones are converted to the respective hydrocarbons in the presence of the sodium ethoxide at 180-200°C for 6-8 h.[121] A review of this reaction covers the literature prior to 1948,[122] however more recent studies have described techniques in which milder conditions were utilized to synthesize oxindole. Soriano described a Wolff-Kishner reduction of isatin under mild conditions for 3-4 h. In this procedure, isatin (**2**) was treated with 55% hydrazine hydrate and heated for 1 h to form the isatin-3-hydrazone **148** which was then treated with sodium ethoxide and acidified with 10% HCl before being recrystallized in water to form the desired oxindole product **3**.[123] However, the drawback to this procedure was that it still required 3-4 h for completion, so Parquet *et al*. utilized microwave technology to make the procedure more efficient. In this modification, isatin was treated with 55% hydrazine hydrate, ethylene glycol and placed in the microwave for 30 s to yield the isatin-3-hydrazone. A mixture of ethylene glycol and potassium hydroxide was then combined with the hydrazone and irradiated in the microwave for 10 s, then acidified with 6 M HCl and extracted with diethyl ether to yield oxindole.[124]



2 **148** **3**

All previous Wolff-Kishner methods for the reduction of isatin required the isolation of the isatin-3-hydrazone **148** until Crestini and Saladino discovered a one-pot synthesis method in 1994.[125] In this one-pot synthesis, it was discovered that *N*-substituted-isatin **124** derivatives reacted readily with hydrazine hydrate to directly produce the corresponding *N*-substituted-oxindole derivative **149** without isolating the intermediate hydrazine and without an additional base.[125] In this reaction synthesis, isatin or *N*-substituted-isatins were added to 98% hydrazine hydrate and refluxed for 15-30 min. The reaction was then poured into cold water and extracted into ethyl acetate. It was believed that the direct decomposition of the isatin hydrazone was due to the ability of the α-ketoamine to give anchimeric assistance in the stage of decomposition of hydrazone.[121]

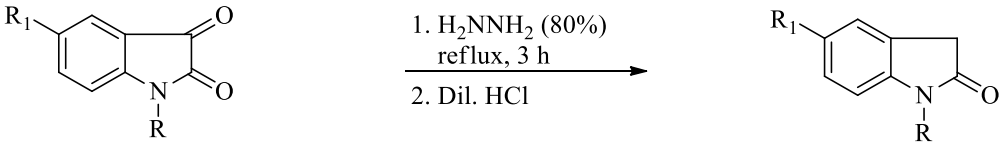


124 **149**

WSU Wolff-Kishner Reduction of Isatins to Oxindoles

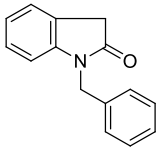
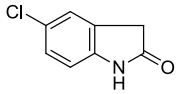
The second step required in the creation of the desired trisubstituted oxindole derivatives (and sub-libraries thereof) involves a Wolff-Kishner type reduction of the C-3 ketone carbonyl on the isatin scaffold. The compounds prepared in this study involved a slight modification of the Crestini and Saladino one-pot protocol,[125] involving heating in hydrazine hydrate (80 wt%), but refluxing for 3 h instead of the reported 15-30 min. Additionally, instead of isolating indoles by extraction, the reaction mixture was acidified and the product was obtained as it precipitated slowly from the aquous solution.

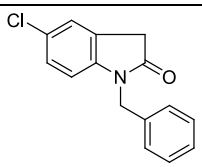
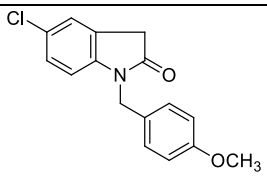
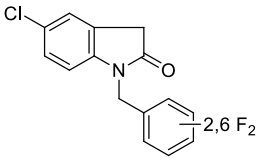
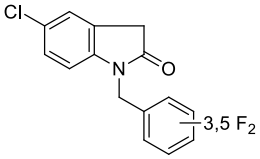
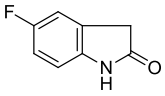
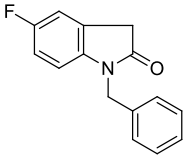
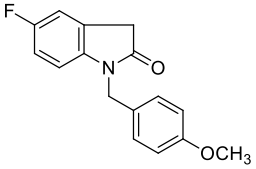
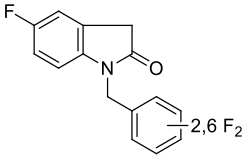
Furthermore, it was found advantageous to wrap aluminum foil around the reaction vessel to facilitate faster/higher heating temperatures of the vessel which allowed for the precipitated hydrazone to 'dissolve' back into solution and for the reaction to continue with loss of nitrogen to afford the desired oxindole.[126] The only exception occurred in the case of 5-fluoroindoline-2-one (**154**), wherein which it was required that the solution be left undisturbed for a week before dark brown crystals would precipitate out of solution. Since the reduction of *N*-substituted isatin derivatives is a relatively neglected area, most of the derivatives created were unknown compounds. The percent yields and melting points for these Wolff-Kishner reactions are reported in **Table 3** below along with the structure of the product of interest.

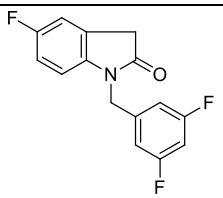


100 **150**

Table 3: Wolff Kishner Reduction of *N*-alkylated isatin derivatives

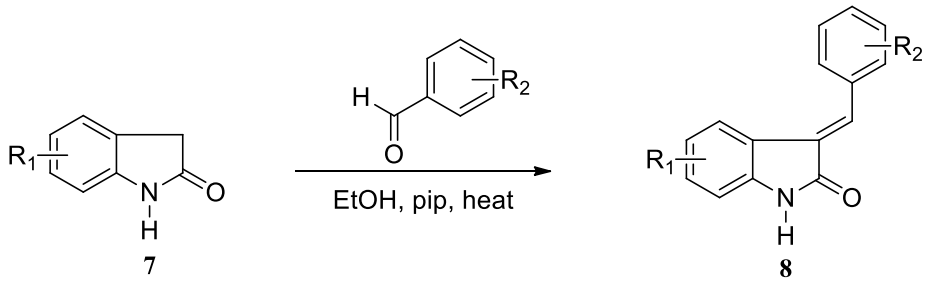
Compound Number	Compound	Melting Point	Lit. Melting Point	Percent Yield
151		84-85°C	77-78[147]	86%
152		199-202°C	195°C[126]	86%

153		90-91˚C	88-89[126]	90%
154		145-147°C	N/R	95%
155		125-129˚C	120-125˚C[126]	88%
156		169-170°C	N/R	91%
157		140-143°C	143-147°C[138]	58%
158		76-78°C	N/R[148]	84%
159		124-127°C	N/R	91%
160		96-98°C	N/R	77%

161		170-173°C	N/R	84%

Aldol Condensation

The most common method in C-3 functionalization of oxindoles is through the use of an aldol (or perhaps more appropriately a Knoevenagel) type reaction. In an aldol condensation, aldehydes or ketones with an α-hydrogen atom undergo a base catalyzed condensation with another carbonyl compound. The aldol reaction occurs by nucleophilic addition of the enolate ion of the donor molecule to the carbonyl group of the acceptor molecule. The resulting tetrahedral intermediate is then protonated to give an alcohol product which can then be followed by dehydration yielding a conjugated enone. A Knoevenagel reaction is a modified version of the aldol condensation that involves a nucleophilic addition of a stabilized enolate anion to a carbonyl group of another molecule followed by spontaneous elimination of water creating an α,β-conjugated enone. The typical method for attaining alkylidene or benzylidene-2-oxindoles **8** involves the use of an oxindole **7** with an aldehyde in refluxing ethanol in the presence of catalytic amounts of piperidine.[10]



An alternative method for the condensation of aldehydes and ketones with oxindole is the dry synthesis method developed by Villemin and Martin.[127] This method utilizes the solid support base of KF/alumina to create arylidene/benzylidene-indolin-2-ones **8** under microwave irradiation. In this condensation, 5-substituted oxindoles **7** and aldehydes were mixed in acetonitrile at room temperature for 5 min, after which the solvent was evaporated by vacuum. The solid was then irradiated and extracted with acetonitrile yielding the desired arylidene/benzylidene-indolin-2-ones **8**.[127]

Such aldol or Knoevenagel type condensations upon oxindoles are the standard means of producing the α,β-unsaturated products characteristic of the "nitrogen-linked Michael acceptor" motif likely responsible for the biological activity of such heterocycles. Considering the previous demonstration of 3-benzylidene-indolin-2-ones as cell death inhibitors it was deemed important to investigate the effects of a 5-fluoro substituent on this inhibition. Initial work focused on implementing an aldol type condensation to attain 3-substituted-5-fluoroindolin-2-ones with a variety of aryl aldehydes. As described earlier, design considerations focused on incorporating some of the structural features of Q-VD-OPh or the SAR of compounds previously prepared and found active from this laboratory. From these previous studies, it was concluded that a an electron-withdrawing substituent at the C-5 position was necessary (or at least

optimal), and that fluorine atoms on the benzylidene aryl substituent were well tolerated but perhaps not necessary. Knowing this information, it was deemed important that a 5-fluoro-indolin-2-one bearing a 2,6-difluoro-benzylidene moiety at the C-3 position be created. Moreover, since Repasky had found that the 5-chloro-indolin-2-one bearing a 2,6-difluoro-benzylidene moiety at the C-3 position was inactive against Jurkat T-cells, it was desireable to ascertain whether the analogous 5-fluoro derivative might retain the activity noticed in the case of the analogous 5-nitro derivative. Additionally, since the N-H derivatives containing 4-pyridyl (*e.g.*, **111)** and *p*-methoxy (*e.g.*, **114)**, synthesized by Abdullah and the 5-chloro N-H derivative containing 4-pyridyl (*e.g.*, **115)** synthesized by Repasky showed 50 μM inhibition, it was decided that making analogs exhibiting hydrogen bond donating motifs would be important since such hydrogen binding between caspase and inhibitor might influence the effectiveness of the inhibitor.

Thus, the aldol type condensations of 5-fluoro-indolin-2-one (**157**) were conducted with the requisite aryl aldehydes (1.2 equiv) in refluxing ethanol (3-6 h) in the presence a catalytic amount of piperidine (0.147 equiv). After that time the reactions were allowed to cool and the products isolated in pure form by crystallization. The percent yield and melting points for these reactions are reported in **Table 4** below along with the structure of the product of interest.

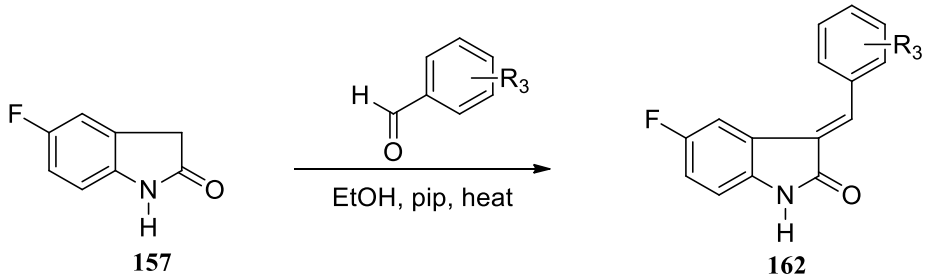
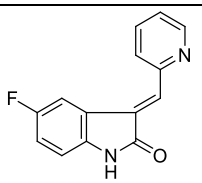
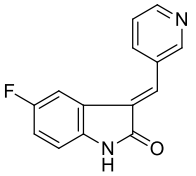
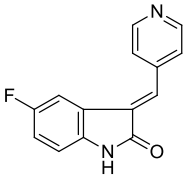
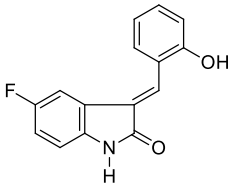
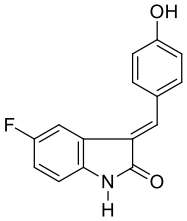
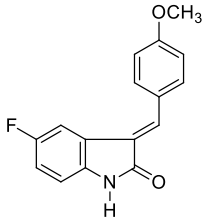
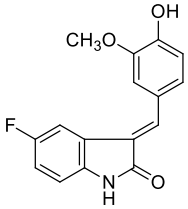
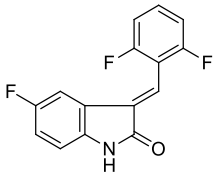
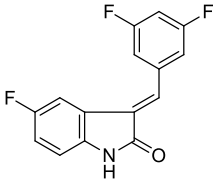
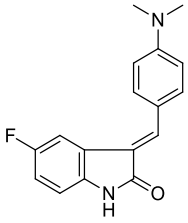


Table 4: 3-Substituted-Indolin-2-ones from Knoevenagel Condensations

	Compound	Percent Yield	Melting Point	Literature Melting Point	Configuration (Z:E)
163		61%	248-253˚C	N/R	(100:0)
164		63%	210-212˚C	N/R	(60:40)
165		81%	229-231˚C	N/R	(90:10)
166		98%	205-208˚C	N/R	(0:100)
167		70%	305-308˚C	N/R	(0:100)

168		58%	192-195°C	N/R[144]	(40:60)
169		81%	218-219˚C	N/R	(0:100)
170		58%	250-255˚C	N/R	(100:0)
171		81%	218-221˚C	N/R	(60:40)
172		43%	220-223˚C	N/R[149]	(60:40)

Since it is known from the work of Mach *et al.*[37] that introduction of an alkyl or benzylic group on the isatin nitrogen results in dramatic improvement in potency for inhibiting caspase-3 activity, it was determined that a library of *N*-substituted oxindole compounds would be synthesized containing a fluorine at the C-5 position of the oxindole scaffold and with an appropriately substituted benzylidene group at the C-3

position.[5] The fluorine was utilized for its high electron withdrawing effect. The first library of compounds was synthesized utilizing the *N*-(2,6-difluorobenzyl)-5-fluoro-indolin-2-one scaffold. This allowed for the incorporation of the 2,6-difluorobenzyl moiety characteristic of Q-VD-OPh and also present in the most active compounds created in the Ketcha lab. The increased inhibition of **118** (possessing a 3-pyridyl group and *N*-2,6-difluorobenzyl substituent) indicated that it might be important to synthesize more molecules that incorporate the available 3 points of variability. This was accomplished in the case of the 5-fluoro and 5-chloro-*N*-2,6-difluorobenzylindolin-2-ones (**173**) in the aldol type condensation reaction with associated aryl-aldehyde (1.2 equiv.) at 90°C for 3-6 h in the presence of catalytic amounts of piperidine (0.147 equiv.) yielding the corresponding arylidene oxindoles **174**. In those cases where the products would not precipitate out of solution, the reaction mixtures were evaporated under reduced pressure and purified by passing through a silica gel column (70:30 hexanes: EtOAc). If necessary, the products could be recrystallized from dichloromethane and hexanes to afford the pure (TLC/GC-MS) product. Moreover, since these compounds had not previously been reported, no literature melting point values could be attained for comparison. The percent yield results and melting points for these reactions are reported in **Table 5** below along with the structure of the product of interest.

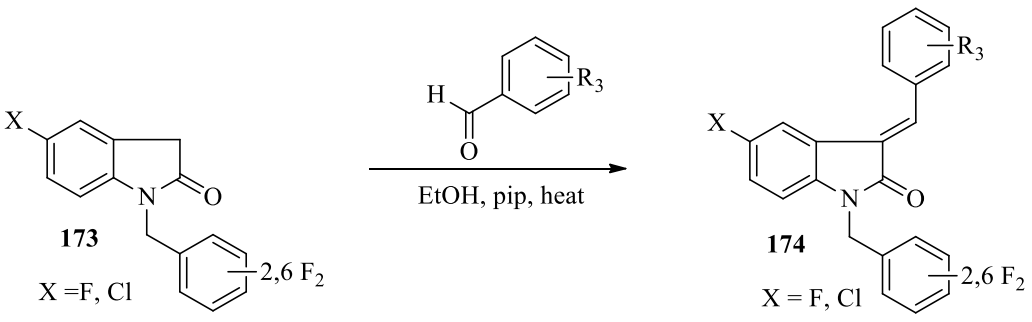
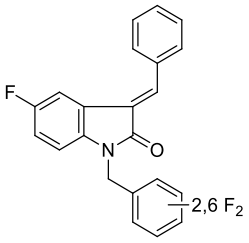
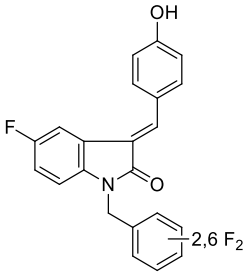
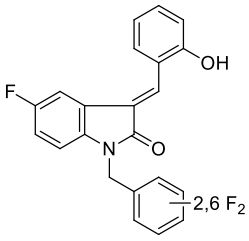
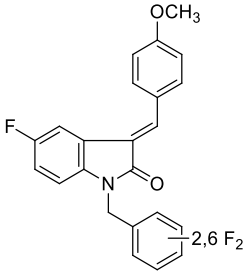
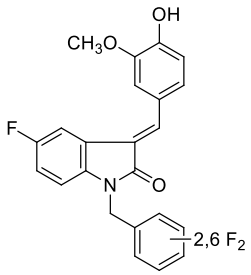
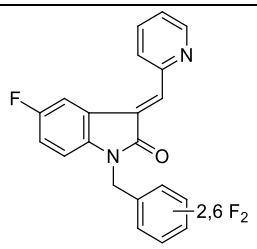
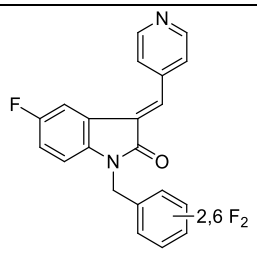
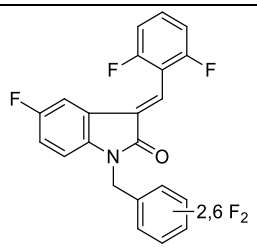
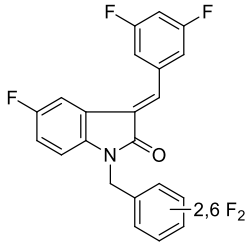
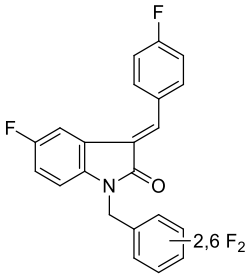
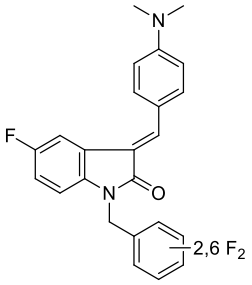
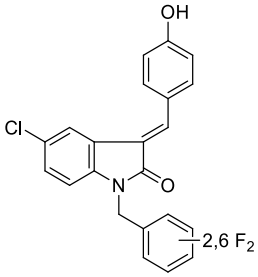
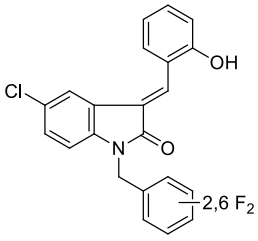


Table 5: *N*-(2,6-Difluorobenzyl)-5-fluoro-3-Substituted-Benzylidene-Indolin-2-ones

	Compound	Percent Yield	Melting Point	Configuration (Z:E)
175		38%	129-131˚C	(0:100)
176		72%	253-255˚C	(0:100)
177		62%	207-211˚C	(0:100)
178		68%	141-144˚C	(40:60)

179		63%	136-140˚C	(30:70)
180		38%	205-208˚C	(0:100)
181		38%	158-161˚C	(40:60)
182		25%	142-144 ºC	(100:0)
183		59%	175-176˚C	(0:100)

184		45%	135-138 ˚C	(0:100)
185		45%	174-176 ˚C	(100:0)
186		69%	250-255 ˚C	(0:100)
187		65%	149-151 ˚C	(0:100)

Although it is hypothesized that the addition of a 2,6-difluorobenzyl substituent at the *N*-position of benzylidene oxindoles is partly responsible for enhanced cell-death inhibition, it is unknown whether the 2,6-difluorobenzyl ring is necessarily required or if the addition of a simple benzyl or alternately substituted benzyl group would suffice. To examine this query, the 5-hydrogen, 5-fluoro and 5-chloro-*N*-benzylindolin-2-ones (**188**)

were reacted in the aldol type condensation reaction with associated aryl-aldehyde (1.2 equiv.) at 90°C for 3-6 h in the presence of catalytic amounts of piperidine (0.147 equiv.) yielding the corresponding arylidene oxindoles **189**. After the appropriate time, the mixture was cooled and the precipitate was collected by vacuum filtration. In some cases, the mixture was cooled but the product would not precipitate out of solution. In these situations, the mixture was evaporated under reduced pressure and silica gel column (70:30 hexanes: EtOAc) was utilized to purify the product. The product was then evaporated under reduced pressure to a solid or oil and recrystallized from dichloromethane and hexanes to afford the pure (TLC/GC-MS) product. The percent yield results and melting points for these reactions are reported in **Table 6** below along with the structure of the product of interest.

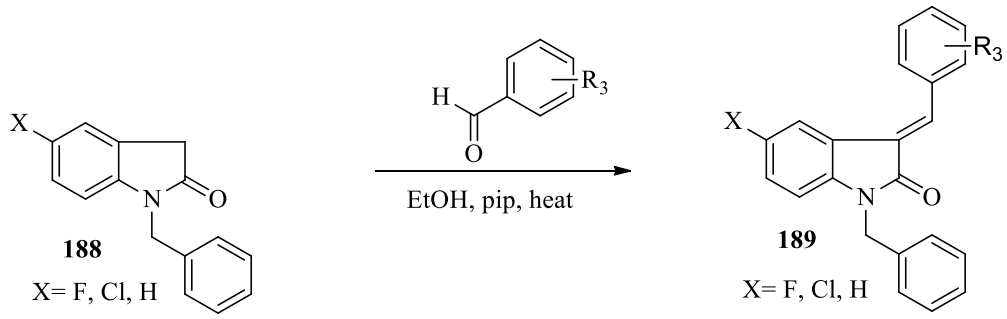
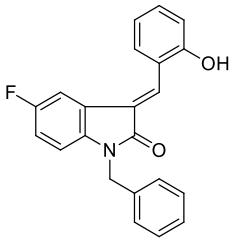
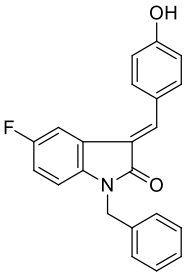
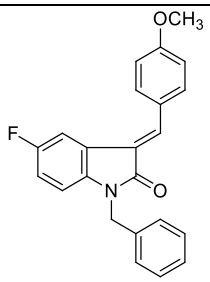
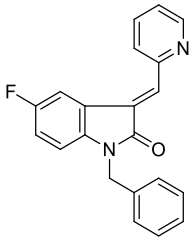
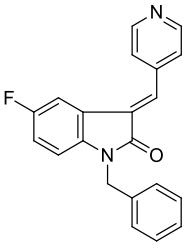
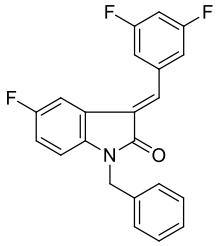
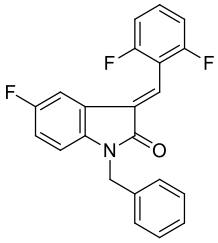
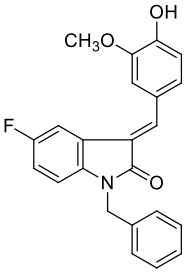
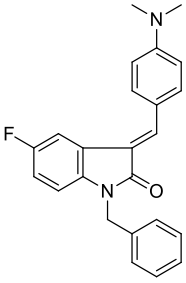
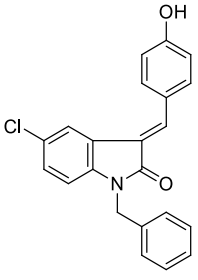
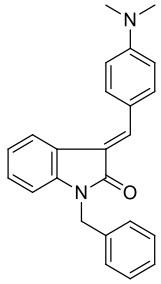


Table 6: *N*-(Benzyl)-5-fluoro-3-Substituted-Benzylidene-Indolin-2-ones

	Compound	**Percent Yield**	**Melting Point**	**Configuration (Z:E)**

190		70%	185-187˚C	(0:100)
191		81 %	236-238 ºC	(0:100)
192		25%	140-142 ºC	(30:70)
193		74%	126-127˚C	(0:100)
194		51%	157-158˚C	(40:60)

195		49%	161-164˚C	(30:70)
196		49%	162-164˚C	(0:100)
197		48%	145-149 ºC	(40:60)
198		76%	139-140˚C	(100:0)
199		82%	255-257 ˚C	(0:100)

200		76%	139-140 ˚C	(70:30)

The orientation of the fluorines on the benzyl ring was also investigated to determine if the substitution pattern had any impact on biological activity. This was accomplished by the incorporation of a 3,5-difluorobenzyl ring at the *N*-position of the isatin scaffold. In this work, 5-fluoro and 5-chloro-*N*-3,5-difluorobenzylindolin-2-ones (**201)** were utilized as substrates in the aldol type condensation reactions with associated aryl-aldehydes (1.2 equiv.) at 90°C for 3-6 h along with catalytic amounts of piperidine (0.147 equiv.) yielding the corresponding arylidene oxindoles (**202**). In some cases, the mixture was cooled but the product would not precipitate out of solution. In these situations, the mixture was evaporated under reduced pressure and a silica gel column (70:30 hexanes: EtOAc) was utilized to purify the product. The product was then evaporated under reduced pressure to a solid or oil and recrystallized from dichloromethane and hexanes to afford the pure (TLC/GC-MS) product. The percent yield results and melting points for these reactions are reported in **Table 7** below along with the structure of the product of interest.

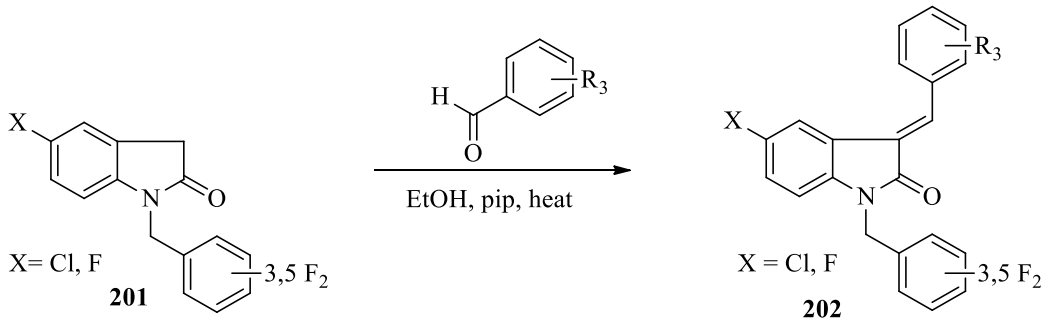
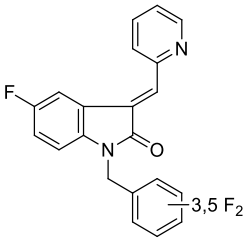
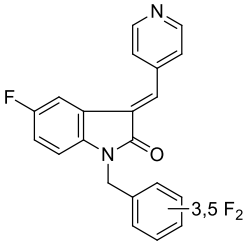
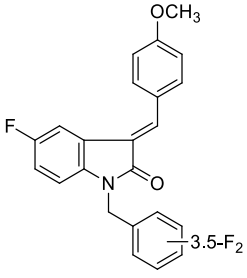
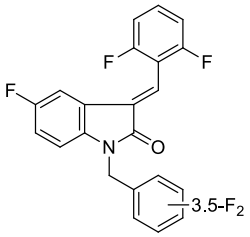
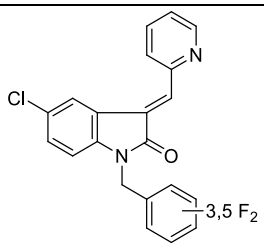
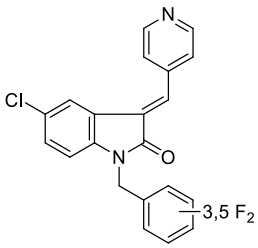
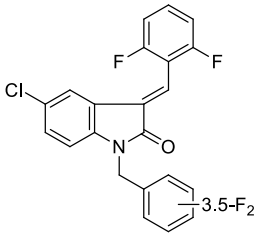


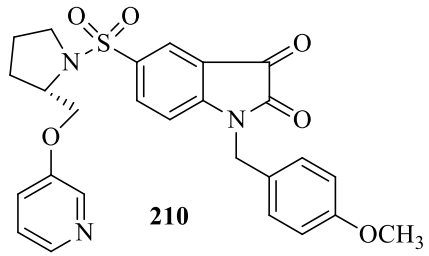
Table 7: *N*-(3,5-Difluorobenzyl)-5-fluoro-3-Substituted-Benzylidene-Indolin-2-ones

	Compound	Percent Yield	Melting Point	Configuration (Z:E)
203		37.1%	204-206˚C	(0:100)
204		59%	138-141 ºC	(40:60)
205		98.9 %	125-126˚C	(40:60)

206		29%	162-164˚C	(100:0)
207		69%	157-159˚C	(0:100)
208		55%	130-133˚C	(40:60)
209		68%	165-167˚C	(0:100)

Additionally, in previous results from the Ketcha Group it had been observed that the incorporation of a moiety with a lone pair of electrons attached to the C-3 benzylidene group (e.g., -OCH_3 or pyridyl-) seemingly enhanced cell-death inhibition presumably through hydrogen bonding to the active site of the caspase. Coincidentally, in the case of isatin sulfonamides, Mach found that 5-(3-pyridin-3-yl-

oxymethyl)pyrrolidine-1-sulfonyl)isatin bearing a 4-methoxy-benzyl ring at the *N*-position (*e.g.*, **210**) was the most potent inhibitor of caspase-3, with IC_{50} of 3.9 μM.[37]



210

Due to these observations, it was hypothesized that incorporating an *N*-substituent with available electrons (*e.g.*, 4-methoxybenzyl-) could potentially increase biological activity in the case of benzylidene oxindoles. This was accomplished by utilizing 5-fluoro or 5-chloro-*N*-(4-methoxybenzyl)-5-fluoro-indolin-2-one (**211**) in the aldol type condensation with associated aryl-aldehyde (1.2 equiv.) at 90°C for 3-6 h in the presence of catalytic amounts of piperidine (0.147 equiv.) yielding the corresponding arylidene oxindoles (**212**). In some cases, the mixture was cooled but the product would not precipitate out of solution. In these situations, the mixture was evaporated under reduced pressure and silica gel column (70:30 hexanes: EtOAc) was utilized to purify the product. The product was then evaporated under reduced pressure to a solid or oil and recrystallized from dichloromethane and hexanes to afford the pure (TLC/GC-MS) product. The percent yield results and melting points for these reactions are reported in **Table 8** below along with the structure of the product of interest.

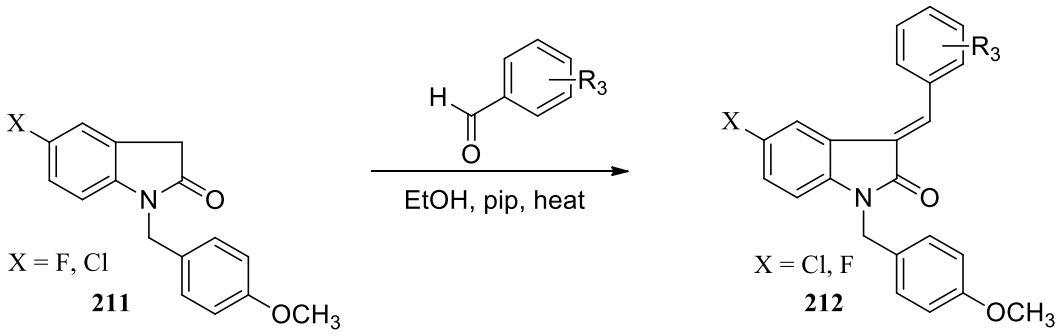
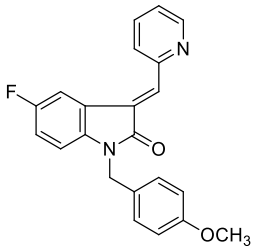
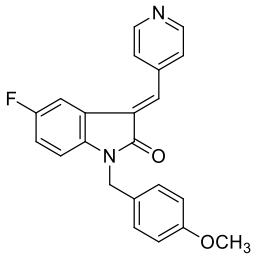
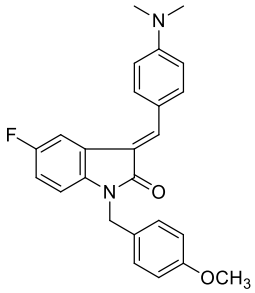
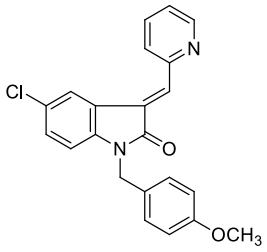
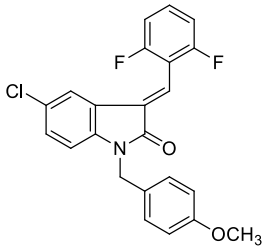


Table 8: *N*-(4-Methoxybenzyl)-3-Substituted-Benzylidene-Indolin-2-ones

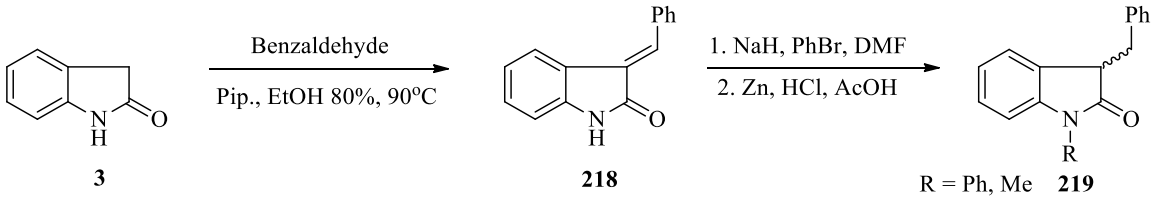
	Compound	Percent Yield	Melting Point	Configuration (Z:E)
213		75%	139-140˚C	(0:100)
214		66%	135-139˚C	(40:60)
215		72%	140-143˚C	(70:30)

216		99%	152-155 ˚C	(0:100)
217		75%	159-162 ˚C	(100:0)

N-Alkylation of Benzylidene Oxindoles

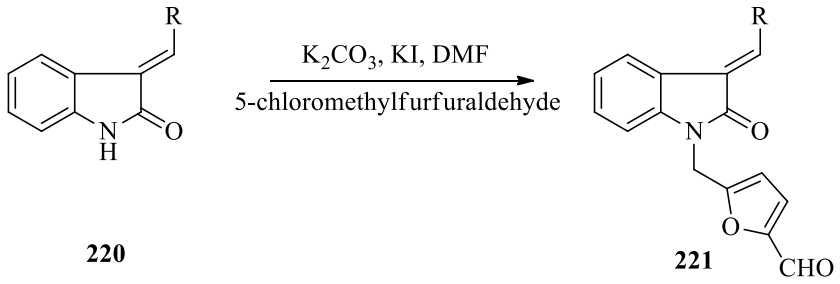
The *N*-alkylation of an arylidene- or benzylidene oxindole is a reaction that has seen only limited usage and sometimes as the first step in a multi-step sequence (without isolation or characterization of the initial alkylated product). In terms of preparing *N*-alkylated beznylidene oxindoles, the standard strategy involves initial *N*-alkylation of the isatin followed by Wolff-Kishner, and ultimately the aldol condensation. However, in order to take full advantage of the *libraries from libraries* concept, we visualized an alternate strategy commencing with the aldol reaction of an oxindole so as to afford a library of *N*-H benzylidene oxindoles (i.e., kinase inhibitors), followed by the *N*-alkylation of this sublibrary. As stated before, this reaction upon benzylidene oxindoles is not well known and just recently Overman *et al.* reported this alkylation as a protocol to *N*-alkylated 3-substituted oxindoles in a sequence involving an initial aldol upon an oxindole to afford a benzylidene oxindole followed by an *N*-alkylation using sodium hydride in DMF immediately followed by reduction of the alkene double bond by zinc

under acidic conditions to yield the C-3 alkylated products(e.g., **219**).[128] A similar approach was employed by Trost and Zhang for the introduction of a MOM protecting group on the nitrogen of benzylidene oxindoles.[129]



3 **218** **219**

In what represents the most extensive study to date, Zhou *et al*. developed an alternative synthesis for the *N*-alkylation of a benzylidene oxindole **220** in the attempt to develop 3-substituted-*N*-(5-formylfurfuryl)-indolin-2-ones **221**.[23] In this synthesis, the benzylidene oxindoles were attained through an aldol condensation with an aldehyde and piperidine in refluxing ethanol for 3-5 h. The resulting benzylidene oxindoles were then alkylated with 5-chloromethylfurfuraldehyde in the presence of K_2CO_3 as well as a catalytic amount of KI in DMF to produce the desired 3-substituted-*N*-(5-formylfurfuryl)-indolin-2-one **221** in varying yields between 5-70%**.**[23]



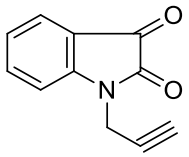
220 **221**

As can be seen from the examples provided above, the *N*-alkylation of benzylidene oxindoles is a fairly new reaction that has not been thoroughly studied. The existing synthetic methods for this reaction sometimes involve working with hazardous

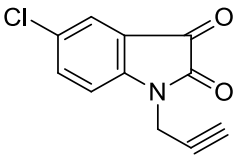
solid bases (*e.g.*, NaH) and/or long drawn out workups due to the use of very polar aprotic solvents like DMF. These workups require multiple extractions with ethyl acetate (EtOAc) or dichloromethane (DCM) and column chromatography. Thus, discovering new methods to *N*-alkylate benzylidene oxindoles appears to be important because of the encouraging medicinal properties of such compounds. The possibility of readily adding variability to *N*-H benzylidene oxindoles can only serve to increase the chances of discovering biologically active compounds based upon this scaffold.

Alternative Route of Synthesis

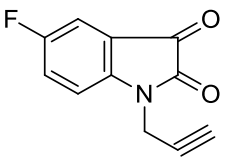
It was originally expected that the synthetic sequence involving initial *N*-alkylation of isatins followed by Wolff-Kishner reduction and final aldol condensation of the *N*-alkylated oxindole could be utilized to create molecules bearing an *N*-propargyl moiety which could be utilized in cycloaddition reactions with azides as a means of preparing PET imaging agents by click chemistry (*vide infra*). To accomplish this task, isatin and 5-substituted isatins (5-chloro-, 5-fluoro-) were *N*-alkylated with KF/alumina in acetonitrile (or DBU in ethanol) with propargyl bromide to yield the corresponding *N*-propargyl derivatives **130, 135,** and **141**, respectively.



130



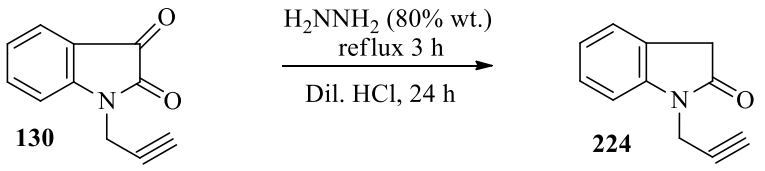
135



141

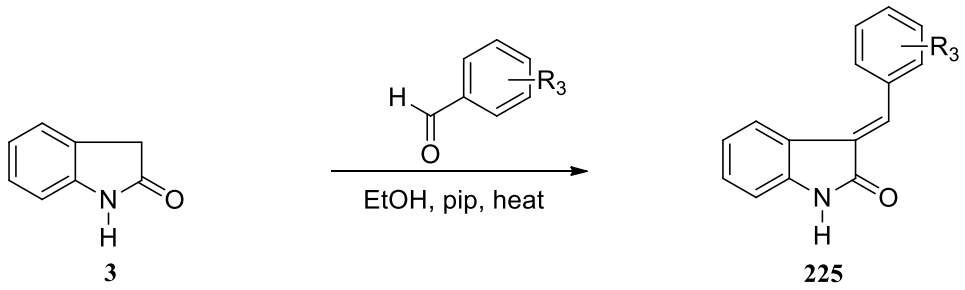
The next step in the proposed sequence involved Wolff-Kishner reduction of the *N*-propargyl-indolin-2,3-dione compounds prior to aldol condensation. However,

attempted Wolff-Kishner reduction of *N*-propargyl-indolin-2,3-one **(130)** (with or without subsequent acidification of the reaction mixture) resulted in degradation of the substrate. In this Wolff-Kishner reaction, *N*-propargyl-indolin-2,3-dione **(130)** and the hydrazine hydrate were placed in a reaction vessel that was wrapped with aluminum foil and the mixture was refluxed for 3 h. After heating, the mixture was poured in to a beaker submerged into an ice bath and acidified with dilute hydrochloric acid and left undisturbed overnight. However, the precipitate that formed from this reaction was black and intractable. A second Wolff-Kishner reaction was performed on *N*-propargyl-indolin-2,3-dione **(130)**, wherein after heating for 3 h, the mixture was poured into a beaker submerged into an ice bath and left undisturbed overnight. In this case, the mixture was not acidified with dilute hydrochloric acid and the precipitate obtained consisted of a small amount of orange, gummy oil. GC-MS analysis of this oil indicated starting material. Upon determining that the C-3 carbonyl of *N*-propargyl-indolin-2,3-dione could not be effectively reduced utilizing the Wolff-Kishner reaction, it was envisioned that an alternate synthesis would be required to create the desired 3 points of variability on the isatin or oxindole scaffold.



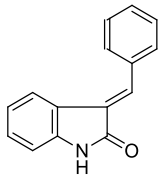
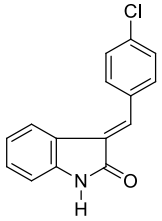
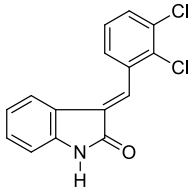
An alternative synthesis was therefore envisioned involving an initial Wolff-Kishner of an isatin substrate followed by an aldol condensation and finally ending with an *N*-alkylation of the resultant benzylidene oxindole. To that end, a variety of *N*-H oxindoles were subjected to aldolization with a variety of aromatic aldehydes to create

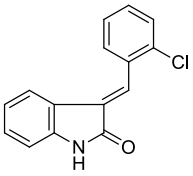
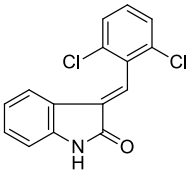
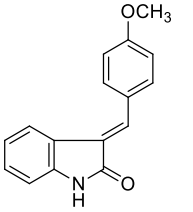
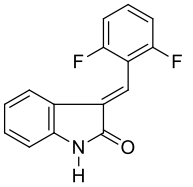
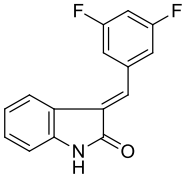
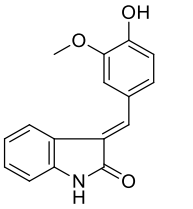
series of sublibraries with various C-5 substituents. For the 5-H series, oxindole was reacted with aryl aldehydes (1.2 equiv.) under standard conditions leading to the compounds shown below in **Table 9.**



3 **225**

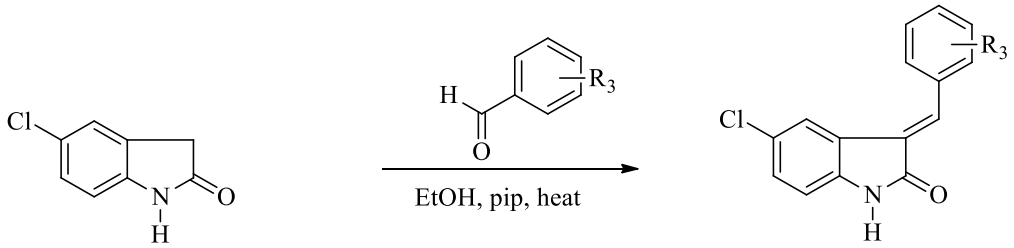
Table 9: 3-substituted-benzylidene-indolin-2-ones

	Compound	Percent Yield	Melting Point	Literature Melting Point	Configuration (Z:E)
226		81%	169-172˚C	175-176˚C[139]	(0:100)
227		83%	186-188˚C	188-190˚C[140]	(0:100)
228		84%	205-207˚C	N/R	(60:40)

229		89%	174-176˚C	N/R	(100:0)
230		29%	181-182˚C	188-189˚C[141]	(0:100)
231		91%	154-157˚C	158-159˚C[142]	(40:60)
232		79%	217-220˚C	213-218˚C[143]	(100:0)
233		54%	197-199˚C	202-205˚C[143]	(100:0)
234		86%	218-220˚C	N/R	(0:100)

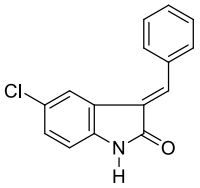
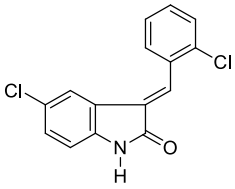
Similarly, a library of benzylidene oxindoles containing an electron-withdrawing group at the 5-position of the oxindole scaffold was constructed. 5-Chloro-oxindole was chosen because it was much easier and efficient to synthesize in large quantities

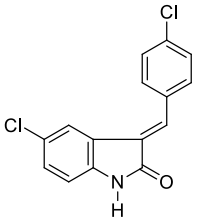
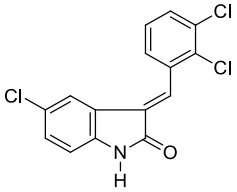
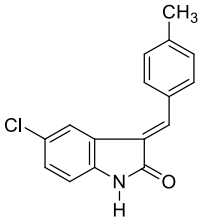
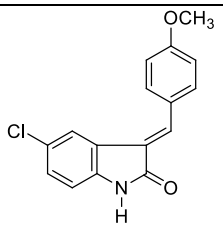
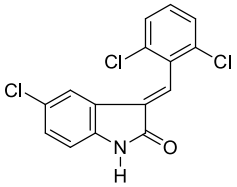
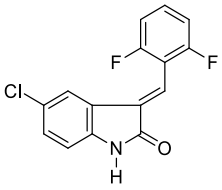
compared to the 5-fluoro analogue. In this aldol reaction, 5-chloro oxindole (**152)** and associated aryl-aldehydes (1.2 equiv.) were mixed at 90°C for 3-6 h in the present catalytic amounts of piperidine (0.147 equiv) yielding **235**. The percent yield results and melting points for these reactions are reported in **Table 10** below along with the structure of the product of interest

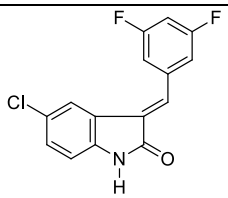
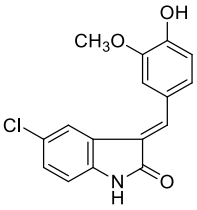
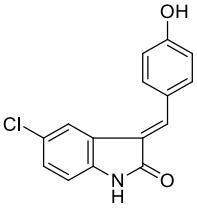
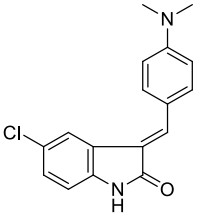


152 **235**

Table 10: 5-chloro-3-substituted benzylidene-indolin-2-ones

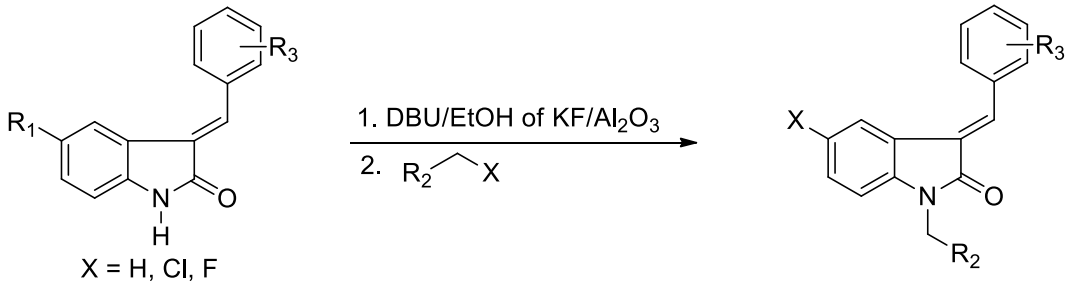
	Compound	Percent Yield	Melting Point	Literature Melting Point	Configuration (Z:E)
236		97%	204-205˚C	210˚C[126]	(0:100)
237		90%	249-250˚C	N/R	(100:0)

238		78%	247-248˚C	252-254˚C[126]	(40:60)
239		85%	273-276˚C	N/R	(80:20)
240		51%	218-220	220-223[150]	(60:40)
241		88%	218-220˚C	219-220˚C[126]	(20:80)
242		64%	215-218˚C	197-199˚C[151]	(0:100)
243		80%	245-246˚C	242-244˚C[126]	(100:0)

244		59%	158-162˚C	N/R	(0:100)
245		83%	227-231˚C	N/R	(80:20)
246		65%	288-290˚C	N/R	(0:100)
247		65%	250-253 ˚C	257-260˚C[151]	(0:100)

The final step of the alternative synthesis, involved an investigation into the *N*-alkylation of the aforementioned benzylidene oxindoles utilizing two different bases; the solid supported base KF/alumina or the soluble liquid base DBU. The *N*-alkylation utilizing KF/alumina as base involved the reaction of various benzylidene oxindoles (1 mmol) along with KF/alumina (6 equiv.) in acetonitrile. The mixture was placed on a heating mantle and allowed to stir at room temperature for 10-15 min, after which time various alkyl halides (3 equiv.) were added to the reaction mixture and the mixture was placed under reflux for 24-36 h. The solid base was then removed via vacuum filtration

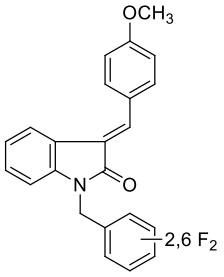
and the filtrate was evaporated under reduced pressure and purified with silica gel column (hexanes: ethyl acetate, 70:30). The product was then evaporated under reduced pressure to a solid or oil and recrystallized from dichloromethane and hexanes to afford the pure (TLC/GC-MS) product.



Benzylidene oxindoles were also *N*-alkylated utilizing the soluble liquid base DBU. This was accomplished through the reaction of various benzylidene oxindoles (1 mmol) and DBU (2 equiv.) in ethanol. The mixtures were placed on a heating mantle and allowed to stir at room temperature for 10-15 min before the addition of an alkyl halide (3 equiv.), after which time the reaction was refluxed for 24 h and the crude reaction mixture was evaporated under reduced pressure and purified with silica gel column (hexanes: ethyl acetate, 70:30). The product was then evaporated under reduced pressure to a solid or oil and recrystallized from dichloromethane and hexanes. The *N*-alkylation of benzylidene oxindoles with DBU was also performed in the CEM microwave. This was performed by placing benzylidene oxindole (1 mmol), DBU (2 equiv.), and alkyl halide (3 equiv.) into a 10 mL microwave vial with ethanol (3 mL) and heating the reaction for 20 min at 180 ^{o}C. The reaction mixture was then evaporated under reduced pressure and purified with silica gel column (hexanes: ethyl acetate, 70:30). The product was then evaporated under reduced pressure to a solid or oil and recrystallized from

dichloromethane and hexanes. The optimal parameters for this process were determined by varying time and temperature in temperature ranges from 120 ^{o}C to 180 ^{o}C, and time ranges from 10 to 20 min. Temperature ranges were increased in 20 ^{o}C increments and time was increased in 5 minute increments. Upon attempting to *N*-alkylate these benzylidene oxindoles, it was discovered that benzylidene oxindoles substituted at the 3-position with a pyridine could not be effectively alkylated. It was hypothesized that when *N*-alkylating the benzylidene oxindole, the nitrogen on the pyridine would compete with the nitrogen in the isatin ring causing the creation of a salt thus preventing the reaction from moving to completion. Therefore, the desirable, biologically active pyridine benzylidene oxindole derivatives could not be *N*-alkylated. The results of the reaction between various benzylidene oxindoles and alkyl halides in the presence of DBU or KF/alumina and utilizing either traditional reflux (R) and microwave heating techniques (M) are presented in **Table 11**. Likewise, results from the the reaction between benzylidene oxindoles and propargyl bromide are shown in **Table 12**..

Table 11: Arylidene Oxindoles via *N*-Alkylation of Benzylidene Oxindole

	Compound	**Percent Yield**	**Melting Point**	**Literature Melting Point**	**Configuration (Z:E)**
248		DBU (R): 45% DBU (M): 41% KF/Alumina (R): 51%	139-141˚C 135-138˚C 139-141˚C	N/R	(40:60)

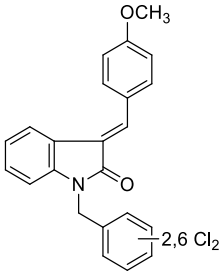
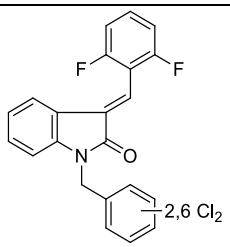
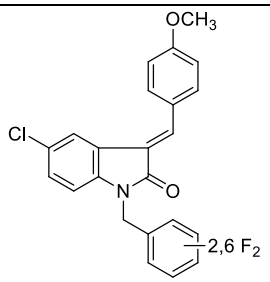
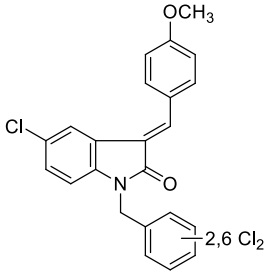
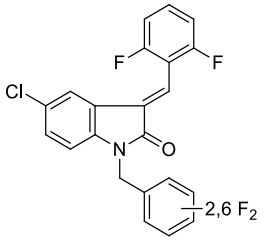
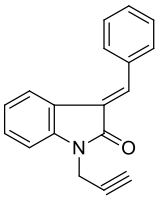
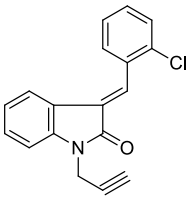
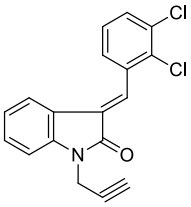
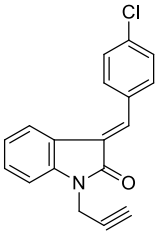
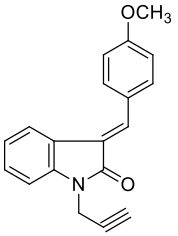
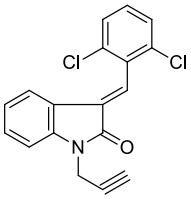
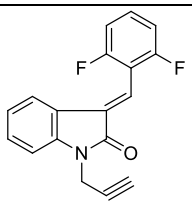
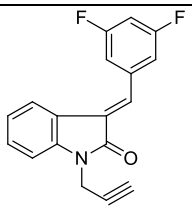
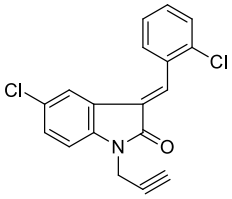
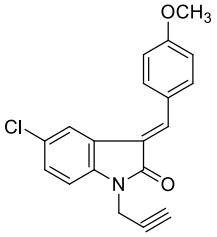
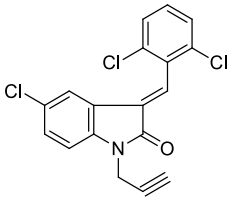
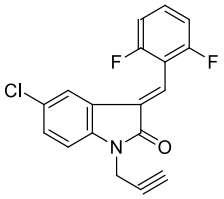
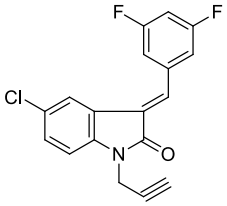
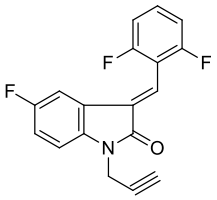
249		DBU (R): 28% DBU (M): 68% KF/Alumina (R): 25%	135-138˚C 140-142˚C 147-149˚C	N/R	(40:60)
250		DBU (R): 59% DBU (M): 62% KF/Alumina (R): 51%	137-139˚C 135-138˚C 139-141˚C	N/R	(100:0)
251		DBU (R): 77% DBU (M): 48% KF/Alumina (R): 73%	167-170˚C 163-165˚C 160-165˚C	N/R	(100:0)
252		DBU (R): 73% DBU (M): 51%	214-216˚C 215-217˚C	N/R	(40:60)
253		DBU (R): 41% DBU (M): 41% KF/Alumina (R): 65%	163-166˚C 169-171˚C 167-169˚C	N/R	(100:0)

Table 12: *N*-Propargyl-3-substituted benzylidene-indolin-2-ones

	Compound	Base	Percent Yield	Melting Point	Configuration (Z:E)
254		DBU (M)	45%	121-124˚C	(10:90)
255		DBU (M)	51%	174-177˚C	(80:20)
256		DBU (M)	33%	151-155˚C	(80:20)
257		DBU (M)	34%	125-128˚C	(30:70)
258		DBU (M)	51%	95-98˚C	(40:60)

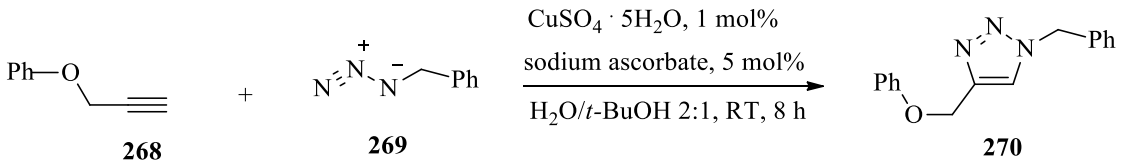
259		DBU (M)	26%	167-169˚C	(0:100)
260		KF/Alumina (R)	17%	123-125˚C	(100:0)
261		DBU (M)	57%	132-134˚C	(60:40)
262		DBU (M)	49%	106-109˚C	(70:30)
263		KF/Alumina (R)	66%	127-129˚C	(40:60)
264		DBU (M)	74.0%	107-110 ˚C	(100:0)

265		DBU (M)	23%	119-121˚C	(100:0)
266		DBU (M) KF/Alumina (R)	62%	177-179˚C	(0:100)
267		KF/Alumina (R)	53%	171-175˚C	(100:0)

"Click Chemistry"

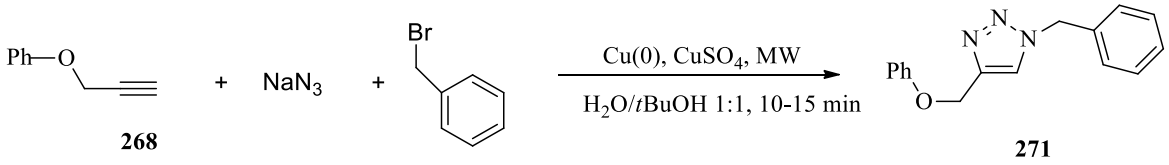
The term "*click chemistry*" was coined by Sharpless *et al*. on the basis of developing an expanding set of powerful, selective, and modular building blocks that add together reliably in both small and large scale applications.[130,131] A set of criteria was developed for a reaction to be considered "click chemistry" which include: the reaction must be modular, wide in scope, give high yields, generate only inoffensive byproducts that can be removed by non-chromatographic methods, and be stereospecific. A "click reaction" should involve simple reaction conditions; have readily available starting materials and reagents, and simple product isolation.[130] Sharpless thought of these reactions as being "*spring loaded*" for a single trajectory because of the speed at which they should proceed. The main focus here will be towards "click chemistry" reactions such as 1,3-dipolar cycloadditions involving two unsaturated reactants which provides

fast access to an enormous variety of five and six-membered heterocycles.[132] Of these 1,3-dipolar cycloaddition reactions, the Huisgen dipolar cycloaddition of azides and alkynes resulting in 1,2,3-triazoles is considered important because of the favorable biological properties they have exhibited.[133] Although azides are rarely utilized in organic synthesis, they are believed to be the most reliable way to introduce a nitrogen substituent through the 1,3-dipolar cycloaddition reaction.[133] The main downside of the original azide/alkyne reaction was that the triazole forming reaction would usually result in a mixture between the 1,4- and 1,5-regioisomers.[132] However, Rostovtsev *et al*. developed a synthesis in which copper (I) was utilized to combine a terminal acetylene **268** and an azide **269** to produce only the 1,4-disubstituted 1,2,3-triazole **270**.[133] It was discovered that making the copper catalyst *in situ* by reduction of copper (II) salts in the form of $CuSO_4 \cdot 5\ H_2O$ worked the best for the reaction and the use of sodium ascorbate allowed for a variety of substituents with high yields. It was also discovered that a variety of solvents like ethanol, *tert*-butyl alcohol, and water could be utilized at ambient temperatures for anywhere between 6-36 h.[133]



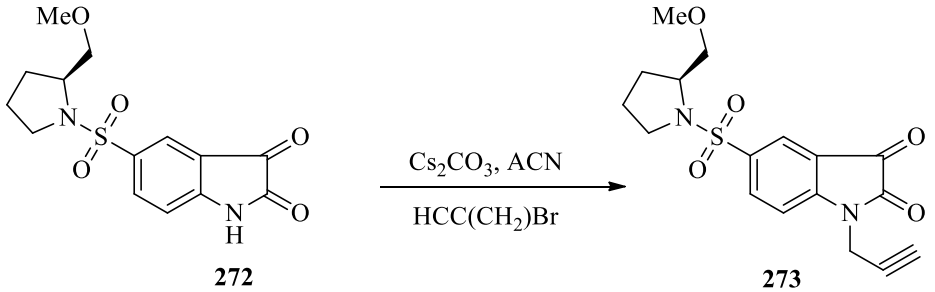
Appukkuttan *et al.* expanded on the click chemistry of 1,2,3-triazoles by developing a more efficient method that shortened reaction times from 6 h to just 10-15 min through the use of microwave irradiation. To optimize this reaction, benzyl bromide and phenylacetylene (**268**) were chosen as the starting materials.[134] These starting

materials along with sodium azide were suspended in a 1:1 mixture of *t*-BuOH and water with the copper (I) catalyst which was prepared *in situ* through the combination of Cu(0) and Cu(II).[134] The mixture was irradiated for 10 min at 100 Watt and 125°C producing the desired product 1-benzyl-4-phenyl-1*H*-1,2,3-triazole (**271**) in 93% yield with no trace of the 1,5-regioisomer.[134] This microwave-enhanced, regioselective reaction allows for an efficient means of synthesizing 1,4-disubstituted-1,2,3-triazoles in a timely manner.



268 **271**

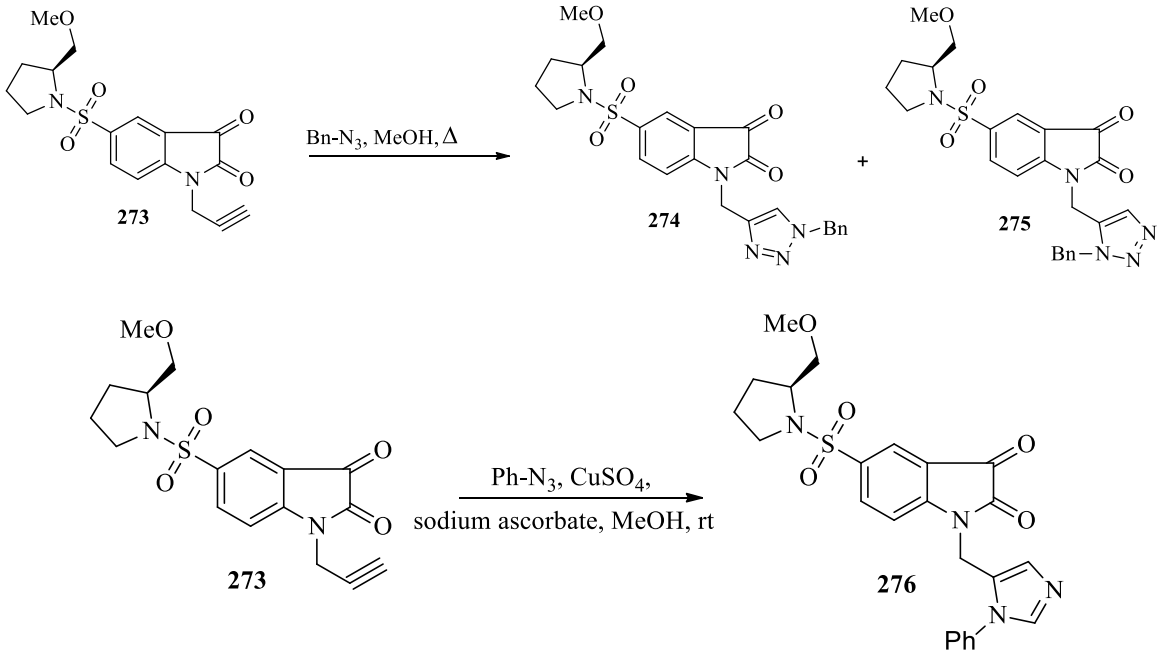
Recently, Jiang *et al.* developed a route towards isatin 1,2,3-triazoles as potential caspase inhibitors.[135] In this synthesis, 5-(*S*)-2-(methoxymethyl)pyrrolidinylsulfonyl isatin (**272**) was alkylated in acetonitrile using 3-bromopropyne in the presence of Cs_2CO_3 to produce 5-(*S*)-2-(methoxymethyl)-pyrrolidinylsulfonyl-*N*-prop-2-ynyl isatin (**273**).[135]



272 **273**

The desired 1,2,3-triazoles were then obtained through 2 different methods involving either a thermal cycloaddition reaction or copper (I) catalyzed cycloaddition reaction. In the thermal cycloaddition reaction, **273** was combined with the benzyl azide

in methanol and heated, yielding both the 1,4- and 1,5-disubstituted triazole isomers (**274, 275)** which were then separated by chromatography. In the copper catalyzed cycloaddition reaction, **273** was placed in a mixture with phenyl azide, $CuSO_4$, and sodium ascorbate in methanol at room temperature yielding only the 1,4-disubstituted–phenyl-triazole **274**.[135]

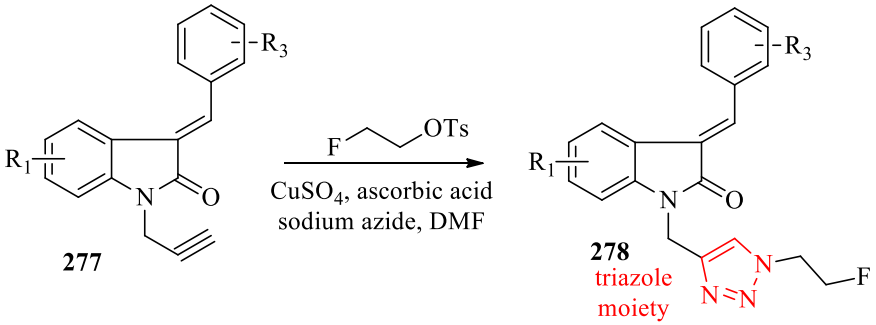


It was determined that the 1,4-disubstituted 1,2,3-triazole **274** was more potent against caspase-3 the the 1,5-regioisomer **275**. Interestingly, in the case of triazoles derived from phenyl azide, the 1,5-disubstituted isomer (e.g., **276)** exhibited 2-fold higher potency than the 1,4-regioisomer derived from benzyl azide (**274)**. Jiang *et al.* was able to conclude that the substitution pattern in the 1,2,3 triazole ring affected the binding affinity towards caspase-3 which explains why the potency of 1,4-disubstituted 1,2,3-triazoles was higher in the case of tyriazoles produced from phenyl azide but 1,5-

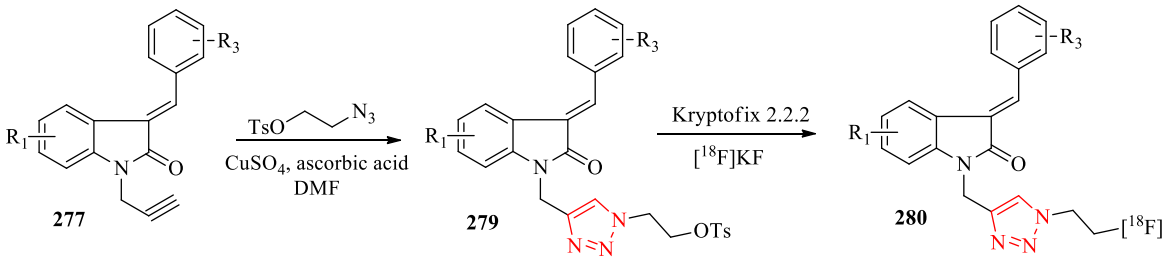
disubstituted 1,2,3-triazoles prepared from benzyl azide exhibited higher potency than that of the 1,4-isomer.[135]

WSU Approach to "Click" chemistry

The main advantage in synthesizing the *N*-alkylated propargyl compounds was their capability to take part in a "click" reaction to create the isatin 1,2,3-triazole which could theoretically be utilized to perform radiochemistry with a [^{18}F]-radiolabel. The goal was to identify a caspase inhibitor in which an ^{18}F radioisotope may be attached through the "click" reaction and utilize this inhibitor as a possible PET imaging agent for apoptosis. To accomplish this task, the *N*-alkynyl derivatives in **Table 12** would be reacted with 2-fluoroethyltosylate in the presence of $CuSO_4$, ascorbic acid and sodium azide in DMF to attain the cold version of the desired triazole targeting agent.



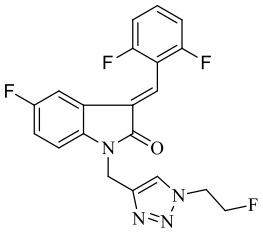
If effective, the radiolabeled [^{18}F]-fluoroethylazide could then be prepared by nucleophilic fluorination of 2-azidoethyl-4-toluenesulfonate with Kryptofix 2.2.2 [^{18}F] KF to create the [^{18}F]-radiolabeled triazole.



The table below shows *three proof of concept* compounds which were synthesized as cold versions of potential cell death imaging agents for PET imaging. Since the most active C-3 pyridyl-derivatives could not be utilized because of the presumed creation of a salt during the *N*-alkylation step, it was decided to utilize the next most potent compound which was 3-(2,6-difluorobenzyl)indolin-2-one. Thus, 3-(2,6-difluorobenzyl)indolin-2-one (**170**) was *N*-alkylated with propargyl bromide and KF/alumina in acetonitrile to produce *N*-propargyl-5-fluoro-3-(2,6-difluorobenzyl)indolin-2-one (**267**). A click reaction was then performed on the *N*-propargyl-5-fluoro-3-(2,6-difluorbenzyl)indolin-2-one (**267**) in a vial with 2-fluoro-ethyl-4-toluenesulfonate (1 equiv), copper (II) sulfate (5 mol%), ascorbic acid (10 mol %) and sodium azide (1.1 equiv) in DMF to create 5-fluoro-1-[1-(2-fluoro-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-3-(2,6-difluorobenzyl)indolin-2-one (**281**).

Table 13: Isatin Triazole

	Compound	**Percent Yield**	**Melting Point**

281		76%	170-174^{o}C

NMR Analysis

NMR Analysis of N-Substituted Isatins

All of the *N*-substituted compounds made by microwave heating using DBU listed in Table 1 were achieved in moderate to high yields. These *N*-alkylated isatins molecules were further characterized by ^{13}C NMR spectroscopy using the Bruker Avance 300 MHz NMR. These samples were compared to previously made derivatives prepared using conventional heating and KF/Alumina. However, since the 5-fluoroisatin molecules had not previously been synthesized in the WSU library, the ^{1}H NMR and ^{13}C NMR spectra were utilized to fully characterize these compounds. Prior to the analysis of the substituted molecules, a thorough analysis of the chemical shifts of 5-fluoroisatin **(Figure 2)** itself was completed and compared to shifts reported in the literature and were found to agree accordingly. All of the following spectra generated were analyzed and expanded using SpinWorks 3.1. The ^{1}H NMR spectrum of 5-fluoroisatin (Sigma-Aldrich) is presented below in **Figure 3** There are clearly four signals which represent the four protons found on the 5-fluoroisatin molecule.

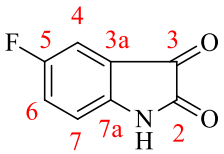


Figure 2.

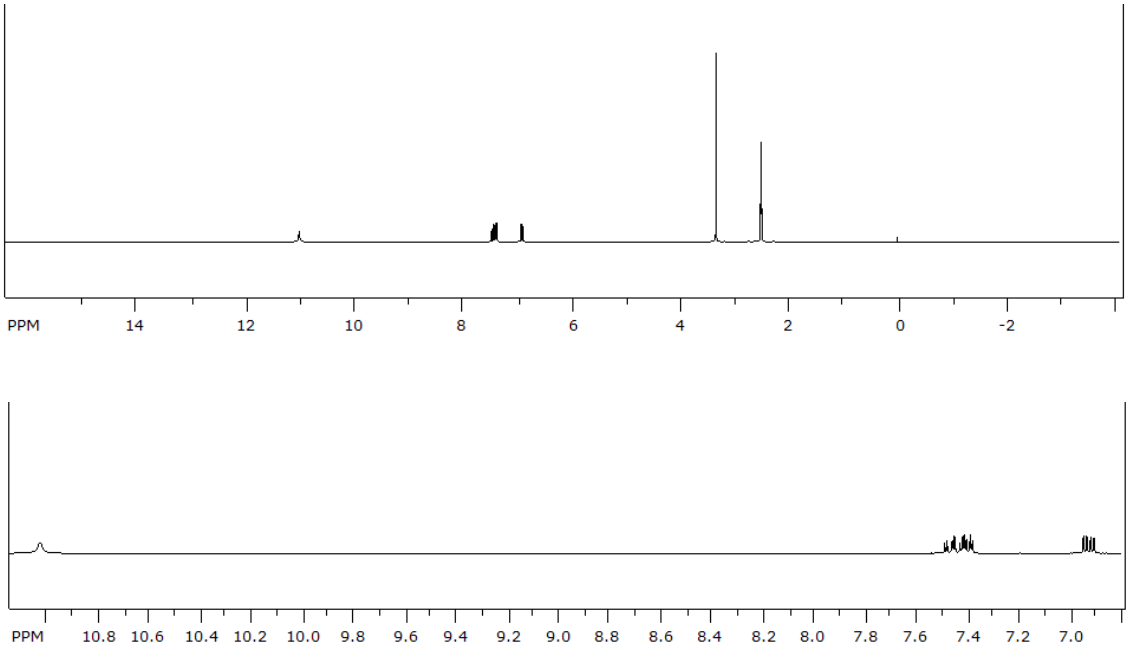


Figure 3.

The signal furthest downfield is a singlet at 11.03 ppm and its downfield shift is due to a strong deshielding caused by the effect of the nitrogen atom bonded to this proton. There is a doublet of doublets at 7.48 ppm (J = 2.7 Hz, J = 17.9 Hz), a doublet of doublets at 7.50 ppm (J = 3.9 Hz, J = 8.5 Hz), and a doublet at 7.40 ppm (J = 7.2 Hz) which are assigned to the protons labeled 4, 6, and 7 respectively. The signals from proton 4 appear further downfield due to the proximity to the carbonyl group. The ^{13}C NMR spectrum **(Figure 4)** of 5-fluoroisatin is presented below and shows eight signals which correspond to the eight carbons in the 5-fluoroisatin molecule. The peak furthest downfield at 183.8 ppm (d, J = 2.3 Hz) correlates to the C-3 ketone carbonyl group of the 5-fluoroisatin molecule, while the amide carbonyl appears at 159.4 ppm (d, J = 1.3 Hz). The C-5 fluoro-carbon exhibits a fairly large splitting pattern appearing at 159.6-156.4 (d, J = 240 Hz) ppm. Carbon 3a appearing at 118.5-118.4 (d, J = 7.1 Hz) ppm and 7a appearing at 146.9 (d, J = 1.6 Hz) ppm like carbons 2 and 3 are quaternary carbons and

consequently do not appear in the 13C DEPT spectrum. The remaining signals for carbons 4, 6, and 7 fall within the aromatic region of the spectrum and appear at 124.6-124.3 (d, J = 24.0 Hz), 111.5-111.1 (d, J = 24.0 Hz), and 113.4-113.3 (d, J = 7.3 Hz) respectively. For reference, the solvent signal appears at 39.6 ppm for DMSO-d_6.

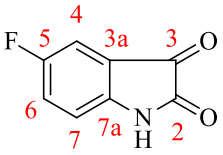


Figure 2.

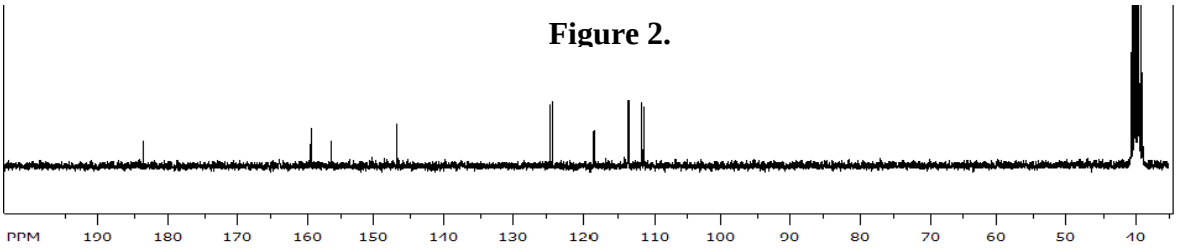
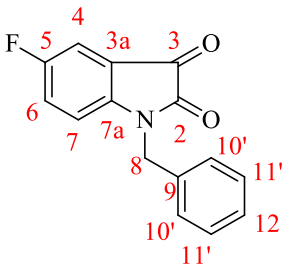
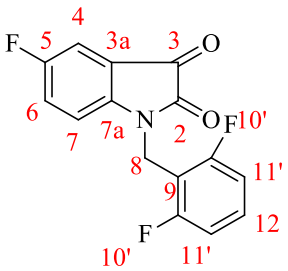


Figure 4.

The NMR spectra for the N-alkylated products were similarly analyzed as well, and as expected there are several shifts of the original carbon peaks due the addition of a group at the nitrogen atom, along with of course the appearance of new proton and carbon signals. The addition of either a benzyl group or a 2,6-difluorobenzyl group at the *N*-position of the 5-fluoroisatin resulted in a shift of the original carbon peaks (*vide infra*).



138



139

The ^{13}C NMR spectrum of **138a** is shown below which gives 13 signals.

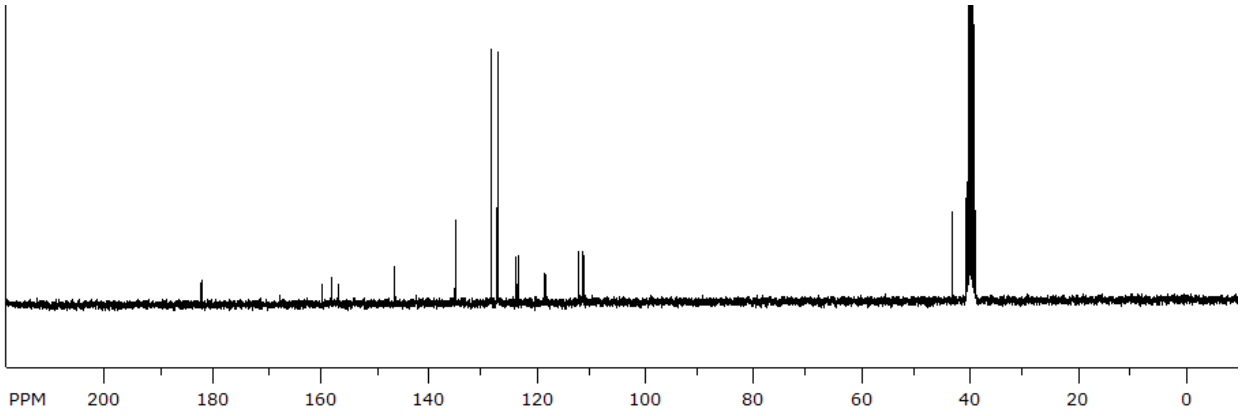


Figure 5

In **Figure 5** above the DMSO-d_6 signal appears at 40.35 ppm. There are two carbonyl peaks which are located at 182.4 ppm for carbon 3 and 158.3 ppm (d, J = 1.4 Hz) for carbon 2. In addition the remaining aromatic peaks for carbons 3a-7a are consistent with those of the 5-fluoroisatin molecule carbon shifts and only change slightly, appearing at 118.7-118.6 (d, J = 7.2 Hz), 123.9-123.6 (d, J = 24.0 Hz), 160.09-156.86 (d, J = 241 Hz), 111.6-111.3 (d, J = 24.3 Hz), 112.4-112.3 (d, J = 7.4 Hz), and 146.5-146.4 (d, J = 1.6 Hz) ppm, respectively. In addition the spectrum includes an aliphatic peak due to carbon 8, which appears at 42.9 ppm. Likewise there are an additional four signals which correspond to the benzyl phenyl ring. The signal for the quaternary carbon 9 appears at 135.3 ppm and does not appear in the 13C DEPT spectrum. The carbons 10’ and 10’ are symmetric and are shown as a large intense peak at 127.3 ppm, as does the signal for carbons 11’ and 11’ which appears at 128.6 ppm. Carbon 12 appears at 127.5 ppm in the spectrum. The ^{13}C NMR spectrum of **139** is shown below in **Figure 6** which gives 13 signals.

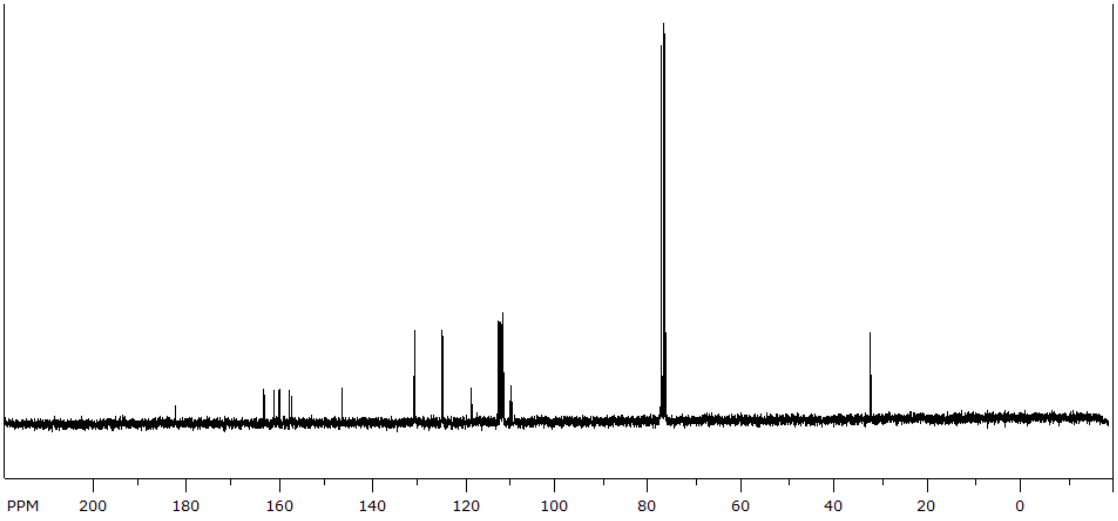


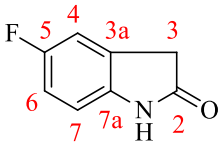
Figure 6

The main difference in the spectrum of compound **139** compared to that of **138** could be seen in carbons C-8, C-9, C-10', C-11', and C-12 in which carbon shifts could be observed at 32.2 (t, *J* = 3.9 Hz), 110.9-110.4 (t, *J* = 18.7 Hz), 162.6-159.2 (dd, *J* = 7.7 Hz), 112.0-111.7 (dd, *J* = 7.3 Hz), and 130.9-130.6 (t, *J* = 10.5 Hz) respectively. The splitting in compounds **138** and **139** could be attributed to the fluorine atom at the C-5 position of the isatin scaffold. Other *N*-substituted 5-fluoroisatins were synthesized in Table 2 through the use of KF/Alumina and ACN under conventional heating conditions. These compounds produced spectra that only slightly deviated from the spectra produced by compound **138** and **139**.

NMR Analysis of 5-fluorooxindoles and *N*-Substituted 5-fluorooxindoles

The compounds synthesized in Table 2 were prepared through the use of a Wolff-Kishner reaction in which hydrazine hydrate was used to reduce the C-3 carbonyl to a methylene. The chemical shifts and splitting patterns in the ^{13}C NMR spectrum for these types of compounds were very similar to that of the 5-fluoroisatin and the *N*-substituted

5-fluoroisatins except for slight chemical shifts. For example the ^{13}C NMR spectrum of **158** is shown below, which gives 8 signals.



157

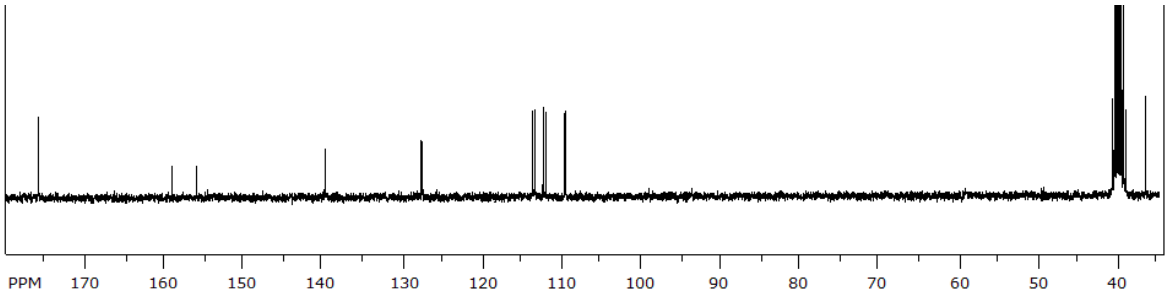
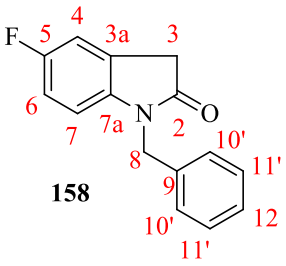


Figure 7

Figure 7 above displays one carbonyl peak which is located at 176.1 ppm for carbon 2. In addition the remaining aromatic peaks for carbons 3a-7a are consistent with those of the 5-fluoroisatin molecule carbon shifts and only change slightly, appearing at 127.6-127.5 (d, J = 9.1 Hz), 113.6-113.5 (d, J = 23.2 Hz), 159.2-156.1(d, J = 235 Hz), 112.3-111.9 (d, J = 24.6 Hz), 109.5-109.4 (d, J = 8.2 Hz), and 139.8 (d, J = 1.6 Hz) ppm respectively.

A variety of oxindole compounds were produced with different substituents at the *N*-position producing numerous spectral results. For example, the ^{13}C NMR spectrum of **159** is shown below which gives 13 signals.



158

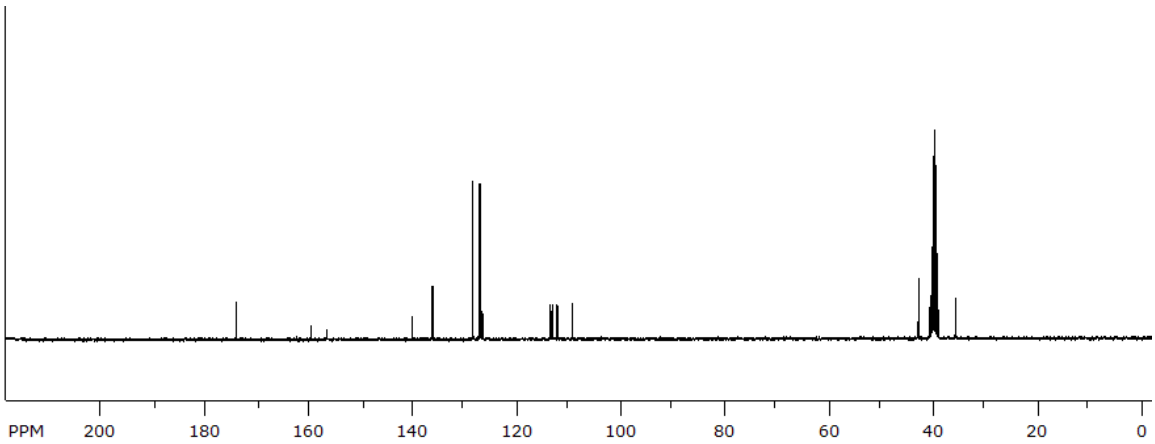
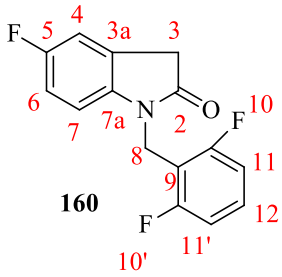
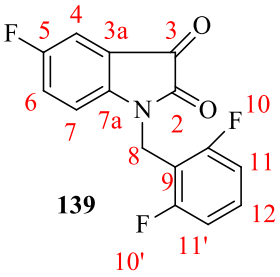


Figure 8

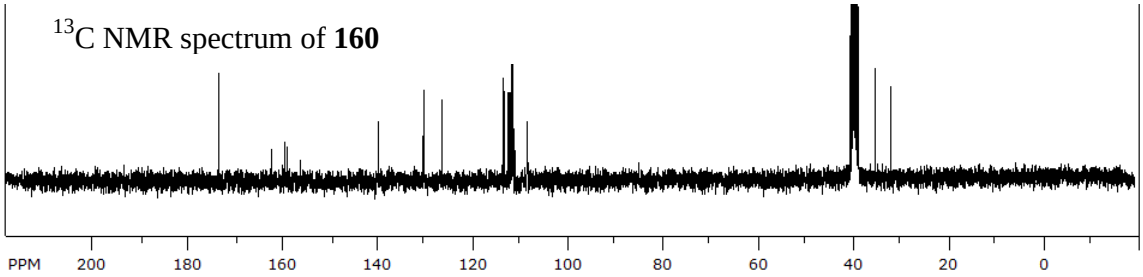
In **Figure 8** above there is one carbonyl peak which is located at 174.2 ppm for carbon 2. In addition the remaining aromatic peaks for carbons 3a-7a are consistent with those of the N-benzyl-5-fluoroisatin molecule carbon shifts, appearing at 126.8-126.7 (d, *J* = 9.2 Hz), 113.5-113.2 (d, *J* = 23.4 Hz), 159.7-156.6 (d, *J* = 236 Hz), 112.4-112.1 (d, *J* = 25.0 Hz), 109.4-109.3 (d, *J* = 8.3 Hz), and 140.1 (d, *J* = 1.6 Hz) ppm, respectively. In addition, the spectrum includes an aliphatic peak due to carbon 8, which appears at 42.6 ppm. Likewise there are an additional four signals which correspond to the benzyl phenyl ring. The signal for the quaternary carbon 9 appears at 136 ppm and does not appear in the ^{13}C DEPT spectrum. The carbons 10' are symmetric and are shown as a large intense peak at 127.2 ppm, as does the signal for carbons 11' which appears at 128.5 ppm. Carbon 12 appears at 127.3 ppm in the spectrum. The ^{13}C NMR spectrum of **160** is very similar to that of **139** as shown below.



160



139



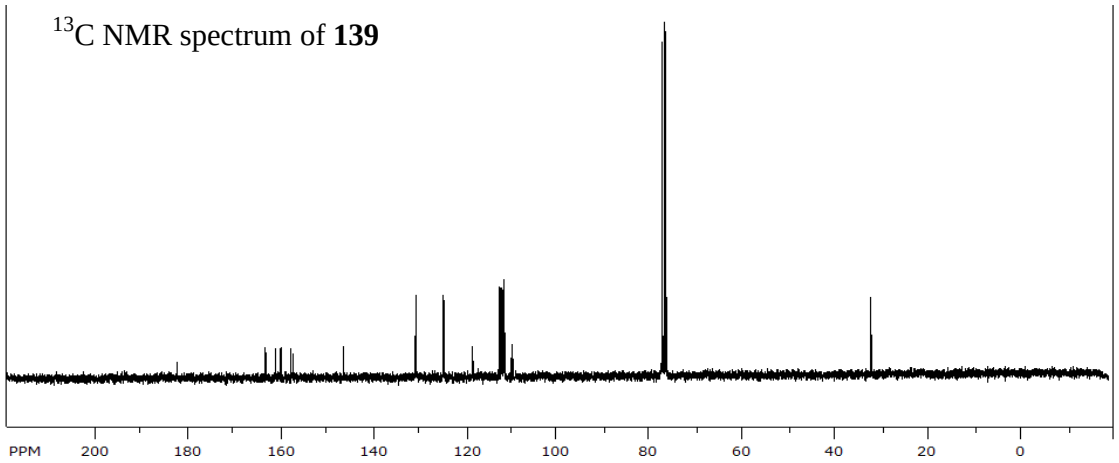


Figure 9 Comparison of ^{13}C NMR spectrum of **160** to ^{13}C NMR spectrum of **139**

Figure 9 shows that there is one carbonyl peak which is located at 173.6 ppm for carbon 2. In addition the remaining aromatic peaks for carbons 3a-7a are consistent with those of the **139a** carbon shifts, appearing at 126.6-126.5 (d, J = 9.2 Hz), 113.7-113.3 (d, J = 23.2 Hz), 159.6-156.5 (d, J = 237 Hz), 112.5-112.2 (d, J = 24.8 Hz), 108.6-108.5 (d, J = 8.3 Hz), and 139.9 (d, J = 1.4 Hz), ppm respectively. In addition the spectrum includes an aliphatic peak due to carbon 8, which appears at 31.9-31.8 (t, J = 3.6 Hz) ppm.

Likewise there are an additional four signals which correspond to the 2,6-difluorobenzyl phenyl ring. The signal for the quaternary carbon 9 appears at 111.2 ppm and does not appear in the 13C DEPT spectrum. The carbons 10' contain fluorines which exhibit as doublet of doublets at 162.6-159.2 (dd, J = 8.0 Hz) ppm. The signal for 11' appear at 111.9-111.5 (dd, J = 7.4 Hz) ppm and carbon 12 appears as a triplet at 130.3 (t, J = 10.4 Hz) ppm in the spectrum.

NMR Analysis of 5-Fluoro-3-substituted indolin-2-ones

Compound **158** from Table 3 was reacted with a variety of aryl aldehydes through an aldol condensation. In this reaction, compound **158** was reacted with a selected aryl aldehyde and piperidine in the presence of ethanol to produce 5-fluoro-3-substituted indolin-2-ones. These 5-fluoro-3-substituted indolin-2-ones existed as either the *E* or *Z* isomer or mixture of the two about the exocyclic double bond. The *E* or *Z* isomer is dependent on the characteristics of the substituents placed at the C-3 position. Through the use of ^{1}H NMR analysis, the *E* or *Z* isomers could be determined due to the chemical shifts of the aromatic protons on the benzylidene ring (H-2' and H-6') and the vinylic proton being significantly influenced by configuration. In the *E*-isomer, the vinylic proton (Hvin) is more deshielded due to the influence of the C-2 carbonyl just as the *ortho*-benzylidene protons (Ho) are shifted downfield due to the C-2 carbonyl. Therefore, in the *Z* isomer, the H-2‟ or H-6‟ protons occur in the range of 7.85-8.53 ppm compared to 7.45-7.84 ppm for the same protons in the *E* isomers.[24] In cases where the H- 2' and H-6' positions were substituted, only the influence of the vinylic proton was used to determine configuration, where the vinylic proton occurs about 7.55 ppm in the *Z*

isomer and 7.84 ppm in the *E* isomer.[136] For example, the ^{1}H NMR spectrum for **170** is presented below in **Figure 10** with the vinylic proton occurring at 7.43 as a singlet resulting in assignment as a Z isomer.

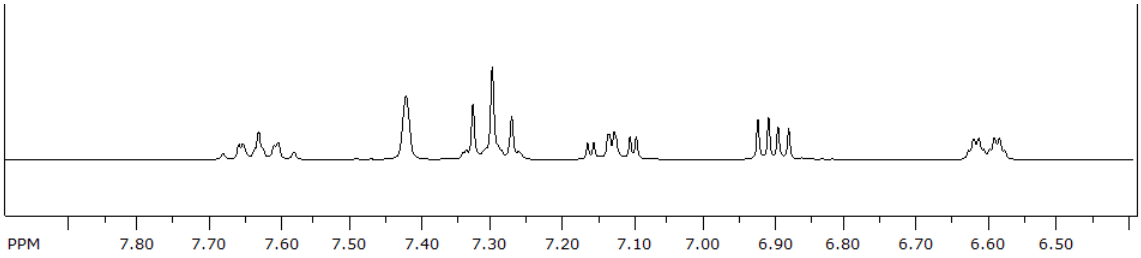


Figure 10

In most cases, the 3-substituted benzylidene compounds favored either 100% E or Z configuration. The compounds exhibiting the *Z* isomer could be attributed to the attraction of the lone pair of electrons at the C-2 carbonyl for partial positive charges created at the carbons attached to electron withdrawing atoms such as –F, and -Cl, upon the benzylidene ring. In addition compounds such as (**164**, **165**, **168**, **179**, **181**) showed a mixture of both E and Z isomers which has also been observed in previous investigations in which polar solvents such as DMSO or methanol has been utilized, or in the presence of light.[10]

Summary and Conclusions

A library of fluorinated derivatives of both oxindoles and isatins were synthesized as potential pharmaceuticals or targeting agents for imaging purposes related to cancer or Alzheimer's disease. These novel compounds have an advantage over other current small molecule inhibitors because of the ability to prepared in a three step synthesis that included the *N*-alkylation of 5 substituted isatin with either solid base of DBU or solid

base support of KF/Al_2O_3 followed by Wolff-Kishner reduction to the corresponding oxindoles and finally Knoevenagel condensation with aryl aldehydes. This allowed for the incorporation of 3 points of variability which was previously shown to increase the potency of oxindoles as caspase inhibitors when the electron-withdrawing chlorine was utilized at C-5 position of the isatin and oxindole scaffold. Additionally, a novel alternative route of synthesis was created to allow for the synthesis of *N*-propargyl benzylidene oxindoles. This novel alternative synthesis involved the Wolff-Kishner reduction of the isatins followed by condensation and finishing with the *N*-alkylation of the aldol products. Finally, the capability to synthesize the *N*-propargyl benzylidene oxindoles allowed for the synthesis of the isatin 1,2,3-triazoles which could be utilized as potential imaging agents for cancer.

Experimental

Chemical Analysis

Melting points were determined via the use of open capillaries with an Electrothermal melting point apparatus and are reported uncorrected. Elemental analyses were performed by Midwest Microlab, Indianapolis, IN. Elemental analysis results are within +0.4% of the theoretical values. The ^{1}H and ^{13}C NMR data were obtained on a Bruker Avance 300 MHz NMR in $CDCl_3$ or DMSO-d_6 solution unless otherwise indicated. The chemical shifts are reported in δ (ppm) downfield from tetramethylsilane as an internal standard; coupling constants (*J*) are in Hz. The following abbreviations are used to describe peak patterns where appropriate: s, singlet; d, doublet, dd, double doublet; t, triplet; q, quartet; dt, double triplet; m, multiplet. GC/MS measurements were performed using Hewlett-Packard 6890 Series GC with auto injection and mass fragments are reported as mass per charge, *m/z*. The GC was coupled with a mass spectrometer with a Hewlett-Packard 5973 mass selective detector/quadrupole system. Flash column (Silica Gel, Premium Rf, 200-400 mesh, Sorbent Technologies) and thin layer chromatography (TLC) were performed on silica gel with indicated solvent systems. All microwave reactions were performed in a monomode Biotage Emery's Creator 300 Watt system and the MARS Glasschem 300 Watt system by CEM. It should be noted that all reactions were run with sample absorption set to "normal". Multiple reactions were carried out using the FIRSTMATE system by Argonaut Technologies.

1-Benzylindolin-2,3-dione (128)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added isatin (0.2943 g, 2.00 mmol), DBU (329 μL, 1.1 eq) and benzyl chloride (257μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 10 min at 140 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was allowed to stand overnight in a freezer and then vacuum filtered to

afford the pure (TLC, GC/MS) product as a light orange solid (0.0536 g, 11.3%): mp 129-134 °C (lit.[119]mp 128-133°C); R_f = 0.41 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure to afford an oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to provide additional product (0. 2895 g) for a combined overall yield of 60.7 %; ^{13}C NMR (300 MHz DMSO-d_6) δ 183.1, 158.3, 150.3, 137.9, 135.5, 128.6, 127.5, 127.3, 124.4, 123.3, 117.7, 111.0, 42.9; MS (*m/z*) 237 (M^+), 146 (100%).

Ethyl 2-(2,3-dioxoindolin-1-yl)acetate (129)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added isatin (0.3111 g, 2.11 mmol), DBU (325 μL, 1.1 eq) and ethyl bromoacetate (257 μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 10 min at 140 °C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was allowed to stand overnight in a freezer and then vacuum filtered to afford the pure (TLC, GC/MS) product as a light orange solid (0.1049 g, 21.2 %): mp 105-110 ºC (lit.[119]mp 124-129 °C); R_f = 0.21 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure to afford an oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to provide additional product (0.1130 g) for a combined overall yield of 46.7%; ^{13}C NMR δ 182.3, 167.1, 158.2, 150.1, 138.5, 124.5, 123.7, 117.3, 111.3, 61.3, 41.1, 14.0 ppm; MS (*m/z*) 233 (M^+), 132 (100%).

1-(Prop-2-yn-1-yl)indolin-2,3-dione (130)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added isatin (0.3064 g, 2.08 mmol), DBU (342 μL, 1.1 eq) and propargyl bromide (210 μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in dynamic mode where the mixture was heated in 5 stages with pressure and high power being held constant throughout the run time at 130 psi and 200 watts, respectively. The first stage of heating was set at 110 °C for 3 min, the second stage of heating was set at 120 °C for 2 min, the third stage of heating was set at 100 °C for 1 min, the fourth stage of heating was set at 120 °C for 2 min, and the final stage of heating was set at 95 °C for 6 min. The first stage had high stirring while the final 4 stages had a low stir setting. The pre-stirring for this setup was high for 1 min. After cooling to rt, the reaction vessel was

placed in the freezer overnight and vacuum filtered to afford the pure product (GCMS/TLC) as an orange solid (0.1277 g, 33.1%): mp 147–149 ^{o}C (lit.[110]mp 157-159 ^{o}C); R_f = 0.46 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure and purified by silica gel chromatography (hexanes/EtOAc, 70:30) to give additional product (0.1844 g) for a combined overall yield of 81%; ^{1}H NMR (300 MHz DMSO-d_6) δ 7.75 (td, *J* = 1.3 Hz, *J* = 7.8 Hz, 1H), 7.61 (dd, *J* = 0.5 Hz, *J* = 1.2 Hz, 1H), 7.58 (dd, *J* = 0.5 Hz, *J* = 1.2 Hz, 1H), 7.24 (d, *J* =7.9 Hz, 1H), 7.21 (dd, *J* = 0.8 Hz, *J* = 15.0 Hz, 1H), 7.19 (d, *J* = 0.7 Hz, 1H), 4.55 (d, *J* = 2.5 Hz, 2H); ^{13}C NMR (300 MHz DMSO-d_6) δ 182.5, 157.3, 149.4, 138.1, 124.5, 123.6, 117.6, 111.1, 77.2, 74.8, 28.9; MS (*m/z*) 185 (M^+), 129 (100%).

1-(Prop-2-yn-1-yl)indolin-2,3-dione (130)

To a solution of isatin (1.0302 g, 7.00 mmol) in acetonitrile (45 mL), was added 6 equiv. of KF/alumina (6.4234 g, 42.0 mmol) and the resulting mixture was stirred at rt for 5 min until the initial orange solution turned to a dark brownish color. To the mixture, propargyl bromide (1.5 equiv, 0.961 mL, 10.5 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 9 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure to afford an orange solid which was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as bright orange crystals (0.9291 g, 69.1%): mp 153-155 ºC (lit.[110]mp 157-159 ºC); R_f = 0.61 (EtOAc/hexanes, 1:1); MS 185 (*m/z*) (M^+), 129 (100%).

1-(2,6-Dichlorobenzyl)indoline-2,3-dione (131)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added isatin (0.3062 g, 2.08 mmol), DBU (342 μL, 1.1 eq) and 2,6-dichlorobenzyl bromide (0.5711 g, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 10 min at 140 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was allowed to stand overnight in a freezer and then vacuum filtered to afford the pure (TLC, GC/MS) product as an orange solid (0.4151 g, 65.3%): mp 181-186 ºC (lit.[116]mp 175-180˚C); R_f = 0.57 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure and purified by silica gel chromatography

(hexanes/EtOAc, 70:30) to provide additional product (0.1787 g) for a combined overall yield of 93.2%; ^{13}C NMR (300 MHz DMSO-d_6) δ 182.7, 157.7, 150.6, 138.4, 135.4, 130.8, 129.7, 129.1, 124.7, 123.4, 117.9, 110.8, 40.4; MS (*m/z*) 305 (M^+), 270 (100%).

1-(2,6-Difluorobenzyl)indoline-2,3-dione (132)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added isatin (0.2962 g, 2.01 mmol), DBU (329 μL, 1.1 eq) and 2,6-difluorobenzyl bromide (0.5278 g, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 10 min at 140 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was allowed to stand overnight in a freezer and then vacuum filtered to afford the pure (TLC, GC/MS) product as yellow-orange solid (0.2469 g, 44.9 %): mp 155-158 ºC (lit.[119]mp 154-156 ˚C); R_f = 0.32 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure to afford an oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to provide additional product (0.2100 g) for a combined overall yield of 83.6%; ^{13}C NMR (300 MHz DMSO-d_6) δ: 182.8, 162.7-159.3 (dd, *J* = 7.8 Hz), 157.6, 150.3, 138.3, 130.8-130.6 (t, *J* = 10.4 Hz), 124.6, 123.4, 117.6, 111.9-111.65 (d, *J* = 7.4 Hz), 110.8, 110.40-110.36 (t, *J* = 7.8 Hz), 32.1; MS (*m/z*): 273 (M^+), 146 (100%).

1-Benzyl-5-chloroindoline-2,3-dione (133)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added 5-chloroisatin (0.3785 g, 2.08 mmol), DBU (329 μL, 1.1 eq) and benzyl chloride (257 μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 20 min at 120 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was placed in the freezer overnight but did not afford a solid. The resulting orange-red liquid was then evaporated under reduced pressure to afford an orange oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to afford a pure (GC-MS/TLC) orange red solid (0.3875 g, 71%): mp 130-133 ºC (lit.[119]mp 134 ˚C); R_f = 0.65 (hexanes:EtOAc, 1:1). ^{13}C NMR δ 181.9, 158.1, 148.8, 136.7, 135.1, 129.7, 128.6, 127.5, 127.2, 123.9, 119.1, 112.6, 42.9; MS (*m/z*) 271 (M^+), 180 (100%).

Ethyl-2-(5-chloro-2,3-dioxoindolin-1-yl)acetate (134)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added 5-chloroisatin (0.3689 g, 2.00 mmol), DBU (329 μL, 1.1 eq) and ethyl bromoacetate (243 μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 20 min at 120 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was allowed to stand overnight in a freezer and then vacuum filtered to afford the pure (TLC, GC/MS) product as a light orange solid (0.1662 g, 31.0%): mp 126-128 ºC (lit.[119]mp 130-135 ˚C); R_f = 0.63 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure to afford an oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to provide additional product (0.1965 g) for a combined overall yield of 67.8%; ^{13}C NMR δ 181.5, 167.3, 157.8, 148.9, 137.3, 127.9, 124.1, 118.6, 112.9, 61.4, 41.3, 13.9; MS (*m/z*) 267 (M^+), 28 (100%).

5-Chloro-1-(prop-2-yn-1-yl)indoline-2,3-dione[150] (135)

To a 10 mL microwave vial charged with ethanol (3mL) and a magnetic stir bar was added isatin (0.3632 g, 2.00 mmol), DBU (329 μL, 1.1 eq) and propargyl bromide (201 μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 20 min at 110 ^{o}C with pre-stirring 30 sec. After cooling to rt, the reaction vessel was placed in the freezer overnight and then vacuum filtered to afford a pure (GCMS/TLC) orange solid (0.0521 g, 11.9%): mp 157-159 ºC; R_f = 0.51 (hexanes/EtOAc, 1:1). The filtrate was then evaporated under reduced pressure to afford an orange oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to give additional product (0.3145 g) for a combined overall yield of 64.3%: ^{1}H NMR (300 MHz DMSO-d_6) δ 7.79 (dd, *J* = 2.2 Hz, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 2.116 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 4.56 (d, *J* = 2.5 Hz, 2H), 4.35 (t, *J* = 5.0, 1H) ^{13}C NMR (300 MHz DMSO-d_6) δ 181.4, 157.1, 147.9, 136.9, 127.8, 124.0, 119.1, 112.8, 77.0, 75.1, 29.1; MS (*m/z*) 219 (M^+), 28 (100 %).

5-Chloro-1-(prop-2-yn-1-yl)indoline-2,3-dione[150] (135)

To a solution of 5-chloro-isatin (1.0302 g, 5.67 mmol) in acetonitrile (45 mL), was added 6 equiv of KF/alumina (6.2751 g, 41.0 mmol) and the resulting mixture was stirred at rt

for 5 min until the initial orange solution turned to a dark brownish color. To the mixture 1.5 equiv. of propargyl bromide (937µL, 7.87 mmol,) was then added dropwise to the stirred solution after which time the mixture was refluxed for 7 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure to afford a dark orange solid which was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as dark orange crystals (0.6503 g, 52.3%): mp 164-168 ºC; R_f = 0.52 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 7.79 (dd, J=2.261 Hz, J=8.455 Hz, 1H), 7.65 (d, J=2.116 Hz, 1H), 7.27 (d, J=8.458 Hz, 1H), 4.56 (d, J=2.511 Hz, 2H), 4.35 (t, J=5.090, 1H) ^{13}C (300 MHz, DMSO-d_6): δ 181.4, 157.1, 147.9, 136.9, 127.8, 124.0, 119.1, 112.8, 77.0, 75.1, 29.1 ; MS (*m/z*): 219 (M^+), 163 (100 %).

1-(2,6-Dichlorobenzyl)-5-chloroindoline-2,3-dione (136)

To a 10 mL microwave vial charged with ethanol (3 mL) a magnetic stir bar was added 5-chloroisatin (0.3658 g, 2.01 mmol), DBU (329 µL, 1.1 eq) and 2,6-dichlorobenzyl bromide (0.5310 g, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 20 min at 120 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was placed in the freezer for 2 h and then vacuum filtered to afford the pure (GC-MS/TLC) product as an orange-red solid (0.5772 g, 84.1 %): mp 232-235 ºC (lit.[119]mp 233-235 ˚C); R_f = 0.60 (hexanes/EtOAc, 1:1). ^{13}C NMR δ 181.6, 157.5, 149.2, 137.3, 135.4, 130.8, 129.5, 129.1, 127.6, 124.2, 118.9, 112.6, 40.4; MS (*m/z*) 339 (M^+), 180 (100%).

1-(2,6-Difluorobenzyl)-5-chloroindoline-2,3-dione (137)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added 5-chloroisatin (0.3650 g, 2.00 mmol), DBU (329 µL, 1.1 eq) and 2,6-difluorobenzyl bromide (0.4554 g, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 20 min at 120 ^{o}C with a pre-stirring 30 sec. After cooling to rt, the reaction vessel was placed in the freezer overnight and then vacuum filtered to afford the pure (TLC, GC/MS) product as a red solid (0.4091 g, 66.3%): mp 171-174 ºC (lit.[119]mp 172-177 ˚C); R_f = 0.56 (hexanes:EtOAc, 1:1). A second crop afforded additional product (0.0339 g) for a combined overall yield of 71.7

%. ^{13}C NMR δ 181.7, 162.7-159.4 (dd, *J* = 8.2 Hz), 157.5, 148.8, 137.1, 130.9-130.6 (t, *J* = 10.5 Hz), 127.6, 124.0, 119.0, 112.2-112.1 (t, *J* = 2.6 Hz), 111.9-111.6 (d, *J* = 17.8 Hz), 110.6, 32.2; MS (*m/z*) 307 (M^+), 127 (100%).

1-Benzyl-5-fluoroindoline-2,3-dione (138)

To a 10 mL microwave vial charged with ethanol (3 mL) and a magnetic stir bar was added 5-fluoroisatin (0.3310 g, 2.00 mmol), DBU (330 μL, 1.1 eq) and benzyl chloride (254 μL, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 25 min at 120 ^{o}C with a pre-stirring of 30 sec. After cooling to room temperature, the reaction vessel was placed in the freezer overnight and then vacuum filtered to afford a pure (GCMS/TLC) dark red solid (0.1408 g, 27.5%): mp 128-131ºC (lit[137]mp 130-133 ˚C); R_f = 0.64 (hexanes:EtOAc, 1:1). The filtrate was then evaporated under reduced pressure to afford a red oil which was purified by silica gel chromatography (hexanes/EtOAc, 70:30) to give a dark red solid (0.3073 g) for a combined overall yield of 87.6%: ^{1}H NMR (300 MHz DMSO-d_6) δ 7.57 ppm (d, *J* = 2.0 Hz, 1H), 7.50 (dd, *J* = 8.2 Hz, 2.2 Hz, 1H), 7.39-7.28 (m, 1H), 6.96 (t, *J* = 8.1 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 4.94 (s, 2H); ^{13}C NMR (300 MHz DMSO-d_6) δ 182.4, 160.09-156.86 (d, *J* = 241 Hz), 158.35-158.33 (d, *J* = 1.4 Hz), 146.5-146.4 (d, *J* = 1.6 Hz), 135.3, 128.6, 127.5, 127.3, 123.9-123.6 (d, *J* = 24.0 Hz), 118.7-118.6 (d, *J* = 7.2 Hz), 112.4-112.3 (d, *J* = 7.4 Hz), 111.6-111.3 (d, *J* = 24.3 Hz), 42.9; MS (*m/z*) 255 (M^+), 164 (100%).

1-Benzyl-5-fluoro-indolin-2,3-dione (138)

To a solution of 5-fluoro-isatin (0.7394 g, 4.48 mmol) in acetonitrile (45 mL), was added 6 equiv. of KF/alumina (4.0994 g, 26.8 mmol) and the resulting mixture was stirred at rt for 5 min until the initial red solution turned to a dark brownish color. To the mixture benzyl chloride (1.5 equiv, 934 μL, 8.12 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 8 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure to afford a red solid which was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as a bright red

powder (0.9336 g, 81.8%): mp (128-129 ºC) (lit[137]mp. 130-133 ˚C); R_f = 0.73 (EtOAc/hexanes, 1:1); MS (m/z) 255 (M_+), 164 (100 %).

1-(2,6-Difluorobenzyl)-5-fluoroindoline-2,3-dione (139)

To a 10 mL microwave vial charged with ethanol (3mL) and a magnetic stir bar was added 5-fluoroisatin (0.3294 g, 1.99 mmol), DBU (329 μL, 1.1 eq) and 2,6-difluorobenzyl bromide (0.4594 g, 1.1 eq). The reaction vessel was sealed and heated under microwave irradiation in standard mode for 25 min at 120 ^oC with a pre-stirring of 30 sec. After cooling to rt, the reaction vessel was placed in the freezer overnight and then vacuum filtered to afford a dark red solid (0.2133 g, 36.7%): mp 129-132 ^oC. The filtrate was then evaporated under reduced pressure to afford a red oil which was purified by silica gel chromatography (hexanes/EtOAc,70:30) to give additional product (0.2612 g) for a combined overall yield of 81.7%; ^1H NMR (300 MHz, DMSO): δ .7.60 (ddd, *J* = 2.8 Hz, *J* = 8.7 Hz, 1H), 7.49 (dd, *J* = 2.7 Hz, *J* = 7.0 Hz, 1H), 7.44 (dd, *J* = 1.7 Hz, *J* = 6.8 Hz, 1H) 7.15 (t, 3H), 7.25 (dd, *J* = 2.7 Hz, *J* = 8.6 Hz, 1H), 6.98 (t, *J* = 8.1 Hz, 1H), 6.91 (dd, *J* = 3.5 Hz, *J* = 8.6 Hz, 1H), 4.96 (s, 2H); ^{13}C NMR (300 MHz DMSO-d_6) δ 182.19-182.16 (d, *J* = 2.1 Hz), 167.7, 162.6-159.2 (dd, *J* = 7.7 Hz), 160.0-156.8 (d, *J* = 241 Hz), 157.73-157.71 (d, *J* = 1.7 Hz), 146.56-146.54 (d, *J* = 1.6 Hz), 130.9-130.6 (t, *J* = 10.5 Hz), 124.41-124.0 (d, *J* = 24.1 Hz), 118.5-118.4 (d, *J* = 7.1 Hz), 111.9-111.7 (d, *J* = 7.3 Hz), 111.6-111.4 (d, *J* = 17.7 Hz), 110.9-110.4 (t, *J* = 18.7 Hz), 32.2 (t, *J* = 3.9 Hz); MS (*m/z*) 291 (M^+), 164 (100%).

1-(2,6-Difluorobenzyl)-5-fluoro-indoline-2,3-dione (139)

To a solution of 5-fluoroisatin (1.0984 g, 6.65 mmol) in acetonitrile (45 mL), was added 6 equiv. of KF/alumina (6.1030 g, 39.9 mmol) and the resulting mixture was stirred at rt for 5 min until the initial red solution turned to a dark brownish color. To the mixture, 2,6-difluorobenzyl bromide (1.5 equiv, 2.1002 g, 10.1 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 15 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure to afford a red solid which was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as bright red

crystals (1.6521 g, 85.3%): mp 143-145 ºC; R_f = 0.61 (EtOAc/hexanes, 1:1);); MS (*m/z*) 291 (M^+), 164 (100%).

1-(4-Methoxy-benzyl)-indoline-2,3-dione[146] (140)

To a solution of isatin (0.5099 g, 2.95 mmol) in acetonitrile (45 mL), was added 6 equiv. of KF/alumina (3.1820 g, 12.1 mmol) and the resulting mixture was stirred at rt for 5 min until the initial orange solution turned to a dark brownish color. To the mixture 4-methoxybenzyl chloride (1.5 equiv, 705 µL, 5.20 mmol) was then added to the stirred solution after which time the mixture was refluxed for 22 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70/30) which was then recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as an orange powder (0.5746 g, 55.1%): mp 150-153 ºC; R_f = 0.64(EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.60 (d, *J* = 1.3 Hz, 1H), 7.58-7.54 (m, 2H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 4.83 (s, 2H), 3.71 (s, 3H); ^{13}C NMR δ: 183.1, 158.6, 158.1, 150.3, 137.9, 128.8, 127.2, 124.4, 123.2, 117.6, 114.0, 111.1, 55.0, 42.3 ppm: MS (*m/z*) 267 (M^+), 146 (100 %).

1-(3,5-Difluorobenzyl)-indoline-2,3-dione (141)

To a solution of isatin (0.6128 g, 4.17 mmol) in acetonitrile (45 mL), was added 6 equiv. of KF/alumina (3.4532 g, 22.6 mmol) and the resulting mixture was stirred at rt for 5 min until the initial orange solution turned to a dark brownish color. To the mixture 1.5 equiv. of 3,5-difluorobenzyl bromide (0.808 mL, 5.85 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 3 h. The mixture was then allowed to cool to rt and the suspended K_2CO_3 was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) afford an orange solid (0.5634 g, 49.5%): mp 157-159 ºC. The solid was recrystallized from DCM/Hexanes to afford the pure (TLC, GC/MS) product as orange-red crystals (0.1866 g, 16.4%): mp. 161-164 ºC; R_f = 0.55 (EtOAc/hexanes, 1:1); ^{1}H (300 MHz, $CDCl_3$): δ 7.68 (d, *J* = 8.2 Hz, 1H), 7.58 (dd, *J* = 1.3 Hz, *J* = 15.6 Hz, 1H),

7.55 (d, *J* = 1.3 Hz,1H), 7.28 (s, 1H), 7.19 (dd, *J* = 0.6 Hz, *J* = 15.0 Hz, 1H), 7.16 (d, *J* =0.4 Hz, 1H), 6.89 (d, *J* = 7.8 Hz, 2H), 6.81 (t, *J* = 2.283 Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 2H), 4.92 (s, 2H); ^{13}C (300 MHz, $CDCl_3$): δ 182.5, 165.1-161.5 (dd, *J* = 12.6 Hz), 158.1, 150.1, 138.4, 125.7, 124.2, 117.7, 110.5, 110.4-110.0 (dd, *J* = 8.3 Hz), 104.1-103.4 (t, *J* = 25.3 Hz, 43.3-43.2 (t, *J* = 1.9 Hz) ppm; MS (*m/z*) 273 (M^+), 28 (100 %).

1-(4-Methoxy-benzyl)-5-chloro-indoline-2,3-dione (142)

To a solution of 5-chloro-isatin (0.8655 g, 4.76 mmol) in acetonitrile (45 mL) was added 6 equiv. of KF/alumina (4.4752 g, 29.2 mmol) and the resulting mixture was stirred at rt for 5 min until the initial orange solution turned to a dark brownish color. To the mixture 4-methoxybenzyl chloride (1.5 equiv, 969 µL, 7.14 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 22 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure to afford a red solid which was then recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as a bright red powder (1.0726 g, 74.5%): mp 148-150 ºC (lit.[145] mp. 152-153°C); R_f = 0.60 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.64 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 7.4 Hz, 1H), 6.91 (d, *J* = 6.6 Hz, 2H), 5.75 (s, 1H), 4.83 (s, 2H), 3.72 (s, 3H); ^{13}C NMR δ: 182.0, 158.7, 158.0, 148.7, 136.7, 128.8, 127.4, 126.9, 123.9, 119.0, 114.0, 112.7, 55.0, 42.4 ppm: MS (m/z) 301 (M^+), 121 (100 %) ; Anal. Calcd for $C_{16}H_{12}ClNO_3$: C, 63.69; H, 4.01; N, 4.64; Found: C, 63.34; H, 4.10; N, 4.67.

1-(3,5-Difluorobenzyl)-5-chloro-indoline-2,3-dione (143)

To a solution of 5-fluoroisatin (0.8604 g, 4.73 mmol) in acetonitrile (45 mL) was added 6 equiv. of KF/alumina (4.3469 g, 28.4 mmol) and the resulting mixture was stirred at rt for 5 min until the initial orange solution turned to a dark brownish color. To the mixture 1.5 equiv. of 3,5-difluorobenzyl bromide (0.919 mL, 6.69 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford an orange solid (0.8110 g, 55.8%): mp 165-167 ºC.

The orange solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as orange-red crystals (0.7477 g, 51.4%): mp 174-177 ºC; R_f = 0.68 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.65 (d, *J*= 4.9 Hz, 1H), 7.61(d, J=2.2 Hz, 1H), 7.25 (dd, J=2.2 Hz, J=8.6 Hz, 1H), 7.17 (tt, J=2.3 Hz, J=9.3 Hz), 6.93 (d, J=8.8 Hz, 1H), 5.75 (s,1H), 4.93 (s, 2H) ; ^{13}C NMR δ: 181.5, 164.2-160.7 (dd, *J* = 13.2 Hz), 158.2, 148.3, 140.1-139.9 (t, *J* = 9.5 Hz), 136.5, 127.5, 123.8, 119.5, 112.4, 110.5-110.2 (dd, *J* = 8.0 Hz), 103.2-102.5 (t, *J* = 25.7 Hz), 42.2 (t, *J* = 1.6 Hz) ppm; MS (*m/z*) 307 (M^+), 127 (100%). Anal. Calcd for $C_{15}H_8ClF_2NO_2$: C, 58.55; H, 2.62; N, 4.55 Found: C, 58.51; H, 2.74; N, 4.48

1-(3,5-Difluorobenzyl)-5-chloro-indoline-2,3-dione (143)

To a solution of 5-fluoroisatin (0.6076 g, 3.35 mmol) in acetonitrile (45 mL) was added 6 equiv. of KF/alumina (3.0696 g, 20.1 mmol) and the resulting mixture was stirred at rt for 5 min until the initial orange solution turned to a dark brownish color. To the mixture 1.5 equiv. of 3,5-difluorobenzyl bromide (0.650 mL, 5.02mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure to afford a red solid. The red solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as orange-red crystals (0.9320 g, 95.4%): mp 173-175 ºC; R_f = 0.68 (EtOAc/hexanes, 1:1); MS (*m/z*) 307 (M^+), 127 (100%).

5-Fluoro-1-(prop-2-yn-1-yl)indoline-2,3-dione (144)

To a solution of 5-fluoro-isatin (0.5975 g, 3.62 mmol) in acetonitrile (45 mL) was added 6 equiv. of KF/alumina (3.3214 g, 21.7 mmol) and the resulting mixture was stirred at rt for 5 min until the initial red solution turned to a dark brownish color. To the mixture 1.5 equiv. of propargyl bromide (500 µL, 5.46 mmol,) was then added dropwise to the stirred solution after which time the mixture was refluxed for 48 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure and recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as red crystals (0.2665 g,

36.3%): mp 124-126 ºC; R_f = 0.59 (EtOAc/hexanes, 1:1); ^{1}H NMR: (DMSO-d_6) δ: 7.63 (ddd, *J*= 2.7 Hz, *J* = 8.6 Hz, 1H), 7.52 (dd, *J* = 2.6 Hz, *J* = 7.1 Hz, 1H), 7.28 (dd, *J* = 3.8 Hz, *J* = 8.6 Hz, 1H), 4.56 (d, *J* = 2.5, 2H), 3.77 (t, *J* = 2.4 Hz,1H); ^{13}CNMR (DMSO-d_6) δ: 181.8 (d, *J* = 2.2 Hz), 160.2-157.0 (d, *J* = 241 Hz), 157.3 (d, *J* = 1.5 Hz), 145.6 (d, *J* = 1.6 Hz), 124.2-123.8 (d, *J* = 24.1 Hz), 118.6-118.5 (d, *J* = 7.4 Hz), 112.6-112.5 (d, *J* = 7.4 Hz), 111.6-111.5 (d, *J* = 24.5 Hz), 77.1, 75.0, 29.1 ppm; MS (*m/z*) 203 (M^+), 147 (100%). Anal. Calcd for $C_nH_nClNO_n$: C, 65.03; H, 2.98; N, 6.89; Found: C, 64.63; H, 3.04 ; N, 6.80.

1-(4-Methoxy-benzyl)-5-fluoro-indoline-2,3-dione (145)

To a solution of 5-fluoro-isatin (0.4896 g, 2.95 mmol) in acetonitrile (45 mL) was added 6 equiv. of KF/alumina (2.7466 g, 17.9 mmol) and the resulting mixture was stirred at rt for 5 min until the initial red solution turned to a dark brownish color. To the mixture 4-methoxybenzyl chloride (1.5 equiv, 603 µL, 4.44 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 19 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70/30) which was then recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as a orange powder (0.3174 g, 41.9%): mp 132-135 ºC (lit.[146] mp. 138-139˚C); R_f = 0.54 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.47 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 2.7 Hz, 2H), 7.37 (d, *J* = 6.8 Hz, 1H), 7.00 (d, *J* = 7.4 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 6.90 (dd, *J* = 2.0 Hz, *J* = 6.6 Hz, 1H), 5.75 (s, 1H), 4.83 (s, 2H), 3.72 (s, 3H) ; ^{13}C NMR δ: 182.5, 160.0, 158.2, 157.7, 146.5, 128.8, 127.0, 123.7, 118.5, 114.0, 112.4, 111.4, 55.0, 42.4 ppm: MS (m/z) 285 (M_+), 121 (100 %). Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; N, 4.91; Found: C, 67.44; H, 4.30 ; N, 4.91

1-(3,5-Difluorobenzyl)-5-fluoro-indoline-2,3-dione (146)

To a solution of 5-fluoroisatin (1.1089 g, 6.72 mmol) in acetonitrile (45 mL) was added 6 equiv. of KF/alumina (6.1608 g, 40.3 mmol) and the resulting mixture was stirred at rt for 5 min until the initial red solution turned to a dark brownish color. To the mixture 1.5

equiv. of 3,5-difluorobenzyl bromide (1.302 mL, 10.1 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 31 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was then evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford a red solid (1.889 g, 96.6%): mp 149-152ºC. The red solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright red crystals (1.697 g, 86.8 %): mp 160-161 ºC; R_f = 0.88 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.51 (dd, *J*=2.5, *J* =7.1 Hz, 1H), 7.48 (ddd, *J* = 2.7 Hz, *J* =8.6 Hz, 1H), 7.26 (dd, *J* =2.2 Hz , *J* =8.6 Hz, 2H), 7.17 (dt, *J* =2.3 Hz, *J* =9.3 Hz, 1H), 6.93 (dd, *J* =3.7 Hz, *J* = 8.6 Hz, 1H), 5.75 (s, 1H), 4.93 (s, 2H): ^{13}C (300 MHz, DMSO-d_6): δ 182.0 (d, *J* = 2.2 Hz), 164.2-160.7 (dd, *J* = 13.2 Hz), 160.1-156.9 (d, *J* = 241 Hz), 158.5 (d, *J* = 1.4 Hz), 146.0 (d, *J* = 1.7 Hz), 140.2-140.0 (t, *J* = 8.9 Hz), 123.7-123.3 (d, *J* = 24.0 Hz), 119.0-118.9 (d, *J* = 7.2 Hz), 112.1-112.0 (d, *J* = 7.5 Hz), 111.5-111.2 (d, *J* = 24.3 Hz), 110.5-110.2 (dd, *J* = 8.0 Hz), 103.2-102.5 (t, *J* = 25.8 Hz), 42.2 (t, *J* = 2.0 Hz) ppm; MS (m/z) 291 (M^+), 28 (100%). Anal. Calcd for $C_{15}H_8F_3NO_2$: C, 61.86; H, 2.77; N, 4.81; Found: C, 61.84; H, 2.80; N, 4.75.

1-Benzyl-indolin-2-one (151)

1-Benzyl-indoline-2,3-dione (0.5585 g, 2.35 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (20 mL). The flask was wrapped in aluminum foil and heated under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 125 mL), placed in a hood uncovered and allowed to sit overnight. A white solid formed and was vacuum filtered to yield a pure product (0.4516 g, 85.9 %); mp 84-85°C (lit.[147] mp. 77-78°C); R_f = 0.62 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.33 (d, *J* = 4.4 Hz, 4H), 7.28 (m, 2H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.00 (m, 1H), 6.87 (d, *J* = 7.7 Hz, 1H), 4.88 (s, 2H), 3.66 (s, 2H) ; ^{13}C NMR δ: 174.5, 143.9, 136.5, 128.5, 127.3, 127.2, 127.2, 124.7, 124.3, 121.8, 108.8, 42.5, 35.1 ppm: MS (*m/z*): 223 (M^+), 28 (100%).

5-Chloro-indolin-2-one (152)

5-Chloro-isatin (1.6720 g, 9.21 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (20 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 150 mL), placed in a hood uncovered and allowed to sit overnight. Brown crystals formed and were vacuum filtered with water to yield a pure product (1.3195 g, 85.5%); mp 199-202°C (lit.[126] mp 195°C); R_f = 0.17 (EtOAc/hexanes, 1:1); MS (*m/z*) 167 (M^+), (100%)

1-(Benzyl)-5-chloro-indolin-2-one (153)

1-(Benzyl)-5-chloro-indoline-2,3-dione (0.5028 g, 1.86 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (27 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 167 mL), placed in a hood uncovered and allowed to sit overnight. A white solid formed and was vacuum filtered to yield a pure product (0.4291 g, 90.0%); mp 90-91°C (lit.[126] mp. 88-89°C); R_f = 0.69 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.34 (m, 5H), 7.31 (s, 1H), 7.25 (d, *J* =8.5 Hz, 2H), 6.87 (d, *J* =8.3 Hz, 1H), 4.88 (s, 2H), 3.71 (s, 2H) ^{13}C NMR δ: 174.1, 142.8, 136.2, 128.5, 127.3, 127.1, 127.1, 127.0, 125.9, 124.5, 110.0, 42.5, 35.1 ppm; MS (*m/z*) 257 (M^+), 121 (100%);

1-(4-methoxy-benzyl)-5-chloro-indolin-2-one (154)

1-(4-Methoxy-benzyl)-5-chloro-indoline-2,3-dione (0.4549 g, 1.51 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (15 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 150 mL) was placed in a hood uncovered and allowed to sit overnight. A white solid formed and was vacuum filtered to yield a pure product (0.4135 g, 95.3%); mp 145-147°C; R_f = 0.55 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.33 (d, *J* =2.0 Hz, 1H), 7.27-7.24 (m, 3H), 7.22 (d, *J* = 2.2 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 4.80 (s, 2H), 3.70 (s, 3H), 3.68 (s, 2H); ^{13}C NMR δ: 174.1, 158.5, 142.8, 128.6, 128.0, 127.0, 125.9, 124.4,

113.9, 110.0, 55.0, 42.0, 35.1 ppm ; MS (m/z) 287 (M_+), 121 (100 %). C, 66.79; H, 4.90; N, 4.87; Found C, 66.90; H, 4.80; N, 4.99.

1-(2,6-Difluorobenzyl)-5-chloro-indolin-2-one (155)

1-(2,6-Difluorobenzyl)-5-chloro-indoline-2,3-dione (0.4395 g, 1.43 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (15 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 155 mL), placed in a hood uncovered and allowed to sit overnight. A brown solid formed and was vacuum filtered to yield a pure product (0.3682 g, 87.8%); mp 125-129°C (lit. [126] mp. 120-125˚C); R_f = 0.60 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.46–7.36 (m, 1H), 7.32 (d, *J* = 1.6 Hz, 1H), 7.30-7.27 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 7.10 (t, *J* = 8.2 Hz, 2H), 6.88 (d, *J* = 8.2 Hz, 1H), 4.94 (s, 2H), 3.62 (s, 2H); ^{13}C NMR δ: 173.4, 162.5-159.2 (dd, *J* = 7.9 Hz), 142.6, 130.6-130.3 (t, *J* = 10.4 Hz), 127.2, 126.8, 125.9, 124.5, 111.8-111.6 (dd, *J* = 7.5 Hz), 111.2, 34.8, 31.8 ppm; MS (m/z) 293 (M^+,100 %).

1-(3,5-Difluorobenzyl)-5-chloroindolin-2-one (156)

1-(3,5-Difluorobenzyl)-5-chloro-indoline-2,3-dione (0.4992 g, 1.63 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (20 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 hr. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 90 mL), placed in a hood uncovered and allowed to sit overnight. A white solid formed and was vacuum filtered to yield a pure product (0.4321 g, 90.7%): mp 169-170°C; R_f = 0.48 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (s, 1H), 7.20 (d, J=8.349, 1H), 6.82 (d, J=6.886 Hz, 1H), 6.76 (d, J=17.797 Hz, 1H), 6.73 (s, 1H), 6.61 (d, J=8.349 Hz, 1H), 4.88 (s, 2H), 4.31 (s, 1H) ppm ^{13}C (300 MHz, DMSO-d_6): 174.3, 165.0-161.5 (dd, *J* = 12.7 Hz), 142.2, 139.6-139.3 (t, *J* = 8.8 Hz), 128.2, 127.9, 125.9, 125.1, 110.2-109.9 (dd, *J* = 8.2 Hz), 109.5, 103.7-103.0 (t, *J* = 25.4 Hz), 43.1 (t, *J* = 2.0 Hz), 35.5 ppm; MS (m/z) 293 (M^+), 28 (100%). Anal. Calcd for $C_{15}H_{10}ClF_2NO$: C, 61.34; H, 3.43; N, 4.77. Found: C, 61.30; H, 3.53; N, 4.97.

5-Fluoro-indolin-2-one (157)

5-Fluoro-isatin (0.3279 g, 1.98 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (30 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 150 mL), placed in a hood uncovered and allowed to sit for 8 days. Brown crystals formed and were vacuum filtered to yield a pure product (0.1749 g, 58.3%); mp 140-143°C (lit.[138] mp 143-147°C); R_f = 0.51 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H, N-H), 7.11 (dd, *J* = 2.6 Hz (H,F), *J* = 8.5 Hz (H,H), 1H, 4-H), 6.99 (ddd, *J* = 8.5 Hz (H,F), *J* = 2.5 Hz (H,H), 1H, 6-H), 6.79 (dd, *J* = 4.5 Hz (H,F), *J* = 8.4 Hz (H,H), 1H, 7-H), 3.49 (s, 2H, 3-H); ^{13}C NMR δ: 176.1, 159.2-156.1 (d, *J* = 24.6 Hz), 109.5-109.4 (d, *J* = 8.2 Hz), 36.1 (d, *J* = 1.6 Hz) ppm; MS (*m/z*) 151 (M^+), 122 (100%).

1-Benzyl-5-fluoro-indolin-2-one[148] (158)

1-Benzyl-5-fluoro-indoline-2,3-dione (0.6933 g, 2.72 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (41 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 hr. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 225 mL), placed in a hood uncovered and allowed to sit overnight. A white powdery solid formed overnight and was vacuum filtered to yield a pure product (0.5468 g, 83.5%): mp 76-78°C; R_f = 0.65 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ : 7.33 (d, *J* = 4.4 Hz, 4H), 7.29-7.22 (m, 1H), 7.20 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H), 7.05 (ddd, *J* = 2.6 Hz, *J* = 9.5Hz, 1H), 6.86 (dd, *J* = 4.4 Hz, *J* =8.5 Hz, 1H), 4.88 (s, 2H), 3.70 (s, 2H); ^{13}C NMR δ: 174.2, 159.7-156.6 (dd, *J* = 236 Hz), 140.1(d, *J* = 1.6 Hz), 136.3, 128.5, 127.3, 127.2, 126.8-126.7 (d, *J* = 9.2 Hz), 113.5-113.2 (d, *J* = 23.4 Hz), 112.4-112.1 (d, d, *J* = 25.0 Hz), 109.4-109.3 (d, *J* = 8.3 Hz), 42.6, 35.5-35.4 (d, *J* = 1.5 Hz) ppm; MS (*m/z*) 241 (M^+), 127 (100%) .

1-(4-Methoxy-benzyl)-5-fluoro-indolin-2-one (159)

1-(4-Methoxy-benzyl)-5-fluoro-indoline-2,3-dione (0.4163 g, 1.46 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (15 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 65 mL), placed in a hood uncovered and allowed to sit overnight. A white solid formed overnight and was vacuum filtered to yield a pure product (0.3596 g, 90.8%); mp 124-127°C; R_f = 0.52 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.28 (dd, *J* = 2.0 Hz , *J* = 6.8 Hz, 1H), 7.19 (dd, *J* = 2.5 Hz, *J* = 8.4 Hz, 1H), 7.05 (ddd, *J* = 2.6 Hz, *J* = 8.6 Hz, 1H), 6.90 (dd, *J* = 2.0 Hz, *J* = 6.7 Hz, 1H), 6.85 (d, *J* = 4.5 Hz), 4.80 (s, 2H), 3.70 (s, 3H), 3.67 (s, 2H) ; ^{13}C NMR δ: 174.1, 159.7-156.5 (dd, *J* = 236 Hz), 158.5, 140.1 (d, , *J* = 1.5Hz), 128.6, 128.2, 126.8-126.7 (d, , *J* = 9.1Hz), 113.9, 113.5-113.2 (d, , *J* = 23.2 Hz), 112.4-112.1 (d, , *J* =24.8Hz), 109.4-109.3 (d, , *J* = 8.3 Hz), 54.9, 42.0, 35.5-35.4 (d, , *J* = 1.5Hz) ppm: MS (*m/z*) 271 (M_+), 121 (100 %). Anal. Calcd for $C_{16}H_{14}FNO_2$: C, 70.84; H, 5.20; N, 5.16; Found C, 70.59; H, 5.33; N, 5.23.

1-(2,6-Difluorobenzyl)-5-fluoroindolin-2-one (160)

1-(2,6-Difluorobenzyl)-5-fluoro-indoline-2,3-dione (0.3726 g, 1.28 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (15 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 h. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 90 mL), placed in a hood uncovered and allowed to sit overnight. A tan clay-like solid formed overnight and was vacuum filtered to yield a pure product (0.2727 g, 76.9%); mp 100-103°C; R_f = 0.81 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.48-7.38 (m, 1H), 7.18 (ddd, *J* = 2.6 Hz , *J* = 8.3 Hz, 1H), 7.12 (t, *J* = 8.3 Hz, 1H), 6.86 (d, *J* = 4.3Hz , *J* = 8.5 Hz, 1H), 4.95 (s, 2H), 3.62 (s, 2H); ^{13}C (300 MHz, DMSO-d_6): δ 173.5, 162.6-159.2 (dd, *J* = 8.0 Hz), 159.6-156.5 (d, *J* = 237 Hz), 140.0 (d, *J* = 1.4 Hz), 130.5-130.3 (t, *J* = 10.4 Hz), 126.6-126.5 (d, *J* = 9.1 Hz), 113.7-113.3 (d, *J* = 23.2 Hz), 112.4-112.1 (d, *J* = 24.6 Hz), 111.9-111.5 (dd, *J* = 7.4 Hz), 111.2, 108.6-108.5 (d, *J* = 8.4 Hz), 35.2-35.1 (d, *J* = 1.5 Hz), 31.9-31.8 (t, *J* = 3.7 Hz) ppm; MS (m/z) 277 (M_+), 127 (100 %). Anal. Calcd for $C_{15}H_{10}F_3NO$: C, 64.98; H, 3.64; N, 5.05; Found: C, 65.02; H, 3.77; N, 5.25.

1-(3,5-Difluorobenzyl)-5-fluoroindolin-2-one (161)

1-(3,5-Difluorobenzyl)-5-fluoro-indoline-2,3-dione (0.5345 g, 1.84 mmol) was added to a 250 mL rbf with hydrazine hydrate 80% (30 mL). The flask was wrapped in aluminum foil and heat under reflux for 3 hr. The reaction mixture was transferred to a beaker submerged in an ice bath and acidified to a pH of 4 with dilute HCl (3M, 148 mL), placed in a hood uncovered and allowed to sit overnight. A white solid formed overnight and was vacuum filtered to yield a pure product (0.4284 g, 84.2%): mp 170-173°C, R_f = 0.63 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.22 (dd, *J* = 2.5 Hz, *J* = 8.4 Hz, 1H), 7.17 (dt, *J* = 2.3 Hz, *J* =9.3 Hz, 1H), 7.08 (dd, *J* = 1.6 Hz, *J* = 8.4 Hz, 1H), 6.88 (dd, *J* = 4.3 Hz, *J* = 8.5 Hz, 1H), 4.91 (s, 2H), 3.72 (s, 2H): ^{13}C (300 MHz, DMSO-d_6): δ 174.4, 164.1-160.7 (dd, *J* = 13.2 Hz), 159.8-156.7 (d, *J* = 236 Hz), 141.1 (t, *J* = 9.1 Hz), 139.8 (d, *J* = 1.6 Hz), 127.0-126.8 (d, *J* = 9.2 Hz), 113.6-113.3 (d, *J* = 23.2 Hz), 112.5-112.2 (d, *J* = 25.0 Hz), 110.4-110.2 (dd, *J* = 8.0 Hz), 109.2-109.1 (d, *J* = 8.3 Hz), 103.2-102.5 (t, *J* = 25.9 Hz), 41.9, 35.5 (d, *J* = 1.4 Hz) ppm; MS (*m/z*) 277 (M^+), 28 (100 %). Anal. Calcd for $C_{15}H_{10}F_3NO$: C, 64.98; H, 3.64; N, 5.05; Found: C, 64.99; H, 3.50; N, 5.07.

5-Fluoro-3-(pyridin-2-ylmethylene)indolin-2-one (163)

To a vial was added 5-fluoro-indolin-2-one (0.1051 g, 0.696 mmol) with 2-pyridincarboxaldehyde (1.2 equiv, 0.846 mmol, 0.0805 mL), piperidine (0.1 equiv, 0.0708 mmol, 0.007 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. A light-green precipitate (0.1031 g, 60.9%) was collected by vacuum filtration and washed with cold EtOH: mp 248-253˚C; R_f = 0.33 (EtOAc/hexanes, 1:1): ^{1}H NMR (300 MHz, DMSO) δ: 10.64 (s, 1H), 8.97 (dd, *J* = 2.7Hz, *J* = 10.3 Hz, 1H), 8.91 (dt, *J* = 1.2Hz, *J* = 8.1 Hz, 1H), 8.00 (ddd, *J* = 1.8 Hz , *J* = 7.6 Hz), 7.92 (d, *J* = 7.7 Hz, 1H), 7.62 (s, 1H), 7.52 (qd, *J* = 1.4 Hz, 4.7 Hz, 1H), 7.17(ddd, *J* = 2.5 Hz, *J* = 8.7, 1H), 6.87 (dd, *J* = 4.6 Hz, *J* = 8.7 Hz, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 169.2, 158.8-155.7 (d, *J* = 234 Hz), 152.8, 149.6, 139.9 (d, *J* = 1.5 Hz), 137.4, 135.0, 129.0, 128.9 (d, *J* = 2.8Hz), 124.4, 122.6-122.4 (d, *J* = 10.2 Hz) , 117.1-116.8 (d, *J* = 23.9 Hz), 115.1-114.7 (d, *J* = 27.2 Hz), 110.1-110.0 (d, *J* = 8.3 Hz)

ppm; MS (m/z) 240 (M^+), 212 (100%). Anal. Calcd for $C_{14}H_9FN_2O$: C, 69.99; H, 3.78; N, 11.66; Found: C, 69.84; H, 3.73; N, 11.63.

5-Fluoro-3-(pyridin-3-ylmethylene)indolin-2-one (164)

To a vial was added 5-fluoro-indolin-2-one (0.1051 g, 0.696 mmol) with 3-pyridincarboxaldehyde (1.2 equiv, 0.831 mmol, 0.0780 mL), piperidine (0.1 equiv, 0.0506 mmol, 0.005 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was placed in the freezer overnight to afford an orange solid. The orange precipitate (0.0864 g, 62.9%) was collected by vacuum filtration and washed with cold EtOH: mp 210-212˚C; R_f = 0.36 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.69 (s, 1H), 9.19 (d, *J* = 2.0 Hz, 1H), 8.91 (dd, *J* = 1.7 Hz, *J* = 8.0 Hz, 1H), 8.90 (d, *J* = 8.3 Hz), 8.61 (dd, *J* = 1.6, J = 4.7 Hz, 1H), 7.90 (s, 1H), 7.70 (s, 1H), 7.66 (dd, *J* = 2.5 Hz, *J* =8.9 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.10 (dd, *J* = 2.5 Hz, *J* = 18.0 Hz, 1H), 7.07 (d, *J* = 2.5 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 4.12 (q, *J* = 5.2 Hz, 1H) ; ^{13}C NMR (300 MHz, DMSO-d_6) δ: 167.0, 159.5-156.3 (d, *J* = 235 Hz), 152.4, 150.3, 137.9, 137.3 (d, *J* = 1.3 Hz), 134.4, 129.6, 128.4 (d, *J* = 3.1 Hz), 125.8-125.7 (d, *J* = 9.0 Hz), 123.7, 115.8-115.5 (d, *J* = 23.8Hz), 110.3-110.2 (d, *J* = 8.1 Hz), 107.8 -107.4 (d, *J* = 25.4 Hz) ppm; MS (m/z) 240 (M^+), 212 (100%). Anal. Calcd for $C_{14}H_9FN_2O$: C, 69.99; H, 3.78; N, 11.66; Found: C, 69.72; H, 3.78; N, 11.58.

5-Fluoro-3-(pyridin-4-ylmethylene)indolin-2-one (165)

To a vial was added 5-fluoro-indolin-2-one (0.2190 g, 1.460 mmol) with 4-pyridincarboxaldehyde (1.2 equiv, 1.762 mmol, 0.166 mL), piperidine (0.1 equiv, 0.2123 mmol, 0.021 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was placed in the freezer overnight to afford a golden yellow solid. The golden yellow precipitate (0.2835 g, 80.6%) was collected by vacuum filtration and washed with cold EtOH: mp 229-231˚C; R_f = 0.34 (EtOAc/hexanes, 1:1);); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.73 (s, N-H), 8.74 (dd, *J* = 1.6 Hz , *J* = 4.6 Hz, 1H), 7.62 (s, 1H), 7.15 (ddd, *J* = 2.6 Hz, *J* = 8.8 Hz, 1H), 7.04 (dd, *J* = 2.4 Hz, *J* = 9.1 Hz,1H), 6.90 (dd, *J* = 4.6 Hz, *J* = 8.5 Hz,1H), 6.84 (d, J= 8.543 Hz, 1H); ^{13}C NMR δ:168.0, 158.7 (d, *J* = 236 Hz), 150.2, 141.9, 139.6 (d, *J* = 1.6 Hz), 133.9, 129.9 (d, *J* = 3.0 Hz), 124.6,

121.1-121.0 (d, *J* = 8.7Hz), 117.4-117.1 (d, *J* = 23.8 Hz), 111.2-111.1 (d, *J* = 8.5 Hz), 110.0-109.7 (d, *J* = 25.7 Hz) ppm; MS (m/z) 240 (M_+), 212 (100%). Anal. Calcd for $C_{14}H_9FN_2O$: C, 69.99; H, 3.78; N, 11.66; Found: C, 69.71; H, 3.72; N, 11.52.

5-Fluoro-3-(2-hydroxybenzylidene)indolin-2-one (166)

To a vial was added 5-fluoro-indolin-2-one (0.1051 g, 0.696 mmol) with salicyclaldehyde (1.2 equiv, 0.847 mmol, 0.090 mL), piperidine (0.147 equiv, 0.1011 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 6 h while stirring. The vial was cooled in an ice bath and placed in the freezer overnight but no product was formed. The mixture was then evaporated under reduced pressure to afford a brown oil. The oil was recrystallized with DCM/Hexanes to afford an orange precipitate (0.1745 g, 98.4%) was collected by vacuum filtration: mp 205-208˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ; 10.58 (s, NH), 10.33 (s, OH), 7.75 (s, 1H), 7.61 (dd, *J* = 1.1 Hz, *J* = 7.6 Hz, 1H), 7.37 (ddd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H), 7.19 (dd, *J* = 2.5 Hz, *J* = 9.3 Hz, 1H), 7.09 (ddd, *J* = 2.5 Hz , *J* = 9.3 Hz, 1H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 6.87 (dd, *J* = 4.6 Hz, *J* = 8.5 Hz, 1H); ^{13}C NMR δ: 168.7, 158.6-155.5 (d, *J* = 234 Hz), 156.4, 138.8, 134.0, 131.9, 129.5, 126.3-126.2 (d, *J* = 2.9 Hz), 122.3-122.2 (d, *J* = 8.7 Hz), 120.9, 118.8, 116.1, 115.9-115.6 (d, *J* = 23.5 Hz), 110.5-110.4 (d, *J* = 8.4 Hz), 109.5-109.2 (d, *J* = 25.6 Hz) ppm; R_f = 0.67 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 256 (M+1).

5-Fluoro-3-(4-hydroxybenzylidene)indolin-2-one (167)

To a vial was added 5-fluoro-indolin-2-one (0.1933 g, 1.280 mmol) with 4-hydroxybenzaldehyde (1.2 equiv, 1.544 mmol, 0.1884 g), piperidine (0.147 equiv, 0.1213 mmol, 0.0103 g, 0.012 mL) and EtOH (4 mL). The vial was placed on a hot plate at 60˚C for 20 h while stirring. The vial was cooled in an ice bath and placed in the freezer overnight to afford a bright yellow product. A bright yellow precipitate (0.2286 g, 70.0%) was collected by vacuum filtration: mp 305-308˚C; R_f = 0.27 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.55 (s, N-H), 7.60 (s, 1H), 7.60 (d, *J* = 8.264 Hz, 3H), 7.38 (dd, J = 9.5Hz, J = 2.4 Hz, 1H), 7.07 (ddd, *J* = 2.5 Hz, *J* = 8.9 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.88 (dd, *J* = 4.7 Hz, *J* = 8.5 Hz, 1H), 3.63 (s, OH); ^{13}C NMR δ: 169.1, 159.5, 158.7-155.6 (d, *J* = 235 Hz), 138.6 (d, *J* = 1.1 Hz), 138.3, 131.8, 124.5,

124.3 (d, *J* = 2.8 Hz), 122.2-122.1 (d, *J* = 8.9 Hz), 115.7, 115.4, 110.6-110.5 (d, *J* = 8.5Hz), 109.0-108.7 (d, *J* = 25.9 Hz) ppm; MS (*m/z*) 255 (M^+,100%). Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49; Found: C, 70.49; H, 3.90; N, 5.60.

5-Fluoro-3-(4-methoxybenzylidene)indolin-2-one[144] (168)

To a vial was added 5-fluoro-indolin-2-one (0.1143 g, 0.757 mmol) with *p*-anisaldehyde (1.2 equiv, 0.921 mmol, 0.112 mL), piperidine (0.1 equiv, 0.0809 mmol, 0.008 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. A golden yellow precipitate (0.1178 g, 57.9%) was collected by vacuum filtration and recrystallized with EtOH: mp 192-195˚C; R_f = 0.44 (EtOAc/hexanes, 1:1);); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.41 (dd, *J*=1.7 Hz, *J*=6.9 Hz, 1H), 7.85 (s, 1H), 7.51 (dd, *J* =2.4 Hz, *J* = 9.2 Hz, 1H), 7.02 (dd, *J* = 2.0 Hz, *J* = 6.8 Hz 1H), 6.98 (ddd, *J* = 2.4 Hz, *J* = 8.7 Hz , 1H), 6.88 (dd, *J* = 4.5 Hz, *J* = 8.5 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR δ: 170.6, 161.2, 159.9-156.8 (d, *J* = 237 Hz), 139.2 , 137.4 (d, *J* = 1.6 Hz), 134.8, 126.7, 125.4-124.4 (d, *J* = 3.1 Hz), 123.0-122.9 (d, *J* = 9.0 Hz), 115.8-115.5 (d, *J* = 24.1 Hz), 114.3, 110.5-110.4 (d, *J* = 8.2 Hz), 110.2-109.9 (d, *J* = 26.0 Hz), 55.4 ppm; MS (*m/z*) 269 (M^+,100%);

5-Fluoro-3-(3-hydroxy-4-methoxybenzylidene)indolin-2-one (169)

To a vial was added 5-fluoro-indolin-2-one (0.1889 g, 1.25 mmol) with vanillin (1.2 equiv, 1.506 mmol, 0.2289 g), piperidine (0.147 equiv, 0.1213 mmol, 12 µL) and EtOH (4 mL). The vial was placed on a hot plate at 60˚C for 22 h while stirring. The vial was cooled to room temperature and placed in the freezer overnight to afford a dark brown solid. The brown precipitate (0.0523 g, 80.6%) was collected by vacuum filtration and washed with cold EtOH: mp 218-219˚C; R_f = 0.20 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.55 (s, 1H), 9.87 (s, 1H), 7.77 (dd, *J* = 1.5 Hz, *J* = 8.5 Hz, 1H), 7.63 (s, 1H), 7.51 (dd, *J* =2.5 Hz, *J* = 9.6 Hz, 1H), 7.33 (d, *J* =1.8 Hz, 1H), 7.29 (dd, *J* = 1.5 Hz, 8.2 Hz, 1H), 7.08 (ddd, *J* = 2.5 Hz, *J* = 9.1 Hz, 1H), 6.82 (dd, *J* = 4.4 Hz, *J* =8.4 Hz, 1H), 3.87 (d, *J* =11.0, 3H); ^{13}C NMR δ: 169.0, 158.6-155.5 (d, *J* = 234 Hz), 149.1, 147.5, 139.5, 138.8 (d, *J* = 1.2 Hz), 136.1, 128.3, 127.2-127.1 (d, *J* = 8.9 Hz), 125.8, 124.4-124.3 (d, *J* = 2.8 Hz), 123.8, 115.9-115.7 (d, *J* = 17.5 Hz), 113.9-113.6 (d, *J* = 24.3

Hz), 110.5-110.4 (d, *J* = 8.4 Hz), 109.2-108.8 (d, *J* = 26.0 Hz), 55.6 ppm; MS (m/z) 285 (M^+, 100%),

5-Fluoro-3-(2,6 difluoro-benzylidene)indolin-2-one (170)

To a vial was added 5-fluoro-indolin-2-one (0.1025 g, 0.679 mmol) with 2,6 difluorobenzaldehyde (1.2 equiv, 0.816 mmol, 0.0881 mL), piperidine (0.1 equiv, 0.0667 mmol, 0.0066 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. A bright yellow precipitate (0.1082 g, 58%) was collected by vacuum filtration and recrystallized with EtOH: mp 250-255˚C; R_f = 0.27 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.75 (s, 1H), 7.68-7.58 (m, 1H), 7.44 (s, 1H), 7.32 (t, *J* = 8.3 Hz, 2H), 7.16 (ddd, *J* = 2.6 Hz, *J* = 8.7 Hz, 1H), 6.92 (dd, *J* = 4.5 Hz, *J* = 8.6 Hz, 1H), 6.61 (dd, *J* =2.1 Hz, *J* = 8.8 Hz, 1H); ^{13}C NMR δ: 167.5, 161.2-157.8 (dd, *J* = 7.0 Hz), 159.0-155.8 (d, *J* = 235 Hz), 139.5 (d, *J* = 1.6 Hz), 138.0, 132.6-132.3 (t, *J* = 10.4Hz), 131.8 (d, *J* = 2.4 Hz), 121.6, 121.5, 117.3-117.0 (d, *J* = 23.6 Hz), 112.4-112.0 (d, *J* = 8.3Hz), 110.2-110.1 (t, *J* = 2.8 Hz), 108.9-108.5 (d, *J* = 25.7 Hz) ppm; MS (m/z) 275 (M^+, 100%). Anal. Calcd for $C_{15}H_8F_3NO$: C, 65.46; H, 2.93; N, 5.09; Found: C, 65.27; H, 2.93; N, 5.10.

5-Fluoro-3-(3,5-difluorobenzylidene)indolin-2-one (171)

To a vial was added 5-fluoro-indolin-2-one (0.1118 g, 0.740 mmol) with 3,5-difluorobenzaldehyde (1.2 equiv, 0.885 mmol, 0.1257 g, 0.097 mL), piperidine (0.147 equiv, 0.111 mmol, 0.0094 g, 0.011 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled in an ice bath and placed in the freezer overnight to afford an orange product. The orange precipitate (0.1657 g, 81.4 %) was collected by vacuum filtration: mp 218-221˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.75 (s, 1H), 10.69 (s, 1H), 8.15 (dd, *J* = 2.1 Hz, *J* = 9.4 Hz, 1H), 7.84 (s, 1H), 7.62 (s, 1H), 7.61 (dd, *J* = 2.6 Hz, *J* = 8.9 Hz, 1H), 7.43 (d, *J* = 6.8 Hz, 2H), 7.39 – 7.33 (m, 2H), 7.11 (dd, *J* = 2.8 Hz, *J* = 9.2 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 1H), 6.83 (dd, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H); ^{13}C NMR δ: 168.1, 164.2-160.9 (dd, *J* = 13.6 Hz), 159.5-156.3 (d, *J* = 236 Hz), 139.6 (d, *J* = 1.4 Hz), 136.8-136.7 (d, *J* = 10.2 Hz), 135.3, 129.2 (d, *J* = 3.0 Hz), 121.2, 117.2-116.9 (d, *J* = 23.4 Hz), 114.7-114.5 (dd, *J* = 8.1 Hz), 111.1-110.9 (d, *J* = 8.0

Hz), 109.8-109.5 (d, *J* = 25.5 Hz), 105.4-104.7 (t, *J* = 26.3 Hz) ppm; R_f = 0.47 (EtOAc/hexanes, 1:1); MS (*m/z*) 275 (M^+) 28 (100%).

5-fluoro-3-(4-dimethylamino-benzylidene)indolin-2-one (172)

To a vial was added 5-fluoro-indolin-2-one (0.0983 g, 0.651 mmol) with 4-dimethylaminobenzaldehyde (1.2 equiv, 0.775 mmol, 0.1156 g), piperidine (0.1 equiv, 0.071 mmol, 0.007 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was placed in the freezer overnight to afford an orange solid. The orange precipitate (0.0.0782 g, 64.8%) was collected by vacuum filtration and washed with cold EtOH: mp 220-223˚C; R_f = 0.14 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ: 10.47 (s, 1H), 8.49 (d, *J* = 11.4 Hz, 1H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.58 (s, 1H), 7.52 (dd, *J* = 2.0 Hz, *J* = 9.6 Hz), 7.06 (ddd, *J* = 2.3 Hz, *J* = 9.1 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.76 (dd, *J* = 4.4 Hz, *J* = 8.8 Hz, 1H), 3.03 (s, 6H) ; ^{13}C NMR (300 MHz, DMSO-*d*$_6$) δ: 169.3, 158.7-155.6 (d, *J* = 234 Hz), 151.6, 138.9, 138.2, 132.0, 129.4, 122.8-122.7 (d, *J* = 8.8 Hz), 121.6, 120.6, 114.8-114.5 (d, *J* = 23.4 Hz), 111.6-111.5 (d, *J* = 10.4 Hz), 110.2-110.1 (d, *J* = 8.5 Hz), 108.6-108.3 (d, *J* = 25.8 Hz), 39.5 ppm; LC-MS *(m/z)* 283 (M+1).

3-Benzylidene-1-(2,6-difluorobenzyl)-5-fluoroindolin-2-one (175)

To a vial was added 1-(2,6 difluorobenzyl)-5-fluoro-indolin-2-one (0.1925 g, 0.695 mmol) with benzaldehyde (1.2 equiv, 0.833 mmol, 0.0851 mL), piperidine (0.147 equiv, 0.1011 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was placed in an ice bath and then the freezer overnight. Solid was not obtained so the solution was evaporated under reduced pressure to afford a brown solid which was recrystallized with EtOH. A brown precipitate (0.0964 g, 38.0%) was collected by vacuum filtration and washed with cold EtOH: mp 129-131˚C; R_f =0.62 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 7.84 (s, 1H), 7.72 (dd, *J* = 1.8 Hz, *J* = 7.4 Hz, 1H), 7.59 (ddd, *J* = 2.7 Hz, *J* = 7.6 Hz, 1H), 7.56 (dd, *J* = 1.6 Hz, *J* = 6.0 Hz, 2H), 7.49-7.38 (m, 1H), 7.25 (d, *J* = 2.6 Hz, *J* = 9.2 Hz, 1H), 7.18 (d, *J* = 2.3Hz, 1H), 7.15 (t, *J* = 8.3 Hz, 2H), 6.96 (dd, *J* = 4.4 Hz, *J* = 8.6 Hz, 1H), 5.05 (s, 2H); ^{13}C NMR δ: 166.6, 162.6-159.2 (dd, *J* = 8.3 Hz), 159.0-155.9 (d, *J* = 236 Hz), 139.1 (d, *J* =

1.3 Hz), 138.6, 133.8, 130.6-130.4 (t, *J* = 10.6 Hz), 130.1, 129.2, 128.8, 125.8, 121.4-121.3 (d, *J* = 8.9 Hz), 116.4-116.1 (d, *J* = 23.5 Hz), 111.9-111.6 (dd, *J* = 7.5 Hz), 111.4, 109.5-109.1 (d, *J* = 26.1 Hz), 109.4-109.3 (d, *J* = 9.6 Hz), 32.0 (d, *J* = 3.5 Hz) ppm ; MS (*m/z*) 365 (M^+), 127 (100%).

1-(2,6-Difluorobenzyl)-5-fluoro-3-(4-hydroxybenzylidene)indolin-2-one (176)

To a vial was added 1-(2,6 difluorobenzyl)-5-fluoro-indolin-2-one (0.2143 g, 0.774 mmol) with 4-hydroxybenzaldehyde (1.2 equiv, 0.928 mmol, 0.1133 g), piperidine (0.147 equiv, 0.1112 mmol, 0.011 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was placed in an ice bath and then the freezer overnight to afford a brown-orange solid. A brown-orange precipitate (0.2131 g, 72.3%) was collected by vacuum filtration and washed with cold EtOH: mp 253-255˚C; R_f = 0.64 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 10.35 (s, 1H), 8.84 (d, *J* = 8.8 Hz, 2H), 7.86 (s, 1H), 7.68 (dd, *J* = 2.6 Hz, *J* = 9.0 Hz, 1H), 7.45-7.35 (m, 1H), 7.11 (ddd, *J* = 2.5 Hz, *J* = 9.4 Hz, 1H), 7.10 (dd, *J* = 4.0 Hz, *J* = 9.5 Hz, 2H), 7.08 (d, *J* = 4.0 Hz, 1H), 6.91 (dd, *J* = 2.8 Hz, *J* = 9.6 Hz, 3H), 5.05 (s, 2H); ^{13}C NMR δ: 165.1, 162.6-159.2 (dd, *J* = 8.5 Hz), 160.7, 159.8-156.7 (d, *J* = 235 Hz), 139.8, 136.2 (d, *J* = 0.8 Hz), 135.3, 130.4-130.1 (t, *J* = 11.3 Hz), 126.3-126.1 (d, *J* = 8.9 Hz), 125.2, 120.6 (d, *J* = 2.6 Hz), 115.3, 113.9-113.6 (d, *J* = 24.0 Hz), 112.0, 111.9-111.5 (dd, *J* = 7.5 Hz), 108.7-108.6 (d, *J* = 8.5 Hz), 106.6-106.3 (d, *J* = 25.7 Hz), 32.0-31.9 (d, *J* = 3.9 Hz), ppm ; LC-MS (*m/z*) 382 (M^+). Anal. Calcd for $C_{22}H_{14}F_3NO_2$: C, 69.29; H, 3.70; N, 3.67; Found: C, 68.96; H, 3.78; N 4.10;

1-(2,6-difluorobenzyl)-5-fluoro-3-(2-hydroxy-benzylidene)indolin-2-one (177)

To a vial was added 1-(2,6 difluorobenzyl)-5-fluoro-indolin-2-one (0.1930 g, 0.697 mmol) with salicylaldehyde (1.2 equiv, 0.838 mmol, 0.089 mL), piperidine (0.147 equiv, 0.101 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 6 h while stirring. The vial was placed in an ice bath and then the freezer overnight to afford an orange solid. The orange precipitate (0.1653 g, 62.3%) was collected by vacuum filtration and washed with cold EtOH: mp 207-211˚C; R_f = 0.54 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 10.31 (s, 1H), 7.85 (s, 1H), 7.62

(dd, *J* = 1.1 Hz, *J* = 7.6 Hz, 1H), 7.48-7.40 (m, 1H), 7.38 (ddd, *J* = 1.5 Hz, *J* = 6.1 Hz, 1H), 7.24 (ddd, *J* = 2.6 Hz, *J* = 9.2 Hz, 1H), 7.14 (t, *J* = 8.3 Hz, 3H), 7.02 (ddd, *J* = 0.8 Hz, *J* = 5.6 Hz, 1H), 6.95 (dd, *J* = 4.5 Hz, *J* = 8.4 Hz, 1H), 5.05 (s, 2H); ^{13}C NMR δ: 166.7, 162.6-159.2 (dd, *J* = 8.3 Hz), 159.0-155.9 (d, *J* = 236 Hz), 156.5, 138.7 (d, *J* = 1.6 Hz), 135.1, 132.1, 130.6-130.3 (t, *J* = 10.8 Hz), 129.6, 124.7-124.6 (d, *J* = 2.5 Hz), 121.9-121.7 (d, *J* = 8.8 Hz), 120.6, 118.9, 116.1, 115.8-115.5 (d, *J* = 23.6 Hz), 111.9-111.6 (dd, *J* = 7.4 Hz), 111.5, 109.6-109.3 (d, *J* = 26.1 Hz 109.2-109.1 (d, *J* = 8.3 Hz), 32.0-31.9 (d, *J* = 3.7 Hz) ppm; MS (*m/z*) 381 (M^+), 127 (100%). Anal. Calcd for $C_{22}H_{14}F_3NO_2$: C, 69.29; H, 3.70; N, 3.67; Found: C, 68.89; H, 3.98; N, 4.04;

1-(2,6-difluorobenzyl)-5-fluoro-3-(4-methoxybenzylidene)indolin-2-one (178)

To a vial was added 1-(2,6 difluorobenzyl)-5-fluoro-indolin-2-one (0.1818 g, 0.656 mmol) with *p*-anisaldehyde (1.5 equiv, 0.984 mmol, 116 µL), piperidine (0.147 equiv, 0.096 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was placed in an ice bath and then the freezer overnight. Solid was obtained but proved to be impure thru TLC so the product was purified with silica gel (hexanes/EtOAc, 70:30) to afford a tan solid (0.1773 g, 68.4 %). The tan solid was recrystallized with DCM/Hexanes to afford the pure (TLC/GCMS) product (0.1562 g, 60.3 %) was collected by vacuum filtration and washed with cold hexanes: mp 141-145˚C; R_f =0.88 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 7.89 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.68 (d, *J* = 2.5 Hz, *J* = 9.0 Hz, 1H), 7.44-7.34 (m,2H), 7.12 (ddd, *J* = 4.0 Hz, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 6.90 (dd, *J* = 4.2 Hz, *J* = 8.5 Hz, 1H), 5.03 (s, 2H), 3.87 (s, 3H); ^{13}C NMR δ: 165.0, 162.6-159.2 (dd, *J* = 7.9 Hz), 161.7, 159.8-156.7 (d, *J* = 235 Hz), 139.2, 136.4, 134.8, 130.4-130.1 (t, *J* = 10.4 Hz), 129.9, 126.5, 126.0-125.9 (d, *J* = 8.9 Hz), 121.7 (d, *J* = 8.1 Hz), 114.3, 114.2-113.9 (d, *J* = 24.1 Hz), 111.9-111.5 (dd, *J* = 7.4 Hz), 111.7, 108.8-108.7 (d, *J* = 8.2 Hz), 106.8-106.5 (d, *J* = 25.8 Hz), 55.3 (d, *J* = 4.8 Hz), 31.9-31.8 (t, *J* = 3.1 Hz) ppm; MS (*m/z*) 395 (M^+), 127 (100%) 281 byproduct.

1-(2,6-difluorobenzyl)-5-fluoro-3-(4-hydroxy-3-methoxy-benzylidene)indolin-2-one (179)

To a vial was added 1-(2,6 difluorobenzyl)-5-fluoro-indolin-2-one (0.2142 g, 0.773 mmol) with vanillin (1.5 equiv, 1.444 mmol, 0.1764 g), piperidine (0.5 equiv, 0.3842 mmol, 0.038 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was placed in an ice bath and then the freezer overnight. Solid was obtained but proved to be impure thru TLC so the product was purified with silica gel (hexanes/EtOAc, 70:30) to afford a tan solid (0.2016 g, 63.4%). The tan solid was recrystallized with DCM/Hexanes to afford the pure (TLC/GCMS) product (0.1433 g, 45.2%) was collected by vacuum filtration and washed with cold hexanes: mp 125-129˚C; R_f =0.38 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 9.99 (s, 1H), 8.64 (d, *J* =1.9 Hz, 1H), 7.86 (s, 1H), 7.85 (dd, *J* = 1.9 Hz, *J* = 8.5 Hz, 1H), 7.74 (s, 1H), 7.67 (dd, *J* = 2.5 Hz, *J* = 9.0 Hz, 1H), 7.44-7.35 (m,2H), 7.29 (dd, *J* = 1.7 Hz, *J* = 8.4 Hz, 1H), 7.08 (ddd, *J* = 2.5 Hz, *J* = 9.4 Hz, 1H), 6.96 (dd, *J* = 8.1 Hz, *J* = 13.8 Hz, 1H), 6.87 (dd, *J* = 4.3 Hz, *J* = 8.5 Hz, 1H), 5.07 (s, 2H), 3.87 (s, 3H); ^{13}C NMR δ: 167.0, 162.6-159.2 (dd, *J* = 7.7 Hz), 150.5, 147.5, 140.3, 138.6, 136.0, 130.4-130.2 (t, *J* = 8.4 Hz), 128.5, 126.3-126.2 (d, *J* = 9.3 Hz), 125.7, 124.0, 122.7 (d, *J* = 2.9 Hz), 116.1-115.2 (t, *J* = 31.0 Hz), 111.5, 113.9-113.6 (d, *J* = 24.3 Hz), 111.9-111.6 (dd, *J* = 7.6 Hz), 108.6-108.5 (d, *J* = 9.0 Hz), 106.5-106.2 (d, *J* = 25.8 Hz), 55.6-55.5 (d, *J* = 7.3 Hz), 31.9 (d, *J* = 2.6 Hz) ppm; MS (*m/z*) 411 (M^+), 127 (100%).

1-(2,6-difluorobenzyl)-5-fluoro-3-(pyridin-2-ylmethylene)indolin-2-one (180)

To a vial was added 1-(2,6-difluorobenzyl)-5-fluoro-indolin-2-one (0.2242 g, 0.930 mmol) with 2-pyridincarboxaldehyde (1.5 equiv, 1.219 mmol, 0.1306 g, 0.1160 mL), piperidine (0.5 equiv, 0.4651 mmol, 0.0396 g, 0.046 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 6 h while stirring. The vial was cooled in an ice bath and placed in the freezer overnight. An orange precipitate (0.1128 g, 38.1%) was collected by vacuum filtration and washed with cold EtOH: mp 205-208˚C; R_f = 0.46 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 9.06 (dd, *J* = 2.7 Hz, *J* = 10.1 Hz, 1H), 8.93 (dt, *J* = 1.2 Hz, *J* = 4.4 Hz, 1H), 8.02 (ddd, *J* = 1.7 Hz, *J* = 7.7 Hz, 1H), 7.97 (dd, *J* = 0.9 Hz, *J* = 6.6 Hz, 1H), 7.96 (d, *J* = 6.6 Hz, 1H), 7.74 (s, 1H), 7.53 (qd, *J* = 2.8 Hz, *J* = 8.8 Hz, 1H), 7.47 – 7.37 (m, 1H), 7.25 (ddd, *J* = 2.8 Hz, *J* = 8.8 Hz, 1H), 7.14 (t, *J* = 8.2 Hz, 2H), 6.93 (dd, *J* = 4.4 Hz, *J* = 8.6 Hz, 1H), 5.07 (s, 2H); ^{13}C NMR δ:

167.4, 162.6-159.2 (dd, *J* = 7.6 Hz), 159.2-156.1 (d, *J* = 235 Hz), 152.5, 149.7, 140.4, 139.7 (d, *J* = 1.6), 137.5, 136.1, 130.6-130.3 (t, *J* = 10.5 Hz), 129.3, 127.3-127.0 (d, *J* = 2.7 Hz), 124.7, 122.0, 117.0-116.6 (d, *J* = 24.2 Hz), 115.2-114.8 (d, *J* = 27.7 Hz), 111.9-111.6 (dd, *J* = 7.4 Hz), 108.8-108.7 (d, *J* = 8.0 Hz), 32.1 ppm; MS (*m/z*) 366.1 (M^+), 127 (100%). Anal. Calcd for $C_{21}H_{13}F_3N_2O$: C, 68.85; H, 3.58; N, 7.65; Found: C, 68.60; H, 3.76; N, 7.87.

1-(2,6-difluorobenzyl)-5-fluoro-3-(pyridin-4-ylmethylene)indolin-2-one (181)

To a vial was added 1-(2,6-difluorobenzyl)-5-fluoro-indolin-2-one (0.2037 g, 0.735 mmol) with 4-pyridincarboxaldehyde (1.2 equiv, 1.168 mmol, 0.1100 mL), piperidine (0.147 equiv, 0.1011 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. A bright yellow precipitate (0.1025 g, 38.1%) was collected by vacuum filtration and washed with cold EtOH: mp 158-161˚C; R_f = 0.38 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 8.76 (dd, *J* = 1.5 Hz, *J* = 4.4 Hz, 2H), 8.09 (dd, *J* = 1.4 Hz, *J* = 4.8 Hz, 1H), 7.94 (s, 1H), 7.77 (s, 1H), 7.74 (d, *J* = 2.5 Hz, 1H), 7.49 -7.37 (m, 1H), 7.26 (ddd, *J* = 2.6 Hz, *J* = 8.9 Hz, 1H), 7.15 (t, *J* = 8.2 Hz, 2H), 6.99 (dd, *J* = 4.2 Hz, *J* = 8.7 Hz, 1H), 5.05 (s, 2H); ^{13}C NMR δ: 166.1, 162.6-159.2 (dd, *J* = 8.0 Hz), 159.9-156.7 (d, *J* = 236 Hz), 150.3, 141.6, 139.5 (d, *J* = 1.2 Hz), 135.7, 130.7-130.4 (t, *J* = 10.0 Hz), 128.7-128.6 (d, *J* = 3.2 Hz), 122.9, 120.7-120.6 (d, *J* = 8.8 Hz), 117.2-116.9 (d, *J* = 24.0 Hz), 111.9-111.6 (dd, *J* = 7.5 Hz), 111.2, 110.1-109.8 (d, *J* = 26.4 Hz), 108.3-108.2 (d, *J* = 8.2 Hz), 32.1-32.0 (d, *J* = 3.9 Hz) ppm: MS (*m/z*) 366.1 (M^+), 207 (100%). Anal. Calcd for $C_{21}H_{13}F_3N_2O$: C, 68.85; H, 3.58; N, 7.65; Found: C, 68.64; H, 3.58; N, 7.61.

1-(2,6-Difluorobenzyl)-3-(2,6-difluorobenzylidene)-5-fluoroindolin-2-one (182)

To a vial was added 1-(2,6-difluorobenzyl)-5-fluoro-indolin-2-one (0.1818 g, 0.6563 mmol) with 2,6-difluorobenzaldehyde (1.5 equiv, 1.0936 mmol, 118 µL), piperidine (0.147 equiv, 0.1112 mmol, 11 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was then placed in an ice bath which caused a yellow precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A yellow solid was collected by vacuum filtration but the product was

impure thru TLC. The product was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford a yellow solid. The yellow solid was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as a golden yellow product (0.0720 g, 25.2%): mp 142-144 ºC; R_f = 0.94 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 7.71-7.61 (m, 1H), 7.55 (s, 1H), 7.48-7.38 (m, 1H), 7.36 (t, *J* =8.3 Hz, 2H), 7.26 (ddd, *J* = 2.7 Hz, *J* = 9.1 Hz, 1H), 7.15 (t, *J* = 8.2 Hz, 2H), 6.98 (dd, *J* = 4.3 Hz, *J* = 8.7 Hz, 1H), 6.69 (dd, *J* =2.1 Hz, *J* = 8.7 Hz, 1H) , 5.06 (s, 2H); ^{13}C NMR δ: 165.6, 162.6-159.2 (dd, *J* = 7.8 Hz), 161.2-157.8 (dd, *J* = 6.7 Hz), 159.3-156.2 (d, *J* = 237 Hz), 139.4 (d, *J* = 1.4 Hz), 132.8-132.5 (t, *J* = 10.6 Hz), 130.7-130.4 (t, *J* = 10.6 Hz), 130.1 (d, *J* = 2.2 Hz), 122.6, 121.1-121.0 (d, *J* = 8.8 Hz), 117.2-116.9 (d, *J* = 23.6 Hz), 112.4-112.1 (dd, *J* = 6.3 Hz), 112.0-111.6 (dd, *J* = 7.3 Hz), 111.4-110.9 (t, *J* = 18.7 Hz), 111.3, 110.3-109.9 (d, *J* = 25.6 Hz), 109.7-109.6 (d, *J* = 7.4 Hz), 32.2-32.1 (t, *J* = 2.7 Hz) ppm; MS (m/z) 401 (M^+), 127 (100%).

1-(2,6-Difluorobenzyl)-3-(3,5-difluorobenzylidene)-5-fluoroindolin-2-one (183)

To a vial was added 1-(2,6-difluorobenzyl)-5-fluoro-indolin-2-one (0.1904 g, 0.687 mmol) with 3,5-difluorobenzaldehyde (1.5 equiv, 1.031 mmol, 0.1130 mL), piperidine (0.5 equiv, 0.3438 mmol, 0.034 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 8 h while stirring. The vial was placed in an ice bath which caused a yellow precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A yellow solid (0.1634 g, 59.3%) was collected by vacuum filtration and washed with cold EtOH: mp 175-176˚C; R_f = 0.79 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 8.13 (dd, *J* = 2.1 Hz, *J* = 9.3 Hz, 2H), 7.94 (s, 1H), 7.69 (dd, *J* = 2.5 Hz, *J* = 8.7 Hz, 1H), 7.44-7.36 (m, 2H), 7.20(ddd, *J* = 2.5 Hz, *J* = 9.3 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 2H), 6.95 (dd, *J* =4.2 Hz, *J* = 8.6 Hz, 1H) , 5.04 (s, 2H); ^{13}C NMR δ: 164.5, 163.6-160.3 (dd, *J* = 13.6 Hz), 162.6-159.2 (dd, *J* = 7.9 Hz), 159.9-156.7 (d, *J* = 236 Hz), 139.1 (d, *J* = 0.8 Hz), 137.4, 136.2-136.1 (d, *J* = 2.6 Hz), 130.6-130.3 (t, *J* = 10.0 Hz), 127.0-126.9 (d, *J* = 3.0 Hz), 124.8-124.7 (d, *J* = 9.1 Hz), 116.1-115.8 (d, *J* = 24.5 Hz), 114.7-114.4 (dd, *J* = 8.3 Hz), 111.9-111.6 (dd, *J* = 7.4 Hz), 111.4, 109.5-109.4 (d, *J* = 8.6 Hz), 107.9-107.5 (d, *J* = 25.8 Hz), 106.4-105.2 (t, *J* = 25.8 Hz), 32.1-32.0 (t, *J* = 3.5 Hz)

ppm; MS (*m/z*) 401 (M^+), 127 (100%); Anal. Calcd for $C_{22}H_{12}F_5NO$: C, 65.84; H, 3.01; N, 3.49; Found: C, 65.89; H, 3.06; N, 3.75.

1-(2,6-difluorobenzyl)-5-fluoro-3-(4-fluoro-benzylidene)indolin-2-one (184)

To a vial was added 1-(2,6-difluorobenzyl)-5-fluoro-indolin-2-one (0.2196 g, 0.792 mmol) with 4-fluorobenzaldehyde (1.2 equiv, 0.951 mmol, 0.102 mL), piperidine (0.147 equiv, 0.121 mmol, 0.012 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization but was unsuccessful. The reaction mixture was then evaporated under reduced pressure to afford an orange oil. The oil was then recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as brown-orange powder (0.1352 g, 44.5 %): mp 135-138 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.51 (dd, *J* = 5.7 Hz, .*J* = 8.8 Hz, 1H), 7.97 (s, 1H), 7.81 (d, *J* = 3.8 Hz , *J* = 4.4 Hz, 1H), 7.72 (dd, *J* = 2.5 Hz, *J* = 8.8 Hz, 1H), 7.47-7.33 (m, 3H), 7.21(ddd, *J* = 2.5 Hz, *J* = 8.8 Hz, 1H), 7.14 (t, (dd, *J* = 8.1, 2H), 6.96 (dd, *J* = 4.25 Hz, *J* = 8.6 Hz, 1H), 5.04 (s, 2H); ^{13}C NMR δ: 166.5, 165.0-164.8 (d, *J* = 16.3 Hz), 162.6-159.2 (dd, *J* = 7.4 Hz), 159.9-156.7 (dd, *J* = 235 Hz), 139.1 (d, *J* = 1.9 Hz), 137.9-137.5 (d, *J* = 34.9 Hz), 136.9, 134.9 (d, *J* = 8.7 Hz), 130.6-130.3 (t, *J* = 10.4 Hz), 130.2 (d, *J* = 3.1 Hz), 124.2-124.1 (t, *J* = 2.7 Hz), 121.3-121.1 (d, *J* = 9.0 Hz), 116.1-115.8 (d, *J* = 21.2 Hz), 115.1-114.8 (d, *J* = 23.9 Hz), 111.9-111.5 (dd, *J* = 8.4 Hz), 109.5-109.0 (d, *J* = 9.8 Hz), 107.4-107.1 (d, *J* = 26.1 Hz), 32.0 ppm: R_f = 0.81 (EtOAc/hexanes, 1:1); MS (*m/z*) 383 (M^+), 28 (100%);

1-(2,6-difluorobenzyl)-5-fluoro-3-(4-dimethylamino-benzylidene)indolin-2-one (185)

To a vial was added 1-(2,6- difluorobenzyl)-5-fluoro-indolin-2-one (0.2061 g, 0.744 mmol) with 4-dimethylaminobenzaldehyde (1.2 equiv, 0.899 mmol, 0.1341 g), piperidine (0.1 equiv, 0.101 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was placed in the freezer overnight to afford an orange solid. The orange precipitate (0.1369 g, 45.1%) was collected by vacuum filtration and washed with cold EtOH: mp 174-176˚C; R_f = 0.81 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.48 (d, *J* = 9.0 Hz, 1H), 7.78 (s, 1H), 7.67 (dd, *J* = 4.5 Hz, *J* = 8.9 Hz, 1H), 7.45-7.35 (m, 2H), 7.13 (ddd, *J* = 4.0 Hz, *J* = 8.0 Hz, 2H), 7.02

(ddd, *J* = 2.5 Hz, *J* = 9.4 Hz, 1H), 6.88 (ddd, *J* = 2.5 Hz , *J* = 6.8 Hz, 1H), 6.80 (t, *J* = 9.4 Hz), 5.05 (s, 1H), 3.05 (s, 6H); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 165.2, 162.6-159.8 (dd, *J* =8.0 Hz), 159.8-156.6 (d, *J* =235 Hz), 151.9, 140.3, 138.0 (d, *J* =1.4 Hz), 135.2, 130.3-130.1 (t, *J* =10.6 Hz), 129.4, 126.9-126.8 (d, *J* =9.3 Hz), 121.5 (d, *J* =4.5 Hz), 120.3, 117.6 (d, *J* =3.0 Hz), 112.8-112.5 (d, *J* =23.7 Hz), 112.0-111.7 (dd, *J* =11.0 Hz), 108.3-108.2 (d, *J* =7.2 Hz), 105.8-105.5 (d, *J* =25.3 Hz), 39.6, 31.9-31.8 (t, *J* =3.9 Hz) ppm; LC-MS *(m/z)* 409 (M+1).

1-(2,6 difluorobenzyl)-5-chloro-3-(4-(hydroxy)-benzylidene)indolin-2-one (186)

To a vial was added 1-(2,6-difluorobenzyl)-5-chloro-indolin-2-one (0.1829 g, 0.624 mmol) with 4-hydroxybenzaldehyde (1.2 equiv, 0.798 mmol, 0.0974 g), piperidine (0.147 equiv, 0.091 mmol, 0.009 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.1700 g, 68.6%) was collected by vacuum filtration and washed with cold EtOH: mp 250-255 ˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.35 (s, 1H), 8.44 (d, *J* = 8.1 Hz, 2H), 7.90 (d, *J* = 16.7 Hz, 2H), 7.73 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.42-7.32 (m, 2H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 3H), 6.93 (t, *J* = 8.6 Hz, 4H), 5.04 (s, 2H) ; ^{13}C NMR δ: 165.8, 162.5, 159.8, 139.8, 138.6, 133.7, 130.3, 127.8, 126.2, 125.4, 124.3, 122.3, 120.0, 119.8, 115.5, 111.7, 109.3, 31.9 ppm: R_f = 0.56 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 398 (M^+);

1-(2,6 difluorobenzyl)-5-chloro-3-(2-hydroxy-benzylidene)indolin-2-one (187)

To a vial was added 1-(2,6 difluorobenzyl)-5-chloro-indolin-2-one (0.1577 g, 0.538 mmol) with salicyclaldehyde (1.2 equiv, 0.650 mmol, 0.069 mL), piperidine (0.147 equiv, 0.081 mmol, 0.008 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. No solid precipitated so the solution was evaporated under reduced pressure and recrystallized with DCM/hexanes. A brown precipitate (0.1389 g, 68.6%) was collected by vacuum filtration and washed with cold EtOH: mp 149-151 ˚C; R_f = 0.56 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 398 (M^+).

1-Benzyl-5-fluoro-3-(2-hydroxybenzylidene)indolin-2-one (190)

To a vial was added 1-(Benzyl)-5-fluoro-indolin-2-one (0.2039 g, 0.846 mmol) with salicylaldehyde (1.5 equiv, 1.271 mmol, 135 µL), piperidine (0.5 equiv, 0.4146 mmol, 41 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 6 h while stirring. The vial was then placed in an ice bath which caused a brown precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A brown solid (0.1756 g, 69.9%) was collected by vacuum filtration and washed with cold EtOH: mp 185-187˚C; R_f = 0.43 (EtOAc/hexanes, 1:1);); ^{1}H NMR (300 MHz DMSO-d_6) δ : 9.00 (s, 1H), 8.59 (d, *J* = 9.4 Hz, 1H), 8.12 (s,1H), 7.71 (d, *J* = 9.4 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.43 (d, *J* = 1.7 Hz, 1H), 7.41-7.36 (m, 1H), 7.35 (d, *J* = 4.1 Hz, 2H), 7.31 (d, *J* = 10.0 Hz, 1H), 7.29 (d, *J* = 8.6, 1H), 7.25 (d, *J* = 9.2 Hz, 1H), 7.13 (dd, *J* = 2.6 Hz, *J* = 17.9 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 7.03 (s, 1H), 7.00-6.93 (m, 1H), 4.99 (s, 2H) ;^{13}C NMR δ: 167.3, 159.1-156.0 (d, *J* = 236 Hz), 156.5, 138.9 (d, *J* = 1.2 Hz), 136.3, 135.3, 132.2, 129.6, 128.6, 127.4, 127.2, 125.0-124.9 (d, *J* = 2.7 Hz), 121.9-121.8 (d, *J* = 8.9 Hz), 120.7, 118.9, 116.1, 115.7-115.4 (d, *J* = 23.8 Hz), 110.0-109.9 (d, *J* = 8.3 Hz), 109.6-109.3 (d, *J* = 25.9 Hz), 42.7 ppm:; MS (*m/z*) 345 (M^+), 91 (100%). Anal. Calcd for $C_{22}H_{16}FNO_2$: C, 76.51; H, 4.67; N, 4.06; Found: C, 76.64; H, 4.68; N, 4.06.

1-Benzyl-5-fluoro-3-(4-hydroxybenzylidene)indolin-2-one (191)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.2927 g, 1.214 mmol) with 4-hydroxybenzaldehyde (1.2 equiv, 1.439 mmol, 0.1758 g), piperidine (0.147 equiv, 0.1719 mmol, 17 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was then cooled to room temperature and placed into the freezer overnight.. A yellow solid was collected by vacuum filtration and washed with EtOH to afford the pure (TLC, GC/MS) product as a bright yellow solid (0.3413 g, 81.4 %): mp 236-238 ºC; R_f = 0.33 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 10.35 (s, 1H), 8.48 (dd, *J* = 2.5 Hz , *J* = 11.3 Hz, 2H), 7.90 (s, 1H), 7.69 (dd, *J* = 2.5 Hz, *J* = 9.0 Hz, 1H), 7.83 (s, 1H), 7.33 (d, *J* = 4.4 Hz, 5H), 7.27 (dd, *J* = 3.3 Hz, *J* = 4.7 Hz, 2H), 7.04 (ddd, *J* = 2.5 Hz, *J* = 8.6 Hz, 1H), 6.91 (dd, *J* = 4.5 Hz, *J* = 8.8 Hz, 1H) ^{13}C NMR δ: 165.5, 160.8, 159.9-156.8 (d, *J* = 235 Hz), 140.0, 136.7, 136.3 (d, *J* = 0.7 Hz), 135.3, 128.6, 127.3, 127.2, 126.3-126.1 (d, *J* = 9.1 Hz), 125.2, 120.9-120.8 (d, *J* = 2.9 Hz),

115.3, 113.9- 113.6 (d, *J* = 24.2 Hz), 109.4-109.3 (d, *J* = 8.2 Hz), 106.6-106.3 (d, *J* =25.2 Hz), 42.6 ppm: MS (m/z) 345 (M_+), 91(100%). Anal. Calcd for $C_{22}H_{16}FNO_2$: C, 76.51; H, 4.67; N, 4.06; Found: C, 76.46; H, 4.62; N, 4.15.

1-Benzyl-5-fluoro-3-(4-methoxybenzylidene)indolin-2-one (192)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.1920 g, 0.797 mmol) with *p*-anisaldehyde (1.5 equiv, 1.1917 mmol, 145 µL), piperidine (0.5 equiv, 0.3944 mmol, 39 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was then placed in an ice bath which caused a yellow precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A yellow solid was collected by vacuum filtration but the product was very gummy. The product was dissolved combined with the filtrate and evaporated under reduced pressure to afford a solid and recrystallized from EtOH to afford the pure (TLC, GC/MS) product as a dark brown product (0.0720 g, 25.2%): mp 140-142 ºC; R_f = 0.63 (EtOAc/hexanes, 1:1);); ^{1}H NMR (300 MHz DMSO-d_6) δ: 7.76 (dd, *J* = 2.6 Hz, *J* = 11.1 Hz, 2H), 7.83 (s, 1H), 7.44 (dd, *J* = 2.5 Hz, *J* = 9.3 Hz, 1H), 7.32 (d, *J* = 4.0 Hz, 5H), 7.09 (dd, *J* = 3.2 Hz, *J*=9.1 Hz, 1H), 7.09 (d, *J*=9.1 Hz, 2H), 7.04 (ddd, *J* =2.4 Hz, *J*=9.4 Hz, 1H), 6.97 (dd, *J* = 4.5 Hz, *J*=8.6 Hz, 1H), 6.92 (d, *J*=8.6 Hz, 1H), 4.98 (s, 2H), 3.85 (s,3H) ^{13}C NMR δ: 167.5, 161.0, 159.1-156.0 (d, *J* = 236 Hz), 138.9, 136.5 (d, *J* =1.3 Hz), 136.3, 131.7, 128.6, 127.3, 127.1, 126.0, 123.9-123.8 (d, *J* = 3.0 Hz), 121.7-121.6 (d, *J* = 9.0 Hz), 115.8-115.4 (d, *J* = 23.5 Hz), 114.4, 110.1-110.0 (d, *J* = 8.3 Hz), 109.2-108.9 (d, *J* =26.0 Hz), 55.4, 42.7 ppm; MS (m/z) 359 (M_+), 91(100%). Anal. Calcd for $C_{23}H_{18}FNO_2$: C, 76.86; H, 5.05; N, 3.90; Found: C, 76.66; H, 4.99; N, 3.96.

1-Benzyl-5-fluoro-3-(pyridin-2-ylmethylene)indolin-2-one (193)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.1889 g, 0.784 mmol) with 2-pyridincarboxaldehyde (1.5 equiv, 1.177 mmol, 0.1120 mL), piperidine (0.5 equiv, 0.3944 mmol, 39 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was then placed in an ice bath which caused a brown precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A brown solid (0.1921 g, 74.3%) was collected by vacuum filtration and washed with cold EtOH: mp 126-127˚C; R_f =0.51 (EtOAc/hexanes, 1:1); %).^{1}H NMR

(300 MHz, DMSO-d_6) δ: 9.06 (dd, J = 2.7 Hz, J = 10.1Hz, 1H), 8.94 (dd, J = 1.8 Hz, J = 8.1 Hz, 1H), 8.03 (dd, J = 1.7 Hz, J = 7.8 Hz, 1H), 7.98 (d, J =1.5 Hz, 1H), 7.80 (s, 1H), 7.54-7.51 (m, 1H), 7.34 (d, J= 4.4Hz, 4H), 7.30-7.23 (m, 1H), 7.20 (ddd, J = 2.8 Hz, J =8.8 Hz,1H), 6.97 (dd, J =4.5 Hz, J = 8.6 Hz, 1H), 5.01 (s, 2H);^{13}C (300 MHz, DMSO-d_6): δ 167.9, 159.3-156.2 (d, J = 235 Hz), 152.6, 149.7, 139.9 (d, J = 1.4 Hz), 137.5, 136.2 (d, J = 4.2 Hz), 129.3, 128.6, 127.6-127.5 (d, J = 2.6 Hz), 127.4, 127.1, 124.7, 122.2-122.0 (d, J = 10.2 Hz), 116.9-116.6 (d, J = 24.1 Hz), 115.2-114.9 (d, J = 27.2 Hz), 109.6-109.5 (d, J = 8.5 Hz), 42.8 ppm; MS (*m/z*) 330.1 (M^+), 91 (100%). Anal. Calcd for $C_{21}H_{15}FN_2O$: C, 76.35; H, 4.58; N, 8.48 Found: C, 76.60; H, 4.67; N, 8.45.

1-Benzyl-5-fluoro-3-(pyridin-4-ylmethylene)indolin-2-one (194)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.2032 g, 0.843 mmol) with 4-pyridincarboxaldehyde (1.2 equiv, 1.274 mmol, 0.1200 mL), piperidine (0.147 equiv, 0.1315 mmol, 0.013 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was then placed in an ice bath which caused a bright yellow precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A bright yellow solid (0.1405 g, 50.5%) was collected by vacuum filtration and washed with cold EtOH: mp 157-158˚C; R_f = 0.29 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 8.77(dd, J = 1.5 Hz, J = 4.4 Hz, 2H), 7.82 (s, 1H), 7.78 (dd, J = 2.5Hz, J=8.7 Hz, 1H), 7.67 (dd, J = 1.5 Hz, J = 6.0 Hz, 1H), 7.35 (d, J = 4.4 Hz, 4H), 7.31-7.25 (m, 1H), 7.13 (ddd, J = 2.6 Hz, J = 9.2 Hz, 1H), 7.01 (dd, J=4.5 Hz, 8.9 Hz, 1H), 7.01-6.92 (m, 1H), 4.99 (d, J = 9.6 Hz, 2H); ^{13}C NMR δ: 166.6, 159.1-156.0 (d, J = 236 Hz), 150.3, 141.7, 139.7 (d, J = 1.4 Hz), 136.2, 135.8, 129.0 (d, J = 2.9 Hz), 127.4 (d, J = 4.0 Hz), 124.7, 124.6-124.5 (d, J = 8.9 Hz), 117.1-116.8 (d, J = 23.5 Hz), 110.6-110.5 (d, J = 8.6 Hz), 110.1 (d, J = 2.5 Hz), 110.0-109.8 (d, J = 14.8 Hz), 108.4-108.0 (d, J = 25.6 Hz), 42.8-42.7 (d, J = 8.1 Hz) ppm; MS (*m/z*) 330.1 (M^+), 207.1 (100%). Anal. Calcd for $C_{21}H_{15}FN_2O$: C, 76.35; H, 4.58; N, 8.48; Found: C, 76.35; H, 4.53; N, 8.59.

1-Benzyl-3-(3,5-difluorobenzylidene)-5-fluoroindolin-2-one (195)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.2169 g, 0.900 mmol) with 3,5-difluorobenzaldehyde (1.5 equiv, 1.351 mmol, 0.1480 mL), piperidine (0.5 equiv, 0.4550 mmol, 45 μL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was then placed in an ice bath which caused a yellow precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A yellow solid (0.1624 g, 49.4%) was collected by vacuum filtration and washed with cold EtOH: mp 161-164˚C; R_f =0.69 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 8.18 (dd, *J* =2.1 Hz, *J* =9.4 Hz, 1H), 7.98 (s, 1H), 7.70 (dd, *J* =2.5 Hz, *J* =8.7 Hz, 1H), 7.43 (dt, *J* =2.3Hz ,*J* =9.4 Hz 1H), 7.35 (d, *J* =4.4 Hz, 4H), 7.31-7.24 (m, 1H), 7.13 (ddd, *J* =2.5 Hz, *J* =9.4 Hz, 1H), 6.96 (dd, *J* =3.9 Hz, *J* =8.6 Hz, 1H), 4.98 (s, 2H) ^{13}C NMR δ: 166.8, 164.2-160.7 (dd, *J* = 13.6 Hz), 159.1-156.0 (d, *J* = 236 Hz), 139.6 (d, *J* = 1.0 Hz), 137.5-137.4 (d, *J* = 10.2 Hz), 136.2, 136.0, 128.6, 127.9 (d, *J* = 2.8 Hz), 127.4 (d, *J* = 3.9 Hz), 127.2, 124.9-124.7 (d, *J* = 9.0 Hz),116.9-116.6 (d, *J* = 23.5 Hz), 114.8-114.5 (d, *J* = 26.2 Hz), 112.4-112.0 (dd, *J* = 8.5 Hz), 110.5-110.4 (d, *J* = 8.2 Hz), 105.6-104.9 (t, *J* = 25.8 Hz), 42.8 ppm; MS (*m/z*) 365 (M^+), 91 (100%).

1-Benzyl-3-(2,6-difluorobenzylidene)-5-fluoroindolin-2-one (196)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.1804 g, 0.749 mmol) with 2,6-difluorobenzaldehyde (1.2 equiv, 0.878 mmol, 0.096 mL), piperidine (0.147 equiv, 0.111 mmol, 11 μL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 6 h while stirring. The vial was then placed in an ice bath which caused a yellow precipitate to form. The vial was placed in the freezer overnight to induce more crystallization. A yellow solid (0.1099 g, 49.4%) was collected by vacuum filtration and washed with cold EtOH: mp 162-164˚C; R_f =0.69 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 7.69 (m,1Ht, *J* =8.2 Hz, 1H), 7.61 (s, 1H), 7.36 (d, *J* =4.4 Hz, 5H), 7.32-7.26 (m, 2H), 7.20 (ddd, *J* = 2.5 Hz, *J* = 8.9 Hz, 1H), 7.02 (dd, *J* = 4.4 Hz, *J* = 8.6 Hz, 1H), 6.71 (dd, *J* = 2.0 Hz, *J* = 8.7 Hz,1H), 4.99 (s, 2H); ^{13}C NMR δ: 166.1, 161.2-157.8 (dd, *J* = 6.9 Hz), 159.4-156.3 (d, *J* = 237 Hz), 139.6 (d, *J* = 1.5 Hz), 136.1, 132.8-132.5 (t, *J* = 10.5 Hz), 130.4 (d, *J* = 2.5 Hz), 128.7, 127.5, 127.2, 122.8, 121.2-121.0 (*J* = 8.8 Hz), 117.1-116.8 (d, *J* = 23.7 Hz), 112.4-112.1 (dd, *J* = 22.7 Hz), 111.6 – 111.4 (d, *J* = 19.6 Hz), 110.5-110.4 (d, *J* = 8.3 Hz), 110.3 -110.0 (d, J = 23.5 Hz), 42.8 ppm; MS (*m/z*) 365 (M^+), 91

(100%). Anal. Calcd for $C_{22}H_{14}F_3NO$: C, 72.32; H, 3.86; N, 3.83 Found: C, 72.22; H, 4.00; N, 3.92.

1-Benzyl-5-fluoro-3-(3-hydroxy-4-methoxybenzylidene)indolin-2-one (197)

To a vial was added 1-(benzyl)-5-fluoro-indolin-2-one (0.1843 g, 0.765 mmol) with vanillin (1.2equiv, 0.914 mmol, 0.1389 g), piperidine (0.147 equiv, 0.121 mmol, 0.0103 g, 12 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to room temperature then placed in the freezer overnight but crystallization did not occur. The solution was evaporated under reduced pressure to afford a red oil. The oil dissolved with DCM and the Biotage Horizon HPFC (hexanes/EtOAc, 70:30) was used to separate the product from impurities to give a yellow solid. The solid was dissolved in DCM then recrystallized from hexanes to afford the pure (TLC, GC/MS) product as a yellow-orange product (0.1387 g, 48.4%): mp 145-149 ºC; R_f = 0.43 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 10.02 (s, 1H), 7.91 (s, 1H), 7.57 (dd, *J* =2.5 Hz, *J* = 9.4 Hz, 1H), 7.46 (dd, *J* =1.8 Hz, *J* = 7.9 Hz, 1H), 7.32 (d, *J* =4.8 Hz, 5H), 7.29 – 7.23 (m, 2H), 7.09 (ddd, *J* =2.5 Hz, *J* =8.3 Hz, 1H), 6.89 (d, *J* = 4.2 Hz, *J* =8.3 Hz, 1H), 5.01 (d, *J* = 7.1 Hz, 2H), 3.87 (s, 3H); ^{13}C NMR δ: 165.6, 159.9-156.8 (d, *J* = 235 Hz), 150.5, 147.6, 140.4, 138.8, 136.6, 128.6, 127.2, 127.1, 126.3, 124.8, 124.1, 123.0-122.9 (d, *J* = 2.9 Hz), 115.9, 115.3-115.2 (t, *J* = 5.4 Hz), 113.8-113.5 (d, *J* = 23.9 Hz), 109.4-109.3 (d, *J* = 8.4 Hz), 106.5-106.2 (d, *J* = 25.9 Hz) 55.5, 42.6 ppm; MS (m/z) 375 (M^+), 28 (100%).

1-(Benzyl)-5-fluoro-3-(4-dimethylamino-benzylidene)indolin-2-one (198)

To a vial was added 1-(benzyl)-5-fluoro- indolin-2-one (0.2131 g, 0.884 mmol) with 4-dimethylaminobenzaldehyde (1.2 equiv, 1.023 mmol, 0.1527 g), piperidine (0.1 equiv, 0.121 mmol, 0.012 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was placed in the freezer overnight to afford a red solid. The orange precipitate (0.2711g, 82.4%) was collected by vacuum filtration and washed with cold EtOH: mp 139-140˚C; R_f = 0.80 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.53 (d, *J* = 9.0 Hz, 2H), 7.82 (s, 1H), 7.64 (dd, *J* =2.4 Hz, *J* = 9.2 Hz, 1H), 7.32 (d, J = 4.3 Hz, 4H), 7.28-7.22 (m, 1H), 6.97 (ddd, *J* =2.5 Hz, *J* =8.7 Hz, 1H), 6.87

(dd, *J* = 4.5 Hz, *J* =8.5 Hz, 1H), 6.80 (d, *J* = 9.1 Hz, 2H), 4.99 (s, 2H), 3.05 (s, 6H); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 165.7, 159.9-156.7 (d, *J* = 235 Hz), 152.2, 140.4, 136.9, 135.7, 135.3, 128.5, 127.2, 127.1, 126.9-126.8 (d, *J* = 9.1 Hz), 121.6, 117.9-117.8 (d, *J* = 3.0 Hz), 112.7-112.4 (d, *J* = 24.0 Hz), 111.0, 109.0-108.9 (d, *J* = 8.4 Hz), 105.8-105.5 (d, *J* = 25.5 Hz), 42.5, 39.5 ppm; LC-MS *(m/z)* 373 (M^+), 100%). Anal. Calcd for $C_{24}H_{21}FN_2O$: C, 77.40; H, 5.68; N, 7.52; Found: C, 77.20; H, 5.74; N, 7.61.

1-Benzyl-5-chloro-3-(4-hydroxy-benzylidene)indolin-2-one (199)

To a solution of 1-(benzyl)-5-chloro-indolin-2-one (0.2833 g, 1.102 mmol) with EtOH (45 mL) was added 4-hydroxybenzaldehyde (1.2 equiv, 1.322 mmol, 0.1615 g) and piperidine (0.147 equiv, 0.1617 mmol, 0.0137 g, 0.016 mL). The mixture was heated under reflux and stirred for 5 h or until the solution went from yellow to orange in color. The solution was cooled to room temperature and evaporated under reduced pressure to afford an orange oil. The orange oil was recrystallized with DCM/Hexanes to afford the pure (TLC, GC/MS) product as a yellow solid (0.3246 g, 68.6%): mp 255-257 ˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.32 (s, 1H), 8.50 (d, *J* =8.8 Hz, 1H), 7.96 (s, 1H), 7.88 (d, *J* = 2.0 Hz, 1H), 7.81 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.34 (d, 3.7 Hz, 1H), 7.32 (d, *J* = 2.6 Hz, 3H), 7.30 (d, *J* = 8.6 Hz, 1H), 7.23 (dd, *J* = 2.1 Hz, *J* = 8.3 Hz, 1H), 6.98 (t, *J* = 4.3 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 1H), 4.99 (s, 2H); ^{13}C NMR δ: 166.3, 160.4, 140.0, 139.9, 136.4, 133.7, 128.6, 127.3, 127.1, 127.0, 126.3, 125.4, 124.4, 121.3, 120.1, 115.5, 110.1, 42.6 ppm: R_f = 0.64 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 362 (M+1);

1-(Benzyl)-3-(4-dimethylamino-benzylidene)indolin-2-one (200)

To a vial was added 1-(benzyl)- indolin-2-one (0.1977 g, 0.886 mmol) with 4-dimethylaminobenzaldehyde (1.2 equiv, 0.952 mmol, 0.1421 g), piperidine (0.1 equiv, 0.121 mmol, 0.012 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was placed in the freezer overnight to afford an orange solid. The orange precipitate (0.2377g, 75.7%) was collected by vacuum filtration and washed with cold EtOH: mp 139-140˚C; R_f = 0 75 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.52 (d, *J* = 9.0 Hz, 2H), 7.75 (s, 1H), 7.70 (d, *J* = 7.0 Hz, 1H), 7.33-7.23 (m, 5H), 7.15 (dd, *J* = 0.9 Hz, *J* = 15.2 Hz, 1H), 7.02 (dd, *J* = 0.7 Hz, *J* = 7.5 Hz, 1H),

6.90 (d, *J* = 7.6 Hz, 1H), 6.79 (d, *J* = 9.0 Hz, 2H), 5.00 (s, 2H), 3.04 (s, 6H); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 165.6, 151.9, 139.5, 138.8, 137.1, 134.9, 128.5, 127.2, 127.2, 126.8, 125.1, 121.8, 121.2, 118.5, 118.2, 111.0, 108.3, 42.4, 39.5 ppm; LC-MS (*m/z*): 355 (M+1). Anal. Calcd for $C_{24}H_{22}N_2O$: C, 81.33; H, 6.26; N, 7.90 Found: C, 81.15; H, 6.20; N, 7.92.

1-(3,5-difluorobenzyl)-5-fluoro-3-(pyridin-2-ylmethylene)indolin-2-one (203)
To a vial was added 1-(3,5-difluorobenzyl)-5-fluoro-indolin-2-one (0.1758 g, 0.635 mmol) with 2-pyridincarboxaldehyde (1.2 equiv, 0.767 mmol, 0.073 mL), piperidine (0.147 equiv, 0.091 mmol, 0.009 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. A tan precipitate (0.0861 g, 37.1%) was collected by vacuum filtration and washed with cold EtOH: mp 165-166˚C; R_f = 0.73 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.05 (dd, *J* = 2.7 Hz, *J* = 10.1 Hz 1H), 8.92 (d, *J* = 5.2 Hz,1H), 8.00 (ddd, *J* =1.8 Hz, *J* =7.6 Hz, 1H), 7.94 (d, *J* = 7.3 Hz), 7.76 (s, 1H), 7.51 (qd, *J* =1.4 Hz , *J* = 4.7 Hz, 1H), 7.20 (ddd, *J* =2.8 Hz, *J* = 8.8 Hz), 7.15 (dd, *J* =2.2 Hz, *J* =9.4 Hz 1H), 7.05 (d, *J* = 8.3 Hz, 2H), 6.87 (dd, *J* =4.6 Hz, *J* = 8.6 Hz, 1H), 5.01 (s, 2H); ^{13}C (300 MHz, DMSO-d_6) δ: 168.1, 164.2-160.7, 159.4-156.3, 152.5, 149.7, 141.1-140.8, 139.5-139.4, 137.4, 136.4, 129.3, 127.3, 124.7, 122.2-122.1, 116.9-116.6 (d, *J* = 24.0 Hz), 115.3-114.9 (d, *J* = 27.5 Hz), 110.4-110.1 (dd, *J* = 8.1 Hz), 109.5-109.4 (d, *J* = 8.4 Hz), 103.3-102.6 (t, *J* = 25.9 Hz), 42.6 MS (*m/z*) 366 (M^+), 127 (100%); Anal. Calcd for $C_{21}H_{13}F_3N_2O$: C, 68.85; H, 3.58; N, 7.65 Found: C, 68.72; H, 3.82; N, 7.49.

1-(3,5-Difluorobenzyl)-5-fluoro-3-(pyridin-4-ylmethylene)indolin-2-one (204)
To a vial was added 1-(3,5-difluorobenzyl)-5-fluoro-indolin-2-one (0.1887 g, 0.681 mmol) with 4-pyridincarboxaldehyde (1.2 equiv, 0.8174 mmol, 77 µL), piperidine (0.147 equiv, 0.1011 mmol, 10 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was then placed in the freezer overnight but crystallization did not occur. The solution was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford a yellow solid (0.1461 g, 58.6%). The yellow solid was recrystallized from DCM/ hexanes to afford the pure (TLC,

GC/MS) product as a yellow-orange product (0.0574 g, 23.0%): mp 138-141 ºC; R_f = 0.25 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.77 (dd, *J* = 1.3 Hz, *J* = 4.5 Hz, 2H), 8.12 (d, *J* = 6.0 Hz, 1H) 7.98 (s, 1H), 7.79 (dd, *J* = 2.5 Hz, *J* = 8.7 Hz, 1H), 7.67 (d, *J* = 5.5 Hz, 2H), 7.15 (ddd, *J* =2.5 Hz, *J* =8.8 Hz, 1H), 7.05 (dd, *J* =2.5 Hz, *J* = 8.8 Hz, 2H), 6.99 (dd, *J* =4.5 Hz, *J* = 8.6 Hz, 1H), 5.01 (s, 2H); ^{13}C (300 MHz, DMSO-d_6) δ: 166.8, 164.2-160.7 (dd, *J* = 13.1 Hz), 159.2-156.1 (d, *J* = 236 Hz), 150.3, 141.6, 140.8, 139.4-139.3 (d, *J* = 1.3 Hz), 135.9, 128.5-128.4 (d, *J* = 3.0 Hz), 122.9, 121.0-120.8 (d, *J* = 8.8 Hz), 117.2-116.9 (d, *J* = 23.5 Hz), 110.6-110.2 (dd, *J* = 7.8 Hz), 110.0-109.9 (d, *J* = 8.9 Hz), 108.4-108.1 (d, *J* = 24.6 Hz), 103.4-102.6 (dt, *J* = 4.7, *J* = 25.8 Hz), 42.1 (d, *J* = 7.0 Hz) ppm; MS (m/z) 366 (M^+) 28 (100%). Anal. Calcd for $C_{21}H_{13}F_3N_2O$: C, 68.85; H, 3.58; N, 7.65; Found: C, 68.78; H, 3.74; N, 7.37.

1-(3,5-difluorobenzyl)-5-fluoro-3-(4-methoxy-benzylidene)indolin-2-one (205)

To a solution of 1-(3,5 difluorobenzyl)-5-fluoro-indolin-2-one (0.3303 g, 1.192 mmol) with EtOH (45 mL) was added *p*-anisaldehyde (1.2 equiv, 1.430 mmol, 0.174 mL) and piperidine (0.147 equiv, 0.1719 mmol, 0.017 mL). The mixture was heated under reflux and stirred for 26 h. The solution was cooled to room temperature and evaporated under reduced pressure to afford a yellow oil which was purified with silica gel (hexanes/EtOAc, 70:30) to produce a yellow oil/solid (0.4662 g, 98.9 %). The yellow oil was recrystallized with EtOH to afford the pure (TLC/GCMS) yellow solid product (0.3664 g, 77.8 %):); ^{1}H NMR (300 MHz DMSO-d_6) δ: 8.54 (d, *J* = 8.9 Hz, 1H), 7.84 (s, 1H), 7.78 (d, 8.5 Hz, 2H), 7.73 (d, *J* = 9.1 Hz, 1H), 7.46 (dd, *J* = 2.5 Hz, *J* = 9.3 Hz, 1H), 7.19 (d, *J* = 4.6 Hz, 1H), 7.14 (ddd, *J* =2.5 Hz, *J* =8.8 Hz, 1H), 7.06 (dd, *J* =2.5 Hz, *J* =8.7 Hz, 2H), 6.98 (dd, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H), 5.02 (s, 2H), 3.86 (s, 3H) ;^{13}C NMR (DMSO-d_6) δ: 167.6, 164.1, 161.0, 157.7, 141.2, 139.2, 138.6, 133.3, 126.3, 123.7, 121.8, 115.7, 114.1, 110.4, 109.9, 109.1, 102.8, 55.4, 42.1 ppm; mp 125-126˚C; R_f = 0.76 (EtOAc/hexanes, 1:1); MS (*m/z*) 395 (M^+), 268 (100%). $C_{23}H_{16}F_3NO_2$; C, 69.87; H, 4.08; N, 3.54; Found C, 69.52; H, 3.91; N, 3.47.

1-(3,5-difluorobenzyl)-5-fluoro-3-(2,6-difluorobenzylidene)-indolin-2-one (206)

To a vial was added 1-(3,5-difluorobenzyl)-5-fluoro-indolin-2-one (0.1931 g, 0.697

mmol) with 2,6 difluorobenzaldehyde (1.2 equiv, 0.834 mmol, 0.090 mL), piperidine (0.147 equiv, 0.081 mmol, 0.008 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. An orange precipitate (0.0802 g, 28.7 %) was collected by vacuum filtration and washed with cold EtOH: mp 162-164˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.86 (dd, *J* =2.0 Hz, *J* =9.0 Hz, 1H), 7.71–7.63 (m, 2H), 7.61 (s, 1H), 7.36 (t, *J* = 8.2 Hz, 3H), 7.19 (ddd, *J* =2.5 Hz, *J* =8.7 Hz, 1H), 6.92 (dd, *J* =4.4 Hz, *J* =8.7 Hz, 1H) 6.72 (dd, *J* = 2.1 Hz, *J* = 8.7 Hz, 1H), 5.01 (s, 2H); ^{13}C NMR δ: 168.3, 164.2-160.7 (dd, *J* = 13.1 Hz), 161.2-157.8 (dd, *J* = 6.9 Hz), 159.5-156.4 (d, *J* = 237 Hz), 140.9-140.7 (t, *J* = 9.0 Hz), 139.3 (d, *J* = 1.4 Hz), 132.8-132.5 (t, *J* = 10.5 Hz), 130.2 (d, *J* = 2.2 Hz), 129.9, 122.9, 121.3-121.2 (d, *J* = 9.2 Hz), 117.2-116.9 (d, *J* = 23.7 Hz), 112.4-112.1 (dd, *J* = 6.8 Hz), 110.6-110.3 (dd, *J* = 8.2 Hz), 110.1-110.0 (d, *J* = 7.9 Hz), 109.1-108.7 (d, *J* = 25.8 Hz), 103.4-102.7 (t, *J* = 25.6 Hz), 42.2-41.8 (d, *J* = 1.8 Hz) ppm: R_f = 0.79 (EtOAc/hexanes, 1:1); MS (*m/z*): 401 (M^+), 127 (100%); Calcd for $C_{22}H_{12}F_5NO$: C, 65.84; H, 3.01; N, 3.49; Found: C, 65.57, H, 3.27, N, 3.48.

5-Chloro-1-(3,5-difluorobenzyl)-3-(pyridin-2-ylmethylene)indolin-2-one (207)

To a vial was added 1-(3,5-difluorobenzyl)-5-chloro-indolin-2-one (0.1842 g, 0.629 mmol) with 2-pyridincarboxaldehyde (1.2 equiv, 0.757 mmol, , 0.072 mL), piperidine (0.147 equiv, 0.1011 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. A green precipitate (0.1661 g, 69.2%) was collected by vacuum filtration and washed with cold EtOH: mp 157-159˚C; R_f = 0.55 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.27 (d, *J* = 2.2 Hz, 1H), 8.95 (d, *J* = 4.7 Hz, 1H), 7.88 (d, *J* = 15.4 Hz, 1H), 7.85 (s, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.41 (dd, *J* = 1.0 Hz, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.28 (s, 1H), 7.26 (d, *J* =8.3Hz, 1H), 6.84 (d, *J* =7.7 Hz, 2H), 6.76 (tt, *J* =8.8 Hz, 1H), 6.61 (d, *J* = 8.3 Hz, 1H), 4.98 (s, 2H); ^{13}C NMR ($CDCl_3$) δ: 168.8, 165.0-161.6 (dd, *J* = 12.7 Hz), 158.4, 153.2, 149.7, 141.7, 141.1, 136.8 (d, *J* = 3.7 Hz), 130.0, 128.7, 128.5, 128.0, 127.7, 124.2, 123.1, 110.1-109.8 (dd, *J* = 8.2 Hz), 109.1, 103.3-102.9 (t, *J* = 25.3 Hz), 43.2 ppm; MS (*m/z*): 382 (M^+), 28 (100%); Anal. Calcd for $C_{21}H_{13}ClF_2N_2O$: C, 65.89; H, 3.42; N, 7.32; Found: C, 65.66; H, 3.24; N, 7.20.

5-chloro-1-(3,5-difluorobenzyl)-3-(pyridin-4-ylmethylene)indolin-2-one (208)
To a vial was added 1-(3,5-difluorobenzyl)-5-chloro-indolin-2-one (0.2077 g, 0.677 mmol) with 4-pyridincarboxaldehyde (1.2 equiv, 0.8492 mmol, 0.0910 g, 80 µL), piperidine (0.147 equiv, 0.1011 mmol, 0.0086 g, 10 µL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was then placed in the freezer overnight but crystallization did not occur. The solution was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford a yellow solid (0.1475 g, 54.5%). The yellow solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as a yellow-orange product (0.0275 g, 10.2%): mp 130-133 ºC; R_f = 0.24 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 8.75 (d, *J* = 5.7 Hz, 2H), 8.11 (d, *J* = 5.7 Hz, 2H), 7.92 (d, *J* = 1.7 Hz, 1H), 7.80 (s, 1H), 7.34 (m, 1H), 7.06 (d, 4H), 7.00 (t, 2H), 4.99 (s, 2H); ^{13}C NMR δ: 166.5, 164.2-160.7 (dd, *J* = 13.6 Hz), 150.2, 141.7, 140.8-140.5 (dd, *J* = 8.9Hz), 140.1, 136.1, 130.2, 128.2, 126.7, 124.9, 124.7, 122.2, 110.9, 110.5-110.2 (d, *J* = 26.0 Hz), 103.9-102.6 (td, *J* = 4.8 Hz, *J* = 25.7 Hz), 42.1-42.0 (d, *J* = 6.8 Hz) ppm; MS (*m/z*): 382 (M^+), 28 (100%); Anal. Calcd for $C_{21}H_{13}ClF_2N_2O$: C, 65.89; H, 3.42; N, 7.32; Found: C, 65.64; H, 3.53; N, 7.12.

1-(3,5-difluorobenzyl)-5-chloro-3-(2,6-difluoro-benzylidene)indolin-2-one (209)
To a vial was added 1-(3,5-difluorobenzyl)-5-chloro-indolin-2-one (0.1237 g, 0.422 mmol) with 2,6-difluorobenzaldehyde (1.2 equiv, 0.556 mmol, 0.060 mL), piperidine (0.147 equiv, 0.090 mmol, 0.008 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A gold-yellow precipitate (0.1204 g, 68.4%) was collected by vacuum filtration and washed with cold EtOH: mp 165-167˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.72 (m, 1H), 7.61 (s, 1H), 7.39 (m, 2H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 2H), 7.15 (m, 3H), 7.01 (d, *J* = 10.2 Hz, 1H), 6.90 (d, *J* = 1.4 Hz, 1H), 5.02 (s, 2H); ^{13}C NMR δ: 166.1, 164.2-160.7 (dd, *J* = 13.0 Hz), 161.2-157.8 (dd, *J* = 6.8 Hz), 141.8, 140.8-140.5 (t, *J* = 8.9 Hz), 140.2, 132.9-132.6 (t, *J* = 10.3 Hz), 130.2-129.7 (d, *J* = 38.7 Hz), 126.7-126.4 (d, *J* = 25.0 Hz), 123.1-122.6 (d, *J* = 31.7 Hz), 121.7, 121.3, 112.4-112.1 (d, *J* = 6.5 Hz), 111.7-111.1 (t, *J* = 19.5 Hz), 110.8, 110.6-110.3 (d, *J*

= 10.1 Hz), 103.4-102.7 (t, *J* = 25.6 Hz), 42.2 ppm: R_f = 0.80 (EtOAc/hexanes, 1:1); MS (*m/z*) 417 (M^+), 28 (100%); Anal. Calcd for $C_{22}H_{12}ClF_4NO$: C, 63.25; H, 2.90; N, 3.35; Found: C, 63.17; H, 3.01; N, 3.35.

1-(4-Methoxy-benzyl)-5-fluoro-3-(pyridin-2-ylmethylene)-indolin-2-one (213)

To a vial was added 1-(4-methoxy-benzyl)-5-fluoro-indolin-2-one (0.1488 g, 0.549 mmol) with 2-pyridinecarboxaldehyde (1.2 equiv, 0.652 mmol, 0.062 mL), piperidine (0.147 equiv, 0.090 mmol, 0.008 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A brown-green precipitate (0.1476 g, 74.7%) was collected by vacuum filtration and washed with cold EtOH: mp 139-140˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.04 (dd, *J* = 2.7 Hz, *J* = 10.1 Hz, 1H), 8.93 (d, *J* = 4.4 Hz, 1H), 8.02-7.94 (m, 2H), 7.79 (s, 1H), 7.53-7.48 (m, 1H), 7.30-7.27 (d, *J* = 8.7 Hz, 2H), 7.19 (ddd, *J* = 2.5 Hz, *J* = 8.6 Hz, 1H), 6.98 (dd, *J* = 4.5 Hz, *J* = 8.6 Hz, 1H), 6.90 (dd, *J* =2.5 Hz, *J* = 8.7 Hz, 2H), 4.92 (s, 2H), 3.70 (s, 3H); ^{13}C NMR δ: 167.9, 159.3-156.1 (d, *J* = 234 Hz), 158.5, 152.6, 149.7, 139.9, 137.3, 136.1, 129.2, 128.6, 128.1, 127.6, 124.6, 122.2-122.0 (d, *J* = 10.0 Hz), 116.8-116.5 (d, *J* = 23.9 Hz), 115.2-114.8 (d, *J* = 27.9 Hz), 114.0, 109.6-109.5 (d, *J* = 8.2 Hz), 55.0, 42.3 ppm: R_f = 0.41 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 361 (M+1); Anal. Calcd for $C_{22}H_{17}FN_2O_2$: C, 73.32; H, 4.80; N, 7.60; Found: C,73.22; H, 4.80; N, 7.60.

1-(4-Methoxy-benzyl)-5-fluoro-3-(pyridin-4-ylmethylene)-indolin-2-one (214)

To a vial was added 1-(4-methoxy-benzyl)-5-fluoro-indolin-2-one (0.1562 g, 0.576 mmol) with 4-pyridinecarboxaldehyde (1.2 equiv, 0.689 mmol, 0.065 mL), piperidine (0.147 equiv, 0.090 mmol, 0.009 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.1370 g, 66.1%) was collected by vacuum filtration and washed with cold EtOH: mp 135-139˚C; R_f = 0.41 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.76 (dd, *J* = 1.4 Hz, *J* = 4.5 Hz, 2H), 8.13 (d, *J* = 4.7 Hz, 1H), 7.80 (s, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.19 (ddd, *J* = 2.5 Hz, *J* = 8.6 Hz, 1H), 6.98 (dd, *J* = 4.5 Hz, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 1H),

4.90 (s, 2H), 3.71 (s, 3H); ^{13}C NMR δ: 166.6, 159.1-156.0 (d, *J* = 235 Hz), 158.6, 150.3, 141.7, 139.7 (d, *J* = 1.5 Hz), 135.7, 128.8, 128.6, 128.0, 124.7, 120.8-120.7, 117.1-116.8 (d, *J* = 23.9 Hz), 114.0 (d, *J* = 2.3 Hz), 110.6-110.5 (d, *J* = 8.2 Hz), 108.3-108.0 (d, *J* = 25.6 Hz), 55.0, 42.2 ppm; LC-MS (*m/z*): 361 (M+1);

1-(4-Methoxy-benzyl)-5-fluoro-3-(4-dimethylamino-benzylidene)indolin-2-one (215)
To a vial was added 1-(4-methoxy-benzyl)-5-fluoro-indolin-2-one (0.1301 g, 0.480 mmol) with 4-dimethylaminobenzaldehyde (1.2 equiv, 0.576 mmol, 0.0861 g), piperidine (0.1 equiv, 0.061 mmol, 0.006 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was placed in the freezer overnight but crystallization did not occur. The solution was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford an orange oil (0.1398 g, 72.4%). The orange oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as a orange product (0.0306 g, 15.8%):mp 140-143˚C; R_f =0.76 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ: 8.63 (d, *J* = 9.0 Hz, 2H), 7.75 (s, 1H), 7.48 (d, *J* = 8.9 Hz, 1H), 7.35 (d, *J* = 4.5 Hz, 3H), 7.19 (ddd, *J* = 2.5 Hz, *J* = 8.6 Hz, 1H), 6.98 (dd, *J* = 4.5 Hz, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 1H), 4.98 (s, 2H), 3.71 (s, 3H), 3.12 (s, 6H); ^{13}C NMR (300 MHz, DMSO-*d*$_6$) δ: 167.0, 160.0-156.8 (d, *J* =235 Hz), 153.4, 140.8, 137.3-137.2 (d, *J* =1.4 Hz), 136.4, 130.0, 129.7, 129.6 (d, *J* =2.1 Hz), 123.2, 119.8-119.7 (d, *J* =2.9 Hz), 118.1, 114.8 (d, *J* =3.4 Hz), 113.5-113.2 (d, *J* =24.0 Hz), 112.4-111.9 (d, *J* =36.1 Hz),109.8-109.7 (d, *J* =8.3 Hz), 106.4-106.0 (d, *J* =25.5 Hz), 55.4, 42.4, 40.0 ppm; LC-MS *(m/z)* 403 (M+1).

1-(4-Methoxy-benzyl)-5-chloro-3-(pyridin-2-ylmethylene)-indolin-2-one (216)
To a vial was added 1-(4-methoxy-benzyl)-5-chloro-indolin-2-one (0.1741 g, 0.607 mmol) with 2-pyridinecarboxaldehyde (1.2 equiv, 0.728 mmol, 0.070 mL), piperidine (0.147 equiv, 0.090 mmol, 0.008 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A brown/green precipitate (0.2281 g, 99.7%) was collected by vacuum filtration and washed with cold EtOH: mp 152-155˚C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ: 9.20 (d, *J* = 2.0 Hz, 1H), 8.90 (d, *J* = 4.4 Hz, 1H), 8.02 (d, *J* =

7.8 Hz, 1H), 7.78 (s, 1H), 7.55-7.48 (m, 1H), 7.37 (d, *J* = 8.3 Hz, 1H), 7.29 (d, *J* =8.6 Hz, 2H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.90 (dd, *J* = 1.9 Hz, *J* = 6.7 Hz, 1H), 4.92 (s, 2H); ^{13}C NMR δ: 167.7, 158.6, 152.5, 149.6, 142.3, 137.5, 136.3, 129.9, 129.3, 128.6, 128.0, 127.5, 127.0, 125.9, 124.7, 122.5, 114.0, 110.3, 55.0, 42.3 ppm: R_f = 0.60 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 377 (M^+); Anal. Calcd for $C_{22}H_{17}ClN_2O_2$: C, 70.12; H, 4.55; N, 7.43; Found: C, 70.06; H, 4.56; N, 7.46.

1-(4-methoxy-benzyl)-5-chloro-3-(2,6-difluoro-benzylidene)indolin-2-one (217)

To a vial was added 1-(4-methoxy-benzyl)-5-chloro-indolin-2-one (0.1858 g, 0.647 mmol) with 2,6-difluorobenzaldehyde (1.2 equiv, 0.768 mmol, 0.084 mL), piperidine (0.147 equiv, 0.101 mmol, 0.010 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.2006 g, 75.4%) was collected by vacuum filtration and washed with cold EtOH: mp 159-162˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.00 (d, *J* = 2.0 Hz, 1H), 7.69-7.64 (m, 1H), 7.61 (s, 1H), 7.38-7.32 (m, 3H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 4.91 (s, 2H), 3.71 (s, 3H); ^{13}C NMR δ: 165.9, 161.2-157.8 (dd, *J* = 6.9 Hz), 158.6 (d, *J* = 5.2 Hz), 142.0, 140.6, 132.9-132.6 (t, *J* = 10.3 Hz), 130.2-129.6 (d, *J* = 43.7 Hz), 128.7-128.6 (d, *J* = 7.4 Hz), 127.8, 126.4-126.1 (d, *J* = 21.5 Hz), 129.9, 122.8-122.5 (d, *J* = 24.7 Hz), F121.6, 121.2, 114.1-114.0 (d, *J* = 5.0 Hz), 112.4-112.1 (d, *J* = 6.7 Hz), 111.4-110.6 (d, *J* = 29.6 Hz) 55.0, 42.3 ppm: R_f =0.68 (EtOAc/hexanes, 1:1); MS (*m/z*) 411 (M^+), 121 (100%); Anal. Calcd for $C_{23}H_{16}ClF_2NO_2$: C, 67.08; H, 3.92; N, 3.40; Found: C, 66.89; H, 4.03; N, 3.15.

3-(Benzylidene)indolin-2-one (226)

To a vial was added indolin-2-one (0.6001 g, 4.51 mmol) with benzaldehyde (1.2 equiv, 5.39 mmol, 0.550 mL), piperidine (0.147 equiv, 0.657 mmol, 0.065 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A golden-yellow precipitate (0.8030 g, 80.6%) was collected by vacuum filtration and washed with cold EtOH: mp 169-172˚C (lit.[139] mp. 175-176); ^{1}H NMR (300 MHz, DMSO-

d$_6$) 10.64 (s, 1H), 7.73 (d, *J* = 6.8 Hz, 2H), 7.67 (s, 1H), 7.57-7.48 (m, 4H), 7.27 (t, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.88-6.83 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR δ: 168.5, 142.9, 135.7, 134.4, 130.1, 129.6, 129.2, 128.7, 127.6, 122.3, 121.0, 120.2, 110.1 ppm: R_f = 0.40 (EtOAc/hexanes, 1:1); MS (*m/z*): 221 (M^+), 28 (100%);

3-(4-chlorobenzylidene)indolin-2-one (227)

To a vial was added indolin-2-one (0.3192 g, 2.400 mmol) with 4-chlorobenzaldehyde (1.2 equiv, 2.876 mmol, 0.4044 g), piperidine (0.147 equiv, 0.354 mmol, 0.035 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. An orange precipitate (0.5084 g, 82.9%) was collected by vacuum filtration and washed with cold EtOH: mp 186-188˚C (lit.[140] mp. 188-190˚C); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ: 10.64 (s, 1H), 8.42 (d, *J* = 8.6 Hz, 1H), 7.75 (d, 8.4 Hz, 2H), 7.60 (d, *J* = 2.1 Hz, 2H), 7.57 (s, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.27 (t, *J* = 7.7 Hz, 1H), 6.90-6.83 (m, 2H), ^{13}C NMR δ: 168.4, 143.0, 134.2, 134.0, 133.3, 131.0, 130.3, 128.8, 128.1, 122.4, 121.1, 120.6, 110.1 ppm: R_f = 0.38 (EtOAc/hexanes, 1:1); MS (*m/z*): 255 (M^+), 28 (100%);

3-(2,3- Dichlorobenzylidene)indolin-2-one (228)

To a vial was added indolin-2-one (0.3304 g, 2.484 mmol) with 2,3-dichlorobenzaldehyde (1.2 equiv, 2.977 mmol, 0.5211 g), piperidine (0.147 equiv, 0.364 mmol, 0.036 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A golden-yellow precipitate (0.6071 g, 84.3%) was collected by vacuum filtration and washed with cold EtOH: mp 205-207˚C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ: 10.71 (s, 1H), 10.58 (s, 1H), 7.94 (dd, *J* = 0.9 Hz, *J* = 7.8 Hz, 1H), 7.80 (s, 1H), 7.78 (d, *J* = 8.0 Hz, 1.5H), 7.74 (d, *J* = 7.7 Hz, 1.5H), 7.67 (dd, *J* = 1.0 Hz, *J* = 8.0 Hz, 1H), 7.56 (s, 1H), 7.54 (t, *J* = 7.9 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.29-7.22 (m, 2H), 7.09 (d, *J* = 7.5 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 1.0 Hz, 1H), 6.81 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H) ; ^{13}C NMR δ: 167.1, 142.4, 135.1, 131.8,

131.2, 130.8, 130.3, 129.7, 128.7, 127.2, 123.2, 122.7, 121.0, 120.2, 109.9 ppm: R_f = 0.45 (EtOAc/hexanes, 1:1); MS (*m/z*): 289 (M^+), 254 (100%);

3-(2-chlorobenzylidene)indolin-2-one (229)

To a vial was added indolin-2-one (0.4797 g, 3.60 mmol) with 2-chlorobenzaldehyde (1.2 equiv, 4.31 mmol, 0.486 mL), piperidine (0.147 equiv, 0.526 mmol, 0.052 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A golden-yellow precipitate (0.8168 g, 88.7%) was collected by vacuum filtration and washed with cold EtOH: mp 174-176˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.69 (s, 1H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.60 (s, 1H), 7.53-7.49 (m, 2H), 7.27 (t, *J* = 7.7 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 7.7 Hz, 1H), 6.84-6.79 (t, *J* = 7.6 Hz, 1H) ; ^{13}C NMR δ: 168.0, 143.1, 132.9, 132.8, 131.4, 131.2, 130.5, 130.2, 129.7, 129.5, 127.3, 122.5, 121.2, 120.4, 110.2 ppm: R_f = 0.43 (EtOAc/hexanes, 1:1); MS (*m/z*): 255 (M^+), 28 (100%);

3-(2,6-Dichlorobenzylidene)indolin-2-one (230)

To a vial was added oxindole (0.5524 g, 4.153 mmol) with 2,6-dichlorobenzaldehyde (1.2 equiv, 4.15 mmol, 0.7269 g), piperidine (0.147 equiv, 0.6067 mmol, 0.060 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A brown precipitate was collected but was shown as impure through TLC. The filtrate was evaporated under reduced pressure to afford a yellow oil and recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product (0.3512 g, 29.3%) was collected by vacuum filtration and washed with cold hexanes; mp. 181-183˚C (lit[141]mp.188-189˚C); R_f = 0.42 (EtOAc/hexanes, 1:1); MS (*m/z*): 289 (M^+), 254 (100%);

3-(4-Methoxybenzylidene)indolin-2-one (231)

To a vial was added oxindole (0.8542 g, 6.422 mmol) with *p*-anisaldehyde (1.2 equiv, 7.79 mmol, 0.948 mL), piperidine (0.147 equiv, 0.940 mmol, 0.093 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 5 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A golden yellow precipitate (1.4619 g, 90.7%) was collected by vacuum filtration and washed with cold

EtOH: mp 154-157˚C (lit[142]mp.158-159˚C); R_f = 0.21 (EtOAc/hexanes,1:1); MS (*m/z*): 251 (M^+), 207 (100%).

3-(2,6-Difluorobenzylidene)indolin-2-one (232)

To a vial was added oxindole (0.8579 g, 6.450 mmol) with 2,6-difluorobenzaldehyde (1.2 equiv, 7.73 mmol, 0.835 mL), piperidine (0.147 equiv, 0.920 mmol, 0.091 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A golden yellow precipitate (1.3029 g, 78.6%) was collected by vacuum filtration and washed with cold EtOH: mp 217-220˚C (lit[143]mp.213-218˚C); R_f = 0.39 (EtOAc/hexanes, 1:1); MS (*m/z*): 257 (M^+), 28 (100%).

3-(3,5-difluorobenzylidene)indolin-2-one (233)

To a vial was added indolin-2-one (0.6357 g, 4.77 mmol) with 3,5 -difluorobenzaldehyde (1.2 equiv, 5.75 mmol, 0.630 mL), piperidine (0.147 equiv, 0.647 mmol, 0.064 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A bright yellow precipitate (0.6652 g, 54.2 %) was collected by vacuum filtration and washed with cold EtOH: mp 197-199˚C (lit[143] mp.202-205˚C); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.66 (s, 1H), 7.54 (s, 1H), 7.40-7.37 (d, *J* = 6.9 Hz, 3H), 7.33-7.29 (dd, *J* = 2.2 Hz, *J* = 9.2 Hz, 1H), 7.26-7.21 (M, 1H), 6.88-6.85 (d, *J* = 8.7 Hz, 2H) ^{13}C NMR δ: 168.1, 164.1-160.6 (dd, *J* = 13.5 Hz), 143.3, 138.2-137.9 (t, *J* = 10.0 Hz), 132.7-132.6 (t, *J* = 2.3 Hz), 130.7, 129.4, 122.6, 121.2, 120.2, 112.3-111.9 (dd, *J* = 8.4 Hz), 110.2, 105.0-104.4 (t, *J* = 25.8 Hz) ppm: R_f = 0.5 (EtOAc/hexanes, 1:1); MS (*m/z*): 257 (M^+,100%);

3-(3-hydroxy-4-methoxybenzylidene)-indolin-2-one (234)

To a vial was added indolin-2-one (0.3151 g, 2.369 mmol) with vanillin (1.2 equiv, 2.840 mmol, 0.4321 g), piperidine (0.147 equiv, 0.344 mmol, 0.034 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.5448 g, 86.0 %) was collected by vacuum filtration and washed with cold EtOH: mp 218-220˚C;

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.86 (s, 1H), 8.72-8.71 (d, *J* = 1.8 Hz, 1H), 7.76-7.73 (d, *J* = 8.4 Hz, 1H), 7.70 (s, 1H), 7.66-7.63 (d, *J* = 7.4 Hz, 1H), 7.19-7.13 (dd, *J* = 7.6 Hz, *J* = 15.2 Hz, 1H), 6.99-6.93 (t, *J* = 7.5 Hz, 1H), 6.89-6.82 (m, 2H), 3.86 (s, 3H); ^{13}C NMR δ:167.5, 149.7, 146.9, 139.9, 137.8, 127.9, 127.8, 126.1, 125.6, 122.9, 120.7, 118.8, 115.7, 115.1, 109.1, 55.4 ppm: R_f = 0.19 (EtOAc/hexanes, 1:1); LC-MS (*m/z*): 268 (M+1).

5-Chloro-3-(benzylidene)-indolin-2-one (236)

To a vial was added 5-chloro-indolin-2-one (0.1886 g, 1.039 mmol) with benzaldehyde (1.2 equiv, 1.352 mmol, 0.230 mL), piperidine (0.147 equiv, 0.162 mmol, 0.016 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.2580 g, 97.4%) was collected by vacuum filtration and washed with cold EtOH: mp 204-205˚C (lit.[126]mp 210^{o}C); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.77 (s, 1H), 8.43 (d, *J* = 7.5 Hz, 1H), 7.95 (s, 1H), 7.86 (d, *J* = 2.0 Hz, 1H), 7.74 (s, 1H), 7.71 (d, 7.5 Hz, 2H), 7.59 – 7.48 (m, 4H), 7.41 (d, *J* = 2.0 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.24 (d, *J* = 2.0 Hz, 1H), 6.91 (d, *J* = 16.9 Hz, 1H) ; ^{13}C NMR δ: 167.1, 140.5, 138.1, 133.8, 131.4, 129.7, 128.9, 128.2, 126.8, 125.5, 123.6, 120.7, 111.0 ppm: R_f = 0.35 (EtOAc/hexanes, 1:1); MS (*m/z*): 255 (M^{+}), 28 (100%);

5-Chloro-3-(2-chlorobenzylidene)-indolin-2-one (237)

To a vial was added 5-chloro-indolin-2-one (0.2849 g, 1.569 mmol) with 2-chlorobenzaldehyde (1.2 equiv, 2.042 mmol, 0.230 mL), piperidine (0.147 equiv, 0.253 mmol, 0.025 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.4470 g, 90.4%) was collected by vacuum filtration and washed with cold EtOH: mp 249-250˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.84 (s, 1H), 7.95 (s, 1H), 7.88 (d, *J* = 2.0 Hz, 1H), 7.79 (d, *J* = 6.4 Hz, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 7.67 (s, 1H), 7.59-7.50 (m, 2H), 7.32 (dd, *J* = 2.1 Hz, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 2.091 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H) ; ^{13}C NMR δ: 167.6, 141.9, 133.3, 132.8, 132.5, 131.6, 130.2, 130.1, 129.9, 128.7, 127.4, 125.0,

122.1, 122.0, 111.6 ppm: R_f = 0.49 (EtOAc/hexanes, 1:1); MS (*m/z*): 289 (M^+), 28 (100%);

5-chloro-3-(4-chlorobenzylidene)-indolin-2-one (238)

To a vial was added 5-chloro-indolin-2-one (0.2925 g, 1.612 mmol) with 4-chlorobenzaldehyde (1.2 equiv, 2.101 mmol, 0.2954 g), piperidine (0.147 equiv, 0.253 mmol, 0.025 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.3980 g, 78.3%) was collected by vacuum filtration and washed with cold EtOH: mp 247-248˚C (lit.[126] mp. 252-254˚C); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.77 (s, 1H), 8.43 (d, *J* = 8.6 Hz, 1H), 7.93 (s, 1H), 7.84 (d, *J* = 2.0 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.68 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* =8.6 Hz, 1H) 7.38 (d, *J* = 1.9 Hz, 1H), 7.31 (dd, *J* =2.0 Hz, *J* = 12.5 Hz, 1H), 7.28 (d, *J* = 12.5 Hz, 1H), 6.91 (d, *J* =18.5 Hz, 2H); ^{13}C NMR δ:167.4, 140.6, 136.6, 134.9, 132.7, 132.4, 129.3, 128.4, 126.9, 125.8, 123.6, 120.9, 111.2 ppm; R_f = 0.44(EtOAc/hexanes, 1:1); MS (*m/z*): 257 (M^+, 100%);

5-chloro-3-(2,3-dichlorobenzylidene)-indolin-2-one (239)

To a vial was added 5-chloro-indolin-2-one (0.3005 g, 1.654 mmol) with 2,3-dichlorobenzaldehyde (1.2 equiv, 2.159 mmol, 0.3779 g), piperidine (0.147 equiv, 0.263 mmol, 0.026 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.4968 g, 85.2%) was collected by vacuum filtration and washed with cold EtOH: mp 273-276˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.79–7.76 (dd, *J* = 0.9 Hz , *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 7.55-7.50 (t, *J* = 7.886 Hz,),7.30-7.26 (d, *J* = 8.3 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.90-6.80 (dd, *J* = 8.3 Hz, *J* = 20.4 Hz,1H) ^{13}C NMR δ:167.5, 142.0, 134.9, 133.0, 132.5, 131.6, 130.8, 130.6, 129.5, 129.1, 128.5, 125.6, 122.2, 121.8, 111.7 ppm; R_f =0.47 (EtOAc/hexanes, 1:1); MS (*m/z*): 323 (M^+), 28 (100%); Anal. Calcd for $C_{15}H_8Cl_3NO$: C, 55.50; H, 2.48; N, 4.32 Found: C, 55.70; H, 2.60; N, 4.32.

5-chloro-3-(4-methyl-benzylidene)-indolin-2-one (240)

To a vial was added 5-chloro-indolin-2-one (0.2876 g, 1.584 mmol) with tolualdehyde (1.2 equiv, 2.205 mmol, 0.260 mL), piperidine (0.147 equiv, 0.253 mmol, 0.025 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A green-yellow precipitate (0.2373 g, 51.2%) was collected by vacuum filtration and washed with cold EtOH: 218-220˚C (lit.[126] mp. 220-223˚C); ^{13}C NMR δ: 168.2, 141.6, 138.8, 138.1, 133.9, 132.6, 130.6, 129.3, 126.8, 125.9, 124.8, 121.7, 111.4, 20.7 ppm: R_f = 0.41 (EtOAc/hexanes, 1:1); MS (*m/z*): 269 (M^+), 28 (100%);

5-Chloro-3-(4-methoxybenzylidene)indolin-2-one (241)

To a solution of 5-chloroindolin-2-one (0.8840 g, 5.29 mmol) in EtOH (3 mL), was added *p*-anisaldehyde (1.2 equiv, 779 µL, 6.40 mmol) and piperidine (0.1 equiv, 53 µL, 0.536 mmol). The solution was placed on a hot plate at 90˚C for 3 h while stirring after which time was cooled to rt and placed in the freezer overnight. A yellow precipitate (1.3347 g, 88%) was collected by vacuum filtration: mp 218-220˚C (lit.[126] mp. 219-220˚C); R_f = 0.24 (EtOAc/hexanes, 1:1); MS (*m/z*) 285 (M^+, 100 %).

5-Chloro-3-(2,6-dichlorobenzylidene)indolin-2-one (242)

To a vial was added 5-chloro-indolin-2-one (0.2204 g, 1.32 mmol) with 2,6-dichlorobenzaldehyde (1.2 equiv, 1.58 mmol, 0.2772 g), piperidine (0.147 equiv, 0.1921 mmol, 0.019 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A bright yellow precipitate (0.2734 g, 64.3 %) was collected by vacuum filtration and washed with cold EtOH: mp 215-218˚C (lit.[148] mp. 197-199˚C);; R_f = 0.42 (EtOAc/hexanes, 1:1); MS (*m/z*) 291(M^+, 100%);

5-Chloro-3-(2,6-difluorobenzylidene)indolin-2-one (243)

To a vial was added 5-chloro-indolin-2-one (0.2374 g, 1.42 mmol) with 2,6-difluorobenzaldehyde (1.2 equiv, 1.71 mmol, 0.1841 mL), piperidine (0.147 equiv, 0.212 mmol, 0.021 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 3 h

while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A bright yellow precipitate (0.3290 g, 79.5 %) was collected by vacuum filtration and washed with cold EtOH: mp 245-246˚C(lit.[126] mp. 242-244˚C); R_f = 0.39 (EtOAc/hexanes, 1:1); MS (*m/z*) 291 (M^+,100%);

5-Chloro-3-(3,5-difluorobenzylidene)indolin-2-one (244)

To a vial was added 5-chloro-indolin-2-one (0.4754 g, 2.84 mmol) with 3,5 -difluorobenzaldehyde (1.2 equiv, 3.41 mmol, 0.375 mL), piperidine (0.147 equiv, 0.414 mmol, 0.041 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A bright yellow precipitate (0.4883 g, 58.9 %) was collected by vacuum filtration and washed with cold EtOH: mp 158-162 ˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ; 10.85 (s, 1H), 8.67 (s, 1H), 8.16 (d, *J* = 9.4 Hz, 1H), 7.89 (s, 1H), 7.79 (d, *J* = 2.0 Hz, 1H), 7.64 (s, 1H), 7.45 (d, *J* = 6.7 Hz, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.36 (t, *J* = 2.3 Hz, 1H), 7.32 -7.26 (m, 2H), 6.91 (d, *J* = 7.7 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR δ: 167.2, 162.1, 140.9, 136.7, 137.6, 135.0, 129.6, 128.4, 125.5, 121.1, 113.3, 111.3, 105.6 ppm: R_f = 0.41 (EtOAc/hexanes, 1:1); MS (*m/z*): 2 peaks both: 291 (M^+), 28 (100%); $C_{15}H_8ClF_2NO$; C, 61.77; H, 2.76; N, 4.80; Found C, 61.66; H, 2.81; N, 4.86.

5-Chloro-3-(3-(hydroxy)-4-(methoxy)-benzylidene)indolin-2-one (245)

To a vial was added 5-chloro-indolin-2-one (0.2224 g, 1.33 mmol) with vanillin (1.2 equiv, 1.29 mmol, 0.1975 g), piperidine (0.147 equiv, 0.192 mmol, 0.019 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. An orange precipitate (0.3342 g, 83.4%) was collected by vacuum filtration and washed with cold EtOH: mp 227-231˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.56 (s, 1H), 8.69 (d, *J* = 1.9 Hz, 1H), 7.79 (s, 1H), 7.78 (d, *J* = 1.9 Hz, 1H), 7.75 (d, *J* = 4.5 Hz, 2H), 7.62 (s, 1H), 7.33 (d, 1.9 Hz, 1H), 7.26 (dd, *J* = 2.1Hz, *J* = 8.3 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.17 (dd, *J* = 2.0 Hz, 8.2 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 2H), 6.83 (d, *J* = 8.3 Hz, 2H), 3.84 (d, *J* = 5.3 Hz, 5H), 3.48 (q, *J* = 7.0 Hz, 2H) ; ^{13}C NMR δ: 168.0, 152.0, 147.6, 139.4, 128.7,

127.9, 125.6, 125.0, 124.2, 122.9, 121.3, 120.1, 118.5, 115.8, 111.5, 55.9 ppm: R_f = 0.29 (EtOAc/hexanes, 1:1); MS (*m/z*): 301 (M^+), 28 (100%);

5-Chloro-3-(4-(hydroxy)-benzylidene)indolin-2-one (246)

To a vial was added 5-chloro-indolin-2-one (0.2004 g, 1.20 mmol) with 4-hydroxybenzaldehyde (1.2 equiv, 1.44 mmol, 0.1758 g), piperidine (0.147 equiv, 0.172 mmol, 0.017 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 4 h while stirring. The vial was cooled to rt and placed in the freezer overnight to induce crystallization. A yellow precipitate (0.2121 g, 65.2%) was collected by vacuum filtration and washed with cold EtOH: mp 288-290˚C; R_f = 0.64 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.67 (s, 1H), 10.31 (s, 1H), 8.45 (d, *J* = 8.7 Hz, 2H), 7.84 (s, 1H), 7.80 (d, *J* = 1.9 Hz, 1H), 7.64 (d, *J* = 10.7 Hz, 1H), 7.20 (dd, *J* = 2.0 Hz, *J* = 8.2 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 2H), 8.82 (d, *J* = 8.2 Hz, 1H) ; ^{13}C NMR δ: 167.1, 160.5, 139.3, 136.6, 135.2 127.5, 127.1, 125.4, 125.1, 121.1, 119.0, 115.2, 110.3 ppm: LC-MS (*m/z*): 272 (M^+).

5-chloro-3-(4-dimethylamino-benzylidene)indolin-2-one (247)

To a vial was added 5-chloro-indolin-2-one (0.2461 g, 1.473 mmol) with 4-dimethylaminobenzaldehyde (1.2 equiv, 1.768 mmol, 0.2641 g), piperidine (0.1 equiv, 0.212 mmol, 0.021 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90˚C for 6 h while stirring. The vial was placed in the freezer overnight to afford an orange solid. The orange precipitate (0.2846 g, 64.8%) was collected by vacuum filtration and washed with cold EtOH: mp 250-253˚C (lit.[151] mp 257-260^{o}C); R_f = 0.28 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.59 (s, 1H), 7.70 (d, *J* = 1.8 Hz, 1H), 7.63 (s, 1H), 7.60-7.59 (d, *J* = 3.3 Hz, 1H), 7.22-7.19 (dd, *J* = 1.9 Hz, *J* = 8.2 Hz, 1H), 6.87-6.79 (m, 3H), 3.02 (s, 2H) ; ^{13}C NMR (300 MHz, DMSO-d_6) δ: 169.0, 151.6, 140.6, 139.1, 132.1, 127.9, 124.6, 123.5, 120.9, 120.8, 120.5, 111.4, 110.9, 39.5 ppm; MS *(m/z)*: 299 (M+1).

1-(2,6-Difluorobenzyl)-3-(4-methoxybenzylidene)indolin-2-one (248)

Route A

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.2332 g, 0.929 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (0.8544 g, 5.59 mmol) and the

resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. To the mixture 1.5 equiv. of 2,6-difluorobenzyl bromide (0.2885 g, 1.39 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.1565 g, 44.7 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0638 g, 18.2%): mp 139-141 ºC; R_f = 0.68 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.51 (d, *J* = 8.8 Hz, 1H), 7.84 (s, 1H) 7.74 (s, 1H), 7.72 (d, *J* = 6.8 Hz, 1H), 7.47-7.37 (m, 1H), 7.29 (dd, *J* = 0.966 Hz, *J* = 15.493 Hz, 1H), 7.27 (d, *J* = 0.8 Hz, 1H), 7.14 (t, *J* = 8.9 Hz, 4H), 6.95-6.90 (m, 2H), 5.05 (s, 2H), 3.85 (s, 3H); ^{13}C NMR δ 167.0, 162.6-159.2 (dd, *J* = 7.9 Hz), 160.6, 142.4, 137.1, 131.6, 130.5-130.2 (t, *J* = 10.5 Hz), 129.5, 126.3, 124.0, 121.9, 121.6, 120.6, 114.2, 111.9-111.6 (dd, *J* = 7.2 Hz), 111.4, 108.4, 55.3, 31.8 ppm; MS (*m/z)*: 377 (M^+, 100 %).

Route B

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.2881g, 1.15 mmol) in EtOH (45 mL) was added DBU (2 equiv, 340 µL, 2.27 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a golden-yellow color. To the mixture 2,6 difluorobenzyl bromide (3 equiv, 0.6912 g, 3.33 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 27 h. The mixture was then allowed to cool to rt and evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.1127 g, 68.3 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1286 g, 41.1%): mp 135-138ºC; R_f = 0.69 (EtOAc/hexanes, 1:1); MS (*m/z*): 377 (M^+, 100 %).

Route C

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.2091 g, 0.833 mmol) in EtOH (3 mL) was added 2.0 equiv. of DBU (0.243 mL, 1.62 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution changed to an orange color. To

the mixture 3.0 equiv. of 2,6-difluorobenzyl bromide (0.5041 g, 2.44 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave at 180ºC for 20 min. The mixture was then allowed to cool to rt and placed in the freezer overnight. A solid precipitate did not form so the mixture was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.2145 g, 50.6 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.3388 g, 40.3%): mp 139-141ºC; R_f = 0.71 (EtOAc/hexanes, 1:1); MS (*m/z*): 377 (M^+, 100 %).

1-(2,6-Dichlorobenzyl)-3-(4-methoxybenzylidene)indolin-2-one (249)

Route A

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.2075 g, 0.827 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (0.7566 g, 4. 94 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. To the mixture 2,6 dichlorobenzyl bromide (1.5 equiv, 0.2988 g, 1.25 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 hr.. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.2137 g, 63.2 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0942 g, 27.9%): mp:135-138ºC; R_f = 0.64 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, *J* = 8.9 Hz, 1H), 7.84 (s, 1H), 7.74 (s, 1H), 7.72 (d, *J* = 7.5 Hz, 3H), 7.52 (d, *J* = 7.5 Hz, 1H) 7.41-7.35 (m, 1H), 7.23 (dd, *J* = 1.0 Hz, 15.5 Hz, 1H), 7.20 (d, *J* = 0.954 Hz, 1H), 7.11 (d, *J* =8.7 Hz, 2H), 6.93 (d, *J* = 0.8 Hz, 1H), 6.90 (dd, *J* =0.9 Hz, *J* = 15.2 Hz, 1H), 6.77 (dd, *J* = 7.7 Hz, *J* = 12.7 Hz, 1H) 5.20 (s, 1H), 3.85 (s, 1H) ; ^{13}C NMR δ: 167.2, 160.6, 142.6, 137.1, 135.3, 131.6, 130.6, 130.4, 129.4, 129.0, 126.3, 123.9, 121.8, 121.5, 120.7, 114.2, 108.8, 55.3, 39.8 ppm; MS (*m/z*) 410 (M^+, 100 %). Anal. Calcd for $C_{23}H_{17}Cl_2NO_2$: C, 67.33; H ,4.18 ; N, 3.41 Found: C, 66.97; H, 4.21; N, 3.46.

Route B

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.2881g, 1.15 mmol) in EtOH (45 mL) was added DBU (2 equiv, 340 µL, 2.27 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a golden-yellow color. To the mixture 2,6 difluorobenzyl bromide (3 equiv, 0.6912 g, 3.33 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 27 h. The mixture was then allowed to cool to rt and evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.1127 g, 68.3 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1286 g, 41.1%): mp 140-142ºC; R_f = 0.66 (EtOAc/hexanes, 1:1); MS (*m/z*): 410 (M^+, 100 %).

Route C

1-(2,6-Dichlorobenzyl)-3-(4-methoxybenzylidene)indolin-2-one (KJK-4-135)

To a solution of 3-(4-methoxybenzylidene)indolin-2-one (0.4694 g, 0.929 mmol) in EtOH (3 mL) was added 1.7 equiv. of DBU (0.475 mL, 3.17 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution changed to an orange color. To the mixture 2.2 equiv. of 2,6-dichlorobenzyl bromide (0.9758 g, 4.08 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave at 130ºC for 40 min. The mixture was then allowed to cool to rt and placed in the freezer overnight. A pure solid precipitate did not form so the mixture was evaporated under reduced pressure to afford a yellow oil and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.3322 g, 43.3 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1914 g, 24.9%): mp 147-149 ºC; R_f = 0.69 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, *J* = 8.9 Hz, 1H), 7.83 (s, 1H), 7.74-7.68 (m, 4H), 7.52 (dd, *J* = 1.0 Hz, *J* = 2.5 Hz, 2H), 7.49 (d, *J* = 2.5 Hz, 1H), 7.40 (dd, *J* = 1.7 Hz, *J* = 2.8 Hz, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.9 Hz, 1H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.92 (t, *J* = 7.6 Hz, 1H), 6.76 (dd, *J* = 7.7 Hz, *J* = 12.7 Hz, 1H), 5.75 (s, 1H), 5.21 (d, *J* = 2.9 Hz, 2H), 3.84 (s, 3H) ^{13}C NMR δ: 166.2, 160.9, 141.4, 137.5, 135.3, 133.0, 130.7, 130.3, 129.2, 128.0, 126.5, 124.2, 121.6, 121.4, 120.2, 113.7, 108.5, 55.3, 38.9 ppm; MS *m/z* 410 (M^+, 100 %).

1-(2,6-Dichlorobenzyl)-3-(2,6-difluorobenzylidene)indolin-2-one (250)
Route A

To a solution of 3-(2,6-difluorobenzylidene)indolin-2-one (0.2853 g, 1.11 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (1.0869 g, 7.11mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. To the mixture 2,6 dichlorobenzyl bromide (1.5 equiv, 0.8162 g, 3.34 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 hr.. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.2736 g, 59.2 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.2644 g, 57.3%): mp:137-139ºC; R_f = 0.67 (EtOAc/hexanes, 1:1); MS (*m/z*) 415 (M^+, 100 %).

Route B

To a solution of 3-(2,6-difluorobenzyl)-indolin-2-one (0.4487 g, 1.75 mmol) in EtOH (45 mL) was added DBU (1.1 equiv, 290 µL, 1.94 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a golden-yellow color. To the mixture 2,6 dichlorobenzyl bromide (1.5 equiv, 0.6283 g, 2.62 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 28 h. The mixture was then allowed to cool to rt and evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.4056 g, 61.8 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0942 g, 14.4%): mp 135-138ºC; R_f = 0.69 (EtOAc/hexanes, 1:1); MS (*m/z*) 415 (M^+, 100 %).

Route C

To a solution of 3-(2,6-difluorobenzylidene)indolin-2-one (0.5202 g, 2.025 mmol) in EtOH (3 mL) was added 2.0 equiv. of DBU (0.605 mL, 4.05 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution changed to an orange color. To the mixture 3.0 equiv. of 2,6-dichlorobenzyl bromide (1.4569 g, 6.07 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave at

125ºC for 30 min. The mixture was then allowed to cool to rt and placed in the freezer overnight. A solid precipitate did not form so the mixture was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.4255 g, 50.6 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.3388 g, 40.3%): mp 139-141ºC; R_f = 0.48 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 – 7.58 (m, 1H), 7.42 (dd, *J* = 7.2 Hz, J = 8.9 Hz, 2H), 7.33 (t, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 6.93 (t, *J* = 7.3 Hz, 1H) 5.21 (s, 2H) ; ^{13}C NMR δ: 165.8, 161.2-157.8 (dd, *J* = 6.8 Hz), 143.2, 135.3, 132.2-132.0 (t, *J* = 10.6 Hz), 130.7, 130.6, 130.5, 130.3, 129.0, 122.9, 122.0, 120.8 (t, *J* = 1.8 Hz), 120.2, 112.3-112.0 (dd, *J* = 6.9 Hz), 111.8, 109.1, 39.9 ppm; MS (*m/z*) 415 (M^+, 100 %). Anal. Calcd for $C_{22}H_{13}F_2Cl_2NO_2$: C, 63.48; H, 3.15; N, 3.36 Found: C, 63.45; H, 3.20; N, 3.37

5-Chloro-1-(2,6-difluorobenzyl)-3-(4-methoxybenzylidene)indolin-2-one (251)

Route A

To a solution of 5-chloro-3-(4-methoxy-benzylidene)indolin-2-one (0.4038 g, 1.42 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (1.294 g, 8.47 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. To the mixture 1.5 equiv. of 2,6 difluorobenzyl bromide (0.4389 g, 1.25 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h.. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.4468 g, 76.7 %). The yellow solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.3479 g, 59.7%): mp 167-170 ºC; R_f = 0.69 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ: 8.52 (d, *J* = 8.9 Hz, 1H), 7.97 (s, 1H), 7.89 (d, *J* = 2.0,1H), 7.78 (s, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.45-7.38 (m, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.30 (dd, *J* = 2.1 *J* = 8.3 Hz, 1H), 7.14-7.06 (m, 4H), 6.98 (d, *J* = 8.3 Hz, 1H), 5.75 (s, 1H), 5.05 (s, 2H), 3.86 (d, *J* = 1.3 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.7, 161.8, 161.0, 141.2, 139.6, 134.9, 130.5-130.2 (t, *J* = 10.6 Hz), 129.0, 126.6, 126.0, 125.6, 123.1, 121.3, 121.0,

114.4, 111.9-111.6 (dd, J = 4.0 Hz), 109.9, 55.4, 32.0 ppm: MS (*m/z)* 411 (M^+), 28 (100 %). Anal. Calcd for $C_{23}H_{16}ClF_2NO_2$: C, 67.08; H, 3.92; N, 3.40 Found: C, 66.85; H, 3.92; N, 3.41

Route B

To a solution of 5-chloro-3-(4-methoxy-benzylidene)indolin-2-one (0.2413 g, 0.847 mmol) in EtOH (45 mL) was added DBU (2.0 equiv, 260 µL, 1.74 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a golden-yellow color. To the mixture 2, 6 dichlorobenzyl bromide (3.0 equiv, 0.5312 g, 2.57 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h. The mixture was then allowed to cool to rt and evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.1675 g, 48.1 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0801g, 23.8%): mp 162-165 ºC; R_f = 0.65 (EtOAc/hexanes, 1:1); MS (*m/z*): 411 (M^+), 28 (100 %).

Route C

To a solution of 5-chloro-3-(4-methoxy-benzylidene)indolin-2-one (0.1899 g, 0.666 mmol) in EtOH (3 mL) was added DBU (2.0 equiv, 260 µL, 1.74 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a golden-yellow color. To the mixture 2, 6 dichlorobenzyl bromide (3.0 equiv, 0.4731 g, 1.97 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave at 180ºC for 20 min. The mixture was then allowed to cool to rt and placed in the freezer overnight. A solid precipitate did not form so the mixture was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.2849 g, 72.6 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0743g, 18.9%): mp 160-165 ºC; R_f = 0.62 (EtOAc/hexanes, 1:1); MS (*m/z*): 411 (M^+), 28 (100 %).

5-Chloro-1-(2,6-dichlorobenzyl)-3-(4-methoxybenzylidene)indolin-2-one (252)

Route B

To a solution of 5-chloro-3-(4-methoxy-benzylidene)indolin-2-one (0.2564 g, 0.900 mmol) in EtOH (45 mL) was added 2.2 equiv of DBU (295 µL, 1.97 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 2.2 equiv. of 2,6-dichlorobenzyl bromide (0.5792 g, 2.41 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h. The mixture was then allowed to cool to rt and evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.2016 g, 50.6 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1083 g, 27.2%): mp (214-216 ºC); R_f = 0.71 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 8.50 (d, J = 8.9 Hz, 1H), 7.97 (s, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.75 (s, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.62 (d, J = 2.1 Hz, 1H), 7.53 (dd, J = 1.0 Hz, J = 2.6 Hz, 1H), 7.50 (d, J = 2.6 Hz, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.39 (dd, J = 3.1 Hz, J = 8.9 Hz, 1H), 7.31 (dd, J = 2.1 Hz, J = 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 15.8 Hz, 2H), 6.80 (d, J = 15.8 Hz, 1H), 5.21 (d, J = 3.8 Hz, 2H), 3.86 (d, J = 2.4 Hz, 3H) ^{13}C (300 MHz, DMSO-d_6): δ 165.9, 161.4, 141.3, 139.2, 138.9, 135.3, 134.8, 130.4, 128.9, 127.3, 126.5, 125.7, 122.6, 120.9, 120.2, 114.1, 109.9, 55.4, 38.6 ppm; LC-MS (*m/z)* 444 (M^+). Anal. Calcd for $C_{23}H_{16}Cl_3NO_2$: C, 62.11; H, 3.63 ; N, 3.15 Found: C, 62.21; H, 3.86; N, 3.06. Exact Mass: 443.02

Route C

To a solution of 5-chloro-3-(4-methoxybenzylidene)indolin-2-one (0.1899 g, 0.666 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (200 µL, 1.34 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an orange color. To the mixture 3.0 equiv. of 2,6 dichlorobenzyl bromide (0.4731 g, 1.97 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave at 180ºC for 20 min and placed in the freezer overnight. A solid precipitate did not form so the mixture was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.2849 g, 73 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0743 g, 18.9 %): mp 215-217 ºC; R_f = 0.43 (EtOAc/hexanes, 1:1); MS (*m/z):* 417 (M^+, 100 %).

5-Chloro-1-(2,6-difluorobenzyl)-3-(2,6-difluorobenzylidene)indolin-2-one (254)
Route A

To a solution of 5-chloro-3-(2,6-diflouro-benzylidene)indolin-2-one (0.2883 g, 0.991 mmol) in EtOH (45 mL) was added 2.2 equiv of DBU (320 µL, 2.14 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an orange color. To the mixture 2.2 equiv. of 2,6 difluorobenzyl bromide (0.4497 g, 2.17 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 hr.. The mixture was then allowed to cool to rt and evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.1693 g, 40.9 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0738 g, 17.9 %): mp 163-166 ºC; R_f = 0.47 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ 7.73 –7.65 (m, 2H), 7.57 (s, 1H), 7.50-7.33 (m, 5H), 7.26 (d, *J* = 7.9 Hz, 1H), 7.16 (t, *J* = 7.9 Hz, 2H), 7.02 (d, J = 8.4 Hz, 1H) 6.89 (s, 1H), 5.08 (s, 2H); ^{13}C NMR δ: 165.4, 162.6-159.2 (dd, *J* = 7.8 Hz), 161.2-157.8 (dd, *J* = 6.7 Hz), 141.9, 132.9-132.6 (t, *J* = 10.8 Hz), 130.7-130.4 (t, *J* = 10.8 Hz), 130.3, 129.6, 126.1, 122.8, 122.5, 121.6, 112.4-112.1 (d, *J* = 24.1 Hz), 112.0-111.6 (dd, *J* = 7.4 Hz), 111.4, 111.1, 110.2, 32.2-32.1 (t, *J* = 2.6 Hz) ppm; MS (*m/z):* 417 (M^+, 100 %). Anal. Calcd for $C_{22}H_{12}ClF_4NO$: C, 63.25; H, 2.90; N, 3.35 Found: C, 62.83; H, 3.02; N, 3.36

Route B

To a solution of 5-chloro-3-(2,6-diflouro-benzylidene)indolin-2-one (0.1944 g, 0.668 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (204µL, 1.34 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an orange color. To the mixture 3.0 equiv. of 2,6 difluorobenzyl bromide (0.4238 g, 2.00 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave at 180ºC for 20 min and placed in the freezer overnight. A solid precipitate did not form so the mixture was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.0825 g, 40.9 %). The yellow oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0452 g, 17.9 %): mp 169-171 ºC; R_f = 0.43 (EtOAc/hexanes, 1:1); MS (*m/z):* 417 (M^+, 100 %).

Route C

To a solution of 5-chloro-3-(2,6-diflouro-benzylidene)indolin-2-one (0.2399 g, 0.824 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (0.7657 g, 5.00 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. To the mixture 3 equiv. of 2,6 difluorobenzyl bromide (0.5120 g, 2.47 mmol,) was then added to the stirred solution after which time the mixture was refluxed for 24 h.. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.2249 g, 65.4 %). The yellow solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1253 g, 36.4%): mp 167-169 ºC; R_f = 0.45 (EtOAc/hexanes, 1:1); MS (*m/z):* 417 (M^+, 100 %).

1-(Propargyl)-3-(benzylidene)indolin-2-one (254)

To a solution of 3-(benzylidene)indolin-2-one (0.3682 g, 1.666 mmol) in EtOH (3 mL) was added 1.7 equiv of DBU (427 µL, 2.85 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 3 equiv. of propargyl bromide (460 µL, 5.02 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 20 min at 180 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel (hexanes/EtOAc, 70:30) to afford the desired product as yellow oil (0.1919 g, 44.5 %). The oil was dissolved in DCM and then recrystallized from hexanes to afford the pure (TLC, GC/MS) product as yellow/gold crystals (0.1002 g, 23.2 %): mp (121-124 °C); R_f = 0.77 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.37 (d, J = 7.4 Hz, 1H), 7.79 (d, J = 5.4 Hz, 1H), 7.77 (s, 1H), 7.59-7.46 (m, 5H), 7.38 (t, J = 7.7 Hz, 1H), 7.14 (d, J = 7.5 Hz, 1H), 6.98 (t, J =7.6 Hz, 1H), 4.63 (d, J = 2.5 Hz, 2H); ^{13}C (300 MHz, DMSO-d_6): δ 166.4, 142.0, 137.6, 133.9, 131.9, 130.1, 129.2, 128.1, 126.1, 122.2, 122.1, 120.2, 109.5, 78.1, 74.2, 28.7 ppm; MS (*m/z)* 2 peaks at 259 (M^+, 100 %).

1-(Propargyl)-3-(2-chloro-benzylidene)indolin-2-one (255)

To a solution of 3-(2-chloro-benzylidene)indolin-2-one (0.3286 g, 1.285 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (326 µL, 2.18 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 3 equiv. of propargyl bromide (360 µL, 3.93 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 20 min at 180 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel (hexanes/EtOAc, 70:30) to afford the desired product as yellow oil (0.1918 g, 50.8 %). The oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1081g, 28.6 %): mp (174-177 °C); R_f = 0.71 (EtOAc/hexanes, 1:1);); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.09 (d, J = 7.6 Hz, 1H), 7.95 (s, 1H), 7.80 (d, J = 6.9 Hz, 1H), 7.72 (s, 1H), 7.68 (d, J =7.3 Hz, 1H), 7.57-7.48 (m, 3H), 7.40 (t, J = 7.7 Hz, 2H), 7.20 (t, J = 8.3 Hz, 2H), 7.13 (d, J =7.6 Hz, 1H), 6.96 (dd, J =0.9 Hz, J =7.5 Hz, 1H), 5.75 (s, 1H), 4.64 (dd, J = 2.4 Hz, J = 25.9 Hz, 2H); ^{13}C (300 MHz, DMSO-d_6) δ: 165.8, 142.2, 132.9, 132.8, 132.4, 131.3, 130.5, 130.2, 129.8, 128.5, 127.3, 126.3, 122.3, 120.1, 109.7, 77.9, 74.3, 28.7 ppm; MS (*m/z)* 293(M^+), 28 (100 %).

1-(Propargyl)-3-(2,3-dichlorobenzylidene)indolin-2-one (256)

To a solution of 3-(2,3-dichloro-benzylidene)indolin-2-one (0.3467 g, 1.20 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (357 µL, 2.39 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an orange color. To the mixture 3 equiv. of propargyl bromide (305 µL, 3.57 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 20 min at 180 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.1282 g, 32.7 %). The oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0497 g, 12.7 %): mp (151-155 °C); R_f = 0.68 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.89 (dd, J =0.5 Hz J = 1.4 Hz, 1H), 7.82-7.66 (m, 2H), 7.61 (s, 1H), 7.54 (t, J =7.9 Hz, 1H), 7.41-7.35 (m, 1H), 7.17 (d, J =7.8 Hz, 1H), 7.13 (d, J =7.5 Hz, 1H), 6.95 (t, J =7.6 Hz, 1H), 4.63 (d, J =2.4 Hz, 2H); ^{13}C NMR δ:

165.7, 142.3, 135.1, 132.4, 132.3, 131.4, 130.7, 130.6, 128.8, 128.5, 122.6, 122.3, 120.8, 119.6, 109.7, 78.8, 74.4, 28.8 ppm: MS (*m/z*) 327(M^+), 292(100 %). $C_{18}H_{11}Cl_2NO$; C, 65.87; H, 3.38; N, 4.27; Found C, 65.76; H, 3.42; N, 4.22.

1-(Propargyl)-3-(4-chlorobenzylidene)indolin-2-one (257)

To a solution of 3-(4-chlorobenzylidene)indolin-2-one (0.3302 g, 1.29 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (397 µL, 2.65 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an yellow color. To the mixture 3 equiv. of propargyl bromide (360 µL, 3.93 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 20 min at 180 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.1269 g, 33.5 %). The oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0923 g, 24.4 %): mp (125-128 °C); R_f = 0.75 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.41 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.71 (s, 1H), 7.61-7.51 (m, 3H), 7.25 (t, *J* = 7.4 Hz, 1H), 7.16 (d, *J* = 7.7 Hz, 1H), 7.02-6.95 (m, 1H), 6.84 (d, *J* = 7.6 Hz, 1H), 4.63 (d, *J* = 2.5 Hz, 2H); ^{13}C NMR δ: 167.0, 142.1, 135.1, 135.0, 133.6, 133.5, 131.1 130.2, 128.8, 128.1, 121.0, 119.9, 109.6, 78.0, 74.2, 28.7 ppm: MS (*m/z*) 293(M^+), 28 (100 %). $C_{18}H_{12}ClNO$;

1-(Propargyl)-3-(4-methoxybenzylidene)indolin-2-one (258)

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.3103 g, 1.236 mmol) in EtOH (3 mL) was added 1.7 equiv of DBU (304 µL, 2.03 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 2.5 equiv. of propargyl bromide (280 µL, 3.05 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 15 min at 150 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel column (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.1815 g, 50.8 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0253 g, 7.08%): mp (95-98 °C); R_f =0.72

(EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.50 (d, J = 8.9 Hz, 1H), 7.88 (s, 1H), 7.76 (d, J = 8.9 Hz, 4H), 7.38-7.28 (m, 2H), 7.16 (s, 1H), 7.12 (d, J = 8.7 Hz, 3H), 7.07 (d, J = 9.0 Hz, 1H), 7.02 (d, J = 0.9, 1H), 7.00 (dd, J = 0.9 Hz, J = 7.6 Hz, 1H), 5.75 (s, 1H), 4.62 (d, J = 2.3 Hz, 2H), 3.85 (s, 3H); ^{13}C (300 MHz, DMSO-d_6): δ 166.7, 160.7, 141.7, 138.1, 134.5, 129.6, 126.7, 124.3, 122.1, 121.9, 114.3, 109.4, 78.3, 74.1, 55.3, 28.6 ppm; MS *m/z* 289 (M^+, 100 %).

1-(Propargyl)-3-(2,6-dichloro-benzylidene)indolin-2-one (259)

To a solution of 3-(4-methoxy-benzylidene)-indolin-2-one (0.3398 g, 1.236 mmol) in EtOH (3 mL) was added 1.7 equiv of DBU (298 µL, 1.99 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 2.7 equiv. of propargyl bromide (290 µL, 3.17 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 15 min at 165 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as yellow oil (0.0974 g, 25.6 %). The solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0882 g, 22.9%): mp (167-169 ºC); R_f = 0.64 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.69 (d, J = 1.0 Hz, 1H), 7.66 (s, 1H), 7.59 (s, 1H), 7.56 (d, J = 1.9 Hz, 1H), 7.53 (d, J = 0.6 Hz, 1H), 7.40 (td, J = 1.0 Hz, J = 15.5 Hz, 1H), 7.38 (d, J = 1.010 Hz, 1H), 7.17 (d, J = 7.8 Hz, 1H), 6.95 (dd, J = 0.8 Hz, J = 15.2 Hz, 1H), 6.93 (d, J = 0.8 Hz, 1H), 6.57 (d, J = 7.3 Hz, 1H), 5.75 (s, 1H), 4.64 (d, J = 2.4Hz, 2H); ^{13}C (300 MHz, DMSO-d_6): δ 165.2, 142.1, 133.1, 132.1, 131.4, 130.8, 130.3, 129.7, 128.6, 122.6, 120.0, 109.7, 77.8, 74.5, 28.8 ppm; MS (*m/z*) 327(M^+), 292 (100 %). Anal. Calcd for $C_{18}H_{11}Cl_2NO$: C, 65.87; H, 3.38; N, 4.27; Found: C, 65.70; H, 3.41; N, 4.32.

1-Propargyl-3-(2,6 difluoro-benzylidene)-indolin-2-one (260)

To a solution of 3-(2,6 difluoro-benzylidene)indolin-2-one (0.4410 g, 1.71 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (1.5727 g, 10.3 mmol) and the

resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. Propargyl bromide 80% (235 µL, 2.57 mmol, 1.5 equiv.) was then added dropwise to the stirred solution of the preformed anion after which time the mixture was refluxed for 24 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid. The solid was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0836 g, 16.5%): mp 123-125 ˚C ; R_f = 0.54 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ 7.87 ppm (d, *J* = 7.95Hz, 1H), 7.74 (s, 1H), 7.69 (t, *J* = 15.054Hz, 1H), 7.67 (d, *J* = 14.9 Hz, 1H), 7.49 (s, 1H), 7.44-7.37 (m,1H), 7.34 (t, *J* = 8.2 Hz, 2H), 7.18 (d, *J* =14.0 Hz, 1H), 7.00 (d, *J* = 5.0 Hz, 2H), 4.63 (d, *J* = 2.5 Hz, 1H) ; ^{13}C NMR δ: 165.3, 161.2-157.8 (dd, *J* = 7.0 Hz), 142.3, 132.3-132.1 (t, *J* = 10.4 Hz), 130.8, 130.7, 123.0-122.9 (t, *J* = 2.2 Hz), 122.5, 121.3, 120.0, 112.3-112.0 (dd, *J* = 6.7 Hz), 111.7, 109.6, 77.8, 74.4, 28.8 ppm; MS (*m/z)* 295 (M^+, 100 %). Anal. Calcd for $C_{18}H_{11}F_2NO$: C, 73.22; H, 3.75; N, 4.74. Found: C, 72.96 ; H, 3.69; N, 4.60 .

1-(Propargyl)-3-(3,5-difluoro-benzylidene)indolin-2-one (261)

To a solution of 3-(3,5-difluoro-benzylidene)indolin-2-one (0.3665 g, 1.236 mmol) in EtOH (3 mL) was added 1.7 equiv of DBU (338 µL, 2.26 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 3 equiv. of propargyl bromide (365 µL, 3.99 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 15 min at 170 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.2387 g, 56.7 %). The solid recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1002 g, 23.8%): mp (107-110 ºC); R_f = 0.77 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz DMSO-d_6) δ 8.15 (d, *J* = 9.5 Hz, 1H), 7.70 (s, 1H), 7.47-7.33 (m, 3H), 7.17-7.11 (m, 2H), 7.02 (td, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 4.62 (d, *J* = 2.4 Hz, 2H) ; ^{13}C NMR δ: 166.0, 164.1-160.7 (dd, *J* = 13.4 Hz), 142.3,

137.9-137.6 (t, *J* = 10.3 Hz), 134.9-134.8 (t, *J* = 2.2 Hz), 130.6, 128.0, 123.2, 122.5, 120.0, 112.4-112.0 (dd, *J* = 8.1 Hz), 109.7, 105.7-105.0 (t, *J* = 26.6 Hz), 77.9, 74.3, 28.7 ppm; MS (*m/z)* 295 (M^+), 28 (100 %). Anal. Calcd for $C_{18}H_{11}F_2NO$: C, 73.22; H, 3.75; N, 4.74. Found: C, 72.88; H, 4.02; N, 4.72.

1-(Propargyl)-5-chloro-3-(2-chloro-benzylidene)indolin-2-one (262)

To a solution of 5-chloro-3-(2-chloro-benzylidene)indolin-2-one (0.3875 g, 1.10 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (330 µL, 2.21 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an orange color. To the mixture 3 equiv. of propargyl bromide (305 µL, 3.33 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 20 min at 180 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil (0.2182 g, 49.8 %). The oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.2015 g, 45.9 %): mp (106-109 °C); R_f = 0.73 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.08 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 8.01 (d, *J* =2.0 Hz, 1H), 7.70 (d, *J* =7.8 Hz, 1H), 7.61-7.51 (m, 2H), 7.48 (dd, *J* =2.1 Hz, *J* = 8.4 Hz, 1H), 7.41 (d, *J* =7.6 Hz, 1H), 7.21 (d, *J* =19.1 Hz, 1H), 7.08 (d, *J* = 2.0 Hz, 1H), 4.65 (dd, *J* = 28.2 Hz, 2H) ; ^{13}C NMR δ: 165.5, 141.0, 134.9, 132.5, 131.6, 130.2, 130.0, 129.0, 127.5, 127.2, 126.7, 126.2, 121.7, 120.7, 111.2, 77.6, 74.6, 28.9 ppm: MS (*m/z*) 327(M^+), 292(100 %). $C_{18}H_{11}Cl_2NO$; C, 65.87; H, 3.38; N, 4.27; Found C, 65.76; H, 3.47; N, 4.30.

5-Chloro-1-(prop-2-yn-1-yl)-3-((4)-methoxy-benzylidene)indolin-2-one (263)

To a solution of 3-(4-methoxy-benzylidene)-5-chloro-indolin-2-one (0.5246 g, 1.83 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (1.6884 g, 11.0 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution stayed a bright yellow color. Propargyl bromide 80% (260 µL, 2.84 mmol, 1.5 equiv.) was then added dropwise to the stirred solution of the preformed anion after which time the mixture was refluxed for 49 h. The mixture was then allowed to cool to rt and the suspended

KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid. The solid was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.3905 g, 66%): mp 127-129 ºC; R_f = 0.54 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 8.85 (d, J = 8.9 Hz, 1H), 8.01 (s, 1H), 7.92 (J = 2.0 Hz, 1H), 7.80 (s, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.45 (dd, J = 2.0 Hz, J = 8.4 Hz, 1H), 7.37 (dd, J = 2.0 Hz, J = 8.3 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.15-7.06 (m, 4H), 4.63 (s, 2H), 3.86 (s, 3H) ^{13}C (300 MHz, DMSO-d_6): δ 166.4, 161.9, 140.4, 140.0, 134.9, 129.0, 126.5-126.3 (t, J = 9.4 Hz), 126.0, 123.0, 121.3, 120.9, 114.4, 110.8, 78.0, 74.4, 55.4, 28.8 ppm; MS (*m/z*) 323 (M^+, 100 %).

1-(Propargyl)-5-chloro-3-(2,6-dichloro-benzylidene)indolin-2-one (264)

To a solution of 5-chloro-3-(2,6-dichloro-benzylidene)indolin-2-one (0.3568 g, 1.336 mmol) in EtOH (3 mL) was added 2.0 equiv of DBU (370 µL, 2.47 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned an orange color. To the mixture 3 equiv. of propargyl bromide (370 µL, 4.04 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 20 min at 180 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified by silica gel (hexanes/EtOAc, 70:30) to afford the desired product as yellow oil (0.2182 g, 74.0 %). The oil was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.2015 g, 46.8 %): mp (106-109 °C); R_f = 0.73 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.97 (s, 1H), 7.72 (m, 3H), 7.61 (dd, J = 0.6 Hz, J = 7.1 Hz, 1H), 7.58 (dd, J = 0.7 Hz, J = 7.1 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 5.75 (s, 1H), 4.65 (d, J = 2.4 Hz, 2H) ; ^{13}C NMR δ: 164.8, 140.9, 133.0, 131.8, 131.7, 131.5, 130.4, 129.4, 128.7, 126.5, 122.1, 121.5, 111.3, 76.1, 29.0 ppm: MS (*m/z*): 362 (M^+), 326 (100 %). $C_{18}H_{10}Cl_3NO$; C, 59.62; H, 2.78; N, 3.86; Found C, 60.01; H, 2.91; N, 3.90.

1-(Propargyl)-5-chloro-3-(2,6-difluoro-benzylidene)indolin-2-one (265)

To a solution of 5-chloro-3-(2,6-difluoro-benzylidene)indolin-2-one (0.3210 g, 1.110 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (1.012 g, 6.62 mmol)

and the resulting mixture was stirred at rt for 5 min., the solution stayed a bright yellow color. Propargyl bromide 80% (303 µL, 3.311 mmol, 3 equiv) was then added dropwise to the stirred solution of the preformed anion after which time the mixture was refluxed for 23 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.0837, 23.1%). The solid was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0536 g, 14.8%): mp 119-121 ºC; R_f = 0.57 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 7.72-7.62 (m, 1H), 7.59 (s, 1H), 7.51 (dd, *J* =2.0 Hz, *J* =8.4 Hz, 1H), 7.38 (t, *J* =8.2 Hz, 3H), 7.22 (d, *J* =8.40 Hz, 1H), 6.91 (d, *J* =1.9 Hz, 1H), 4.64 (d, *J* =2.4 Hz, 2H);^{13}C NMR δ: 165.0, 161.2-157.8 (dd, *J* = 7.1 Hz), 141.1, 133.0-132.7 (t, *J* = 10.5 Hz), 130.3, 129.6, 126.5, 123.3, 122.5, 121.6, 112.4-112.1 (dd, *J* =6.8 Hz), 111.3, 111.1, 77.5, 74.7, 29.0 ppm; MS (*m/z*) 329 (M^+), 28 (100 %). Anal. Calcd for $C_{18}H_{10}ClFNO$: C, 65.57; H, 3.06; N, 4.25; Found: C, 65.58; H, 3.20; N, 4.51.

1-(Propargyl)-5-chloro-3-(3,5-difluoro-benzylidene)indolin-2-one (266)

To a solution of 5-chloro-3-(3,5-difluoro-benzylidene)-indolin-2-one (0.4061 g, 1.396 mmol) in EtOH (3 mL) was added 1.7 equiv of DBU (355 µL, 2.56 mmol) and the resulting mixture was allowed to stir at rt for 5 min, the solution turned a orange color. To the mixture 3 equiv. of propargyl bromide (383 µL, 4.19 mmol,) was then added to the stirred solution after which time the mixture was placed in the microwave for 15 min at 170 ºC. The mixture was then allowed to cool to rt and placed in the freezer overnight. The solution was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow oil/solid (0.2860 g, 62.3 %). The oil/solid was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.0554 g, 12.1%): mp (177-179 ºC); R_f = 0.76 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 8.14 (dd, *J* = 2.1 Hz, *J* = 9.3 Hz, 2H), 8.05 (s, 1H), 7.93 (d, *J* = 2.0 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 4.63 (d, *J* = 2.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ: 166.5, 163.6-160.1 (dd, *J* = 10.3 Hz), 139.0, 136.7-136.3 (t, *J* = 10.8 Hz), 129.1, 128.6, 126.0,

125.1, 120.2, 114.8-114.3 (dd, *J* = 8.4 Hz), 110.6, 106.1-105.8 (t, *J* = 25.6 Hz), 77.7, 74.4, 28.8 ppm; MS (*m/z*) 329(M^+), 28 (100 %).

1-(Propargyl)-5-fluoro-3-(2,6-difluoro-benzylidene)indolin-2-one (267)

To a solution of 5-fluoro-3-(2,6-difluoro-benzylidene)indolin-2-one (0.2704 g, 0.983 mmol) in acetonitrile (45 mL) was added 6 equiv of KF/alumina (0.9146 g, 5.98 mmol) and the resulting mixture was stirred at rt for 5 min., the solution stayed a bright yellow color. Propargyl bromide 80% (270 µL, 2.94 mmol, 3 equiv) was then added dropwise to the stirred solution of the preformed anion after which time the mixture was refluxed for 27 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was removed by vacuum filtration. The filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a yellow solid (0.1636, 53.2%). The solid was then recrystallized from EtOH to afford the pure (TLC, GC/MS) product as bright yellow crystals (0.1280 g, 41.6%): mp 171-175 ºC; R_f = 0.69 (EtOAc/hexanes, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6): 7.71-7.61 (m, 1H), 7.57 (s, 1H), 7.36 (t, *J* =8.3 Hz, 2H), 7.33 (d, *J* =2.6 Hz, 1H), 7.30 (d, *J* =9.2 Hz, 1H) 7.20 (dd, *J* =4.4 Hz, *J* =8.6 Hz, 1H), 6.71 (dd, *J* =2.0 Hz, *J* =4.5 Hz, 1H), 4.64 (d, *J* =2.4 Hz, 2H) ;^{13}C NMR δ: 165.2, 161.2-157.8 (dd, *J* =6.9 Hz), 159.6-156.4 (d, *J* =237 Hz), 138.7 (d, *J* =1.3 Hz), 132.9-132.6 (d, *J* =10.6 Hz), 130.2-130.1 (t, *J* =1.0 Hz), 123.1, 121.1-121.0 (d, *J* =9.0 Hz), 117.3-116.9 (d, *J* =23.7 Hz), 112.4-112.1 (dd, *J* =6.8 Hz), 111.5-111.3 (t, *J* =19.3 Hz), 110.6-110.5 (d, *J* =8.3 Hz), 110.4-109.9 (d, *J* =25.5 Hz), 77.7, 74.5, 28.9 ppm; MS (*m/z*) 313 (M^+), 28 (100 %). Anal. Calcd for $C_{18}H_{10}F_3NO$: C, 69.01; H, 3.22; N, 4.47; Found: C, 68.86; H, 3.27; N, 4.60.

3-(2,6-Difluoro-benzylidene)-5-fluoro-1-[1-(2-fluoro-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-1,3-dihydro-indol-2-one (284)

To a solution of 2-fluoroethyltosylate (1 equiv., 0.224 mmol, 0.0488 g) in N, N-dimethylformamide (5 mL) was added sodium azide (1.1 equiv., 0.2456 mmol, 0.0159 g) and the resulting mixture was heated at 50 ºC for 30 min. in which the solution stayed clear. After 30 min., 5 mol % 1M Cu (II) sulfate (12 µL) and 10 mol % (5 mg) sodium ascorbate were added to the mixture turning the solution brown/orange. The mixture was

stirred for 1-2 min. in which the solution changed back to a clear color. 1-(Propargyl)-5-fluoro-3-(2,6-difluoro-benzylidene)indolin-2-one (1 equiv., 0.0700g, 0.223 mmol) was added to the mixture and heated at 80 ºC for 24 h. The reaction was cooled in an ice bath and transferred to a beaker with 30 mL cold water after which 10% NH_4OH (5 mL) was added to the reaction with stirring. The reaction mixture then washed with DCM (30 mL) and brine solution (30 mL). The solution was dried with sodium sulfate and the filtrate was evaporated under reduced pressure and purified with silica gel (hexanes/EtOAc, 70:30) to afford the desired product as a orange oil (0.0709 g, 78.9 %). The oil was then recrystallized from DCM/Hexanes to afford the pure (TLC, GC/MS) product as orange/brown solid (0.0679 g, 75.5%): mp 171-174ºC; R_f = 0.30; MS (*m/z*) 402 (M^+), 28 (100 %).

References

1) a) Kodadek, T. "The rise, fall and reinvention of combinatorial chemistry." *Chem. Commun.* **2011**, *47*, 9757-9763. b) Kennedy, J.P.; Williams, L.; Bridges, T.M.; Daniels, R.N.; Weaver, D.; Lindsley, C.W. "Application of combinatorial chemistry science on modern drug discovery." **2008**, *10*, 345-354.
2) Maclean, D.; Baldwin, J.J.; Ivanov, V.T.; Kato, Y.; Shaw, A.; Schneider, P. "Glossary of terms used in combinatorial chemistry." *J. Comb. Chem.* **2000**, *2*, 562-578
3) Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W. L.; Lundell, G.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; Kling, P.J.; Kunkel, K.A.; Springer, J.P.; Hirshfield, J. "Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists" *J. Med. Chem*. **1988**, *31*, 2235-2246.
4) De Sa Alves, F.R.; Barreiro, E.J.; Fraga Manssour, C.A. "From nature to drug discovery: the indole scaffold as a 'privileged structure'." *Mini-Rev. Med. Chem.* **2009**, *9*, 782-793.
5) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. "Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain, falcipain-2, and rhodesain." *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3527-3530.

6) Baeyer, A., *Berichte Deutchen Chemischen G.* **1879**, 582.

7) Sumpter, W.C. "The Chemistry of Oxindole." *Chem Rev.* **1945,** 443.

8) a) Liu, Y.; Wang, H.; Wan, J. "Recent advances in diversity orientedsynthesis through isatin-based multicomponent reactions" *Asian J. Org. Chem*. **2013**, *2*, 374-386. b) Singh, G.S.; Desta, Z.Y. "Isatins as privileged molecules in design and synthesis of spirocyclic cyclic frameworks." *Chem. Rev*. **2012**, *112*, 6104-6155.; c) Guo, H.; Tian, J. "New Progress in the synthesis of spirocyclic compound via multicomponent reactions involving isatin." *Chin. J. Org. Chem*. **2011**, *31*, 1752-1760; d) MacDonald, J.P.; Badillo, J.J.; Arevalo, G.E.; Silva-Garcia, A.; Franz, A.K. "Catalytic stereoselective synthesis of diverse oxindoles and spirooxindoles from isatins."*ACS Comb. Sci*. **2012**, *14*, 285-293.

9) a) Cohen, P. "Protein Kinases the Major Drug Targets of the Twenty First Century?" *Nature Rev,. Drug Discov.* **2002**, *1*, 309-315; b) Prakash, C.R.; Theivendren, P.; Raja, S. "Indolin-2-ones in clinical trials as potential kinase inhibitors: a review." *Pharmacology & Pharmacy* **2012**, *3*, 62-71; c) Noble, M.E.M.; Endicott, J.A.; Johnson, L.N. "Protein kinase inhibitors: insights into drug design from structure" *Science* **2004**, *303*, 1800-1805.

10) Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; McMahon, G.; Tang, C. "Synthesis and biological evaluations of 3-substituted indolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity toward particular receptor tyrosine kinases" *J. Med. Chem*. **1998**, *41*, 2588-2603.

11) Sun, L.; Tran, N.; Liang, C.; Tang, F.; Rice, A.; Schreck, R.; Waltz, K.; Shawver, L.K.; McMahon, G.; Tang, C. "Design, synthesis, and evaluations of substituted

3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenel]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF receptor tyrosine kinases" *J. Med. Chem*. **1999**, *42*, 5120-5130. Sun, L.; Tran, N.; Liang, C.; Hubbard, S.; Tang, F.; Lipson, K.;Schreck, R.; Zhou, Y.; McMahon, G.; Tang, C. "Identification of substituted 3-[(4,5,6,7-tetrahydro-1H-indol-2-yl)methylene]-1,3-dihydroindol-2-ones as growth factor receptor inhibitors for VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-Rb tyrosine kinases" *J. Med. Chem.* **2000**, *43*, 2655-2663.

12) Sun, L.; Liang, C.; Shirazian, S.; Zhou, Y.; Miller, T.; Cui, J.; Fukuda, J.Y.; Chu, J.Y.; Nematalla, A.; Wang, X.; Chen, H.; Sistla, A.; Luu, T.C.; Tang, F.; Wei, J.; Tang, Cho. *J. Med. Chem.* **2003**, *46*, 1116-1119.

13) a) Lackey, K.; Cory, M.; Davis, R.; Frye, S.V.; Harris, P.A.; Hunter, R. N.; Jung, D.K.; McDonald, O.B.; McNutt, R.W.; Peel, M.R.; Rutkowske, R. D.; Veal, J.M.; Wood, E.R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 223-226. b) Wood, E.; Crosby, R.M.; Dickerson, S.; Frye, S.V.; Griffin, R.; Hunter, R.; Jung, D.K.; McDonald, O.B.; McNutt, R.; Mahony, W.B.; Peel, M.R.; Ray, J.; Lackey, K. *Anti-Cancer Drug Design* **2001**, *16*, 1-6.

14) a) Chin, P.C.; Liu, L.; Morrison, B.E.; Siddiq, A.; Ratan, R.R.; Bottiglieri, T.; D' Mello, S.R. *Journal of Neurochemistry* **2004**, *90*, 595-608. b) Balderamos, M.; Ankati, H.; Akubathini, S.K.; Patel, A.V.; Kamila, S.; Mukherjee, C.; Wang, L.; Biehl, E.R.; D'Mello, S.R. *Exp. Biol. Med.* **2008**, *233*, 1395-1402. c) Ankati, H.; Akubathini, S.K.; Kamila, S.; Mukherjee, C.; D'Mello, S.R.; Biehl, E.R.; d) D'Mello, S.R.; Biehl, E.A. *U.S. Pat. Appl. Publ.* **2009**, US 20090068105 A1 20090312.

15) Li, X.; Huang, P.; Cui, J.J.; Zhang, J.; Tang, C. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1939-1942.

16) Woodard, C.L.; Li, Z.; Kathcart, A.K.; Terrell, J.; Gerena, L.; Lopez-Sanchez, M.; Kyle, D.E.; Bhattacharjee, A.K.; Nichols, D.A.; Ellis, W.; Prigge, S.T.; Geyer, J.A.; Waters, N.C. *J. Med. Chem.* **2003**, *46*, 3877-3882.

17) Davis, S. T.; Benson, B. G.; Bramson, H. N. et al. *Science* **2001**, *291*, 134-137.

18) Johnson, K.; Liu, L.; Majdzadeh, N.; Chavez, C.; Chin, P.C.; Morrison, B.; Wang, L.; Park, J.; Chugh, P.; Chen, H.M.; D'Mello, S.R. *Journal of Neurochemistry* **2005**, *93*, 538-548.

19) Flajolet, M.; He, G.; Heiman, M.; Lin, A.; Nairn, A. C.; Greengard, P. *PNAS* **2007**, *104*, 4159.

20) Hottecke, N.; Liebeck, M.; Baumann, K.; Schubenel, R.; Winkler, E.; Steiner, H.; Schmidt, B. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2958-2963.

21) Guan, H.; Laird, A.D.; Blake, R.A.; Tang, C.; Liang, C. *Bio*org. *Med. Chem. Lett.* **2004**, *14*, 187- 190.

22) a) Olgen, S.; Akaho, E.; Nebioglu, D. *Il* Farmaco **2005**, *60*, 497-506. b) Olgen, S.; Gorz, C.; Jose, J. *Biol. Pharm. Bull.* **2007**, *4*, 715-718

23) Zhou, F.; Zheng, J.; Dong, X.; Zhang, Z.; Zhao, L; Sha, X.; Li, L.; Wen, R. *Letters in Organic Chemistry* **2007,** *4*, 601-605.

24) Zhang, W.; Go, M. L. "Functionalized 3-benzylidene-indolin-2-ones: Inducers of NAD(P)H-quinone oxidoreductase 1 (NQO1) with antiproliferative activity" *Bioorg. Med. Chem.* **2009**, *17*, 2077-2090.

25) a)Jiang, T.; Kuhen, K. L.; Wolff, K.; Yin, H.; Bieza, K.; Caldwell, J.; Bursulaya, B.; Wu, T. Y.-H.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2105-2108 b)Jiang, T.; Kuhen, K. L.; Wolff, K.; Yin, H.; Bieza, K.; Caldwell, J.; Bursulaya, B.; Tuntland, T.; Zhang, K.; Karanewsky, D.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2109-2112.

26) 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006, *Design, synthesis and evaluation of indole and oxindole aspartic acid derivatives as potential caspase inhibitors.* Mofford, D. M..; Brush, E. J., Department of Chemical Sciences, Bridgewater State College, Bridgewater, MA, USA. Abstracts of Papers, CHED-667

27) Joaquim F.M. da Silva; Simon J. Garden; Angelo C. Pinto. "The chemistry of isatins: a review from 1975 to 1999." *J. Braz. Chem. Soc.* **2001**, *12*, 273-324.

28) Ischia, M.; Palumbo, A.; Prota, G.; *Tetrahedron* **1988**, *44*, 6441.

29) Varma, R. S.; Khan, I. A. *Polish J. Pharmacol. Pharm.* **1977**, *29*, 549.

30) Cane, A.; Tournaire, M. C.; Barritault, D.; Crumeyrolle-Arias, M. *Biochem. Biophys. Res. Commun.* **2000**, *276*, 379.

31) Mason, J.S.; Morize, I.; Menard, P.R.; Cheney, D.L.; Hulme, C.; Labaudiniere, R.F. "New 4-point pharmacophore method for molecular similarity and diversity applications: overview of the method and applications, including a novel approach to the design of combinatorial libraries containing priviledged substructures" *J. Med. Chem.* **1999**, *42*, 3521-3264.

32) Iyer, R. A.; Hanna, P. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 89.

33) Webber, S. E.; Tikhe, J.; Worland, S. T.; Fuhrman, S. A.; Hendrickson, T.F.; Matthews, D. A.; Love, R. A.; Patick, A. K.; Meador, A. W.; Ferre, R. A.,Brown, E. L.; DeLisle, D. M.; Ford, S. E.; Binford, S. L. *J. Med. Chem.* **1996,** *39*, 5072.

34) Bridges T. M.; Marlo, J. E., Niswender, C. M.; Jones, C. K.; Satyawan, J. B.; Gentry, P. R.; Plumley, H. C.; Weaver, D. C; Conn J. P.; Lindsley, C. W. *J. Med. Chem.* **2009,** *52*, 3445.

35) Bridges, T. M.; Kennedy, J. P.; Cho, H. P.; Breininger, M. L.; Gentry, P. R.; Hopkins, C. R.; Conn, P. J.; Lindsley, C. W. *Bio. Med. Chem. Lett.* **2010**, *20*, 558-562.

36) Zhou, L.; Ying, L.; Weilin, Z.; Ping, W.; Changkang, H.; Jianfeng, P.; Yaxia, Y.; Luhua, L. *J. Med. Chem.* **2006**, *49*, 3440.

37) Chu, W.; Zhang, J.; Zeng, C.; Rothfuss, J.; Tu, Z.; Chu, Y.; Reichert, D.; Welch, M.J.; Mach, R.H. *J. Med. Chem.* **2005,** *48*, 7637

38) a) Thornberry, N. A.; Lazebnik, Y. "Caspases: enemies within" *Science* **1998**, *281*, 1312-1316.

39) Hengartner, M.O. "The biochemistry of apoptosis" *Nature* **2000**, *407*, 770-776.

40) Thornberry, N.A. "Caspases: key mediators of apoptosis" *Chem. Biol*. **1998**, *5*, R97-R103.

41) Maly, D.J.; Huang, L.; Ellman, J.A. *ChemBioChem*.**2002**, *3*, 16-37.

42) Nicholson, D.W.; All, A.; Thornberry, N.A.; Vaillancourt, J.P.; Ding, C.K.; Gallant, M.; Gareau, Y.; Griffin, P.R.; Labelle, M.; Lazebnik, Y.A.; Munday, N.A.; Raju, S.M.; Smulson, M.E.; Yamin, T.T.; Yu, V.L.; Miller, D.K. "

Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis" *Nature* **1995**, *376*, 37-43.

43) Calvo-Garcia, M.; Peterson, E.P.; Leiting, B.; Ruel, R.; Nicholson, D.W.; Thornberry, N.A. "Inhibition of Human Caspases by Peptide-based and Macromolecular Inhibitors" *The Journal of Biological Chemistry* **1998**, *273*, 32608-32613.

44) Van Noorden, C.J. *Acta Histochem* **2001**, *103*, 241-251.

45) Mersmann, J.; Zacharowski, P.A.; Schmitz, I.; Zacharowski, K. *Resuscitation* **2008**, *79*, 468-474.

46) Braun, J.S.; Prass, K.; Dirnagl, U.; Meisel, A.; Meisel, C.; *Experimental Neurology* **2007**, *206*, 183-191.

47) Caserta, T.M.; Smith, A.N.; Gultice, A.D.; Reedy, M.A.; Brown, T.L. *Apoptosis* **2003**, *8*, 345-352.

48) Haberkorn, U.; Kinscherf, R.; Krammer, P.H.; Mier, W.; Eisenhunt, M. *Nuclear Medicine and Biology* **2001**, *28*, 793–798.

49) Bohn, P.; Mouchard, F.; Rouvet, J.; de Boisgrollier, A.C.; Vera, P. "^{99m}Tc-(Me)FGCDEVD, a potential tracer for apoptosis detection." *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1375-1378.

50) Lee, D.; Long, S.A.; Murray, J.H.; Adams, J.L.; Nuttall, M.E.; Nadeau, D.P.; Kikly, K.; Winkler, J.D.; Sung, C.M.; Ryan, M.D.; Levy, M.A.; Keller, P.M.; DeWolf Jr., W.E. "Potent and selective nonpeptide inhibitors of caspases 3 and 7." *J. Med. Chem.* **2001**, *44*, 2015-2026.

51) Mittl, P.R.E.; Di Marco, S.; Krebs, J.F.; Bai, X.; Karanewsky, D.S.; Priestle, J.P.; Tomaselli, K.J.; Grutter, M.G. "Structure of recombinant human CPP32 in complex with the tetrapeptide acetyl-asp-val-ala-asp fluoromethyl ketone." *J. Biol. Chem.* **1997**, *272*, 6539-6547.

52) Rotonda, J.; Nicholson, D.W.; Fazil, K.M.; Gallant, M.; Gareau, Y.; Labelle, M.; Peterson, E.P.; Rasper, D.M.; Ruel, R.; Vaillancourt, J.P.; Thornberry, N.A.; Becker, J.W.; "The 3-dimensional structure of apopain/CPP32, a key mediator of apoptosis." *Nat. Struct. Biol.* **1996**, *3*, 619-625.

53) Somasekhara, S.; Dighe, V.S.; Suthar, G.K.; Mukherjee, S.L. "Chlorosulphonatiion of isatin" *Curr. Sci.* **1965**, 508.

54) Martinez, F.; Naarmann, H. "New isatin derivatives: Synthesis and reactions" *Synth. Met*. **1990**, *39*, 195-203.

55) Lee, D.; Long, S.A.; Adams, J.L.; Chan, G.; Vaidya, K.S.; Francis, T.A.; Kikly, K.; Winkler, J.D.; Sung, C.; Debouck, C.; Richardson, S.; Levy, M.A.; DeWolf, Jr.,W.E.; Keller, P.M.; Tomaszek, T.; Head, M.S.; Ryan, M.D.; Haltiwanger, R.C.; Liang, P.; Janson, C.A.; McDevitt, P.J.; Johanson, K.; Concha, N.O.; Chan, W.; Abdel-Meguid, S.S.; Badger, A.M.; Lark, M.W.; Nadeau, D.P.; Suva, L.J.; Gowen, M.; Nuttall, M.E. "Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality" *J. Biol. Chem.* **2000**, *275*, 16007-16014.

56) Chu, W.; Zhang, J.; Zeng, C.; Rothfuss, J.; Tu, Z.; Chu, Y.; Reichert, D.; Welch, M.J.; Mach, R.H. "*N*-benzylisatin sulfonamide analogues as potent caspase-3

inhibitors: synthesis, in vitro activity, and molecular modeling studies." *J. Med. Chem.* **2005,** *48,* 7637-7647.

57) Chu, W.; Rothfuss, J.; d'Avignono, A.; Zeng, C.; Zhou, D.; Hotchkiss, R.S.; Mach, R.H. "Isatin sulfonamide analogs containing a Michael addition acceptor: a new class of caspase 3/7 inhibitors." *J. Med. Chem.* **2007**, *50*, 3751-3755.

58) Chu, W.; Rothfuss, J.; Chu, Y.; Zhou, D.; Mach, R.H. "Synthesis and in vitro evaluation of sulfonamide isatin Michael acceptors as small molecule inhibitors of caspase-6." *J. Med. Chem.* **2009**, *52*, 2188-2191.

59) a) Juweid, M.E.; Cheson, B.D. "Positron-Emission Tomography and assessment of cancer therapy"*N. Engl. J. Med*. **2006**, *354*, 496-507. b) Weber, W.A.; Wieder H. "Monitoring chemotherapy and radiotherapy of solid tumors" *Eur. J. Nuc. Med. Mol. Imaging* **2006**, *33*(Suppl), 27-37.

60) Mankoff, D.A.; Eary, J.F.; Link, J.M, Muzi, M.; Rajendran, J.G.; Spence, A.M.; Krohn, K.A. "Tumor-specific positron emission tomography imaging in patients: [^{18}F] Fluorodeoxyglucose and Beyond" *Clin. Cancer Res.* **2007**, *13*, 3460-3469.

61) Tait, J.F. "Imaging of apoptosis" *J. Nucl. Med*. **2008**, *49*, 837-840.

62) Zeng, W.; Miao, W. "Development of small molecule probes for the molecular imaging of apoptosis" Anti-Cancer Agents in Medicinal Chemistry **2009**, *9*, 986-995.

63) Reshef, A.; Shirvan, A.; Akselrod-Ballin, A.; Wall, A.; Ziv, I. "Small-molecule biomarkers for clinical PET imaging of apoptosis" *J. Nucl. Med*. **2010**, *51*, 837-840.

64) Niu, G.; Chen, X. "Apoptosis imaging: beyond Annexin V" *J. Nucl. Med.* **2010**, *51*, 1659-1662.

65) Smith, B.A.; Smith, B.D. "Biomarkers and molecular probes for cell death imaging and targeted therapeutics" *Bioconjugate Chem.* **2012**, *23*, 1989−2006.

66) Hanahan, D.; Weinberg, R.A. "The Hallmarks of Cancer" *Cell* **2000**, 100, 57-70. b) Hanahan, D.; Weinberg, R.A. "Hallmarks of Cancer: The Next Generation" *Cell* **2011**, 144, 646-674.

67) Wang, M.W.; Wang, F.; Zheng, Y.J.; Zhang, Y.J.; Zhang, Y.P.; Zhoa, Q.; Kwang-Fu Shen, C.; Wang, Y.; Sun, S.H. "An in vivo molecular imaging probe 18F-Annexin B1 for apoptosis detection by PET/CT: preparation and preliminary evaluation." *Apoptosis* **2013**, *18*, 238-247

68) Zhou, D.; Chu, W.; Rothfuss, J.; Zeng, C.; Xu, J.; Jones, L.; Welch, M.J.; Mach, R.H. "Synthesis, radiolabeling, and in vivo evaluation of an ^{18}F-labeled isatin analog for imaging caspase-3 activation in apoptosis." *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5041-5046.

69) Yoo, J.; Dence, C.S.; Sharp, T.L.; Katzenellenbogen, J.A.; Welch, M.J. *J. Med. Chem.***2005**, *48*, 6366

70) Chen, D.L.; Zhou, d.; Chu, W.; Herrbrich, P.E.; Jones, L.A.; Rothfuss, J.M.; Engle, J.T.; Geraci, M.; Welch, M.J.; Mach, R.H. "Comparison of radiolabeled isatin analogs for imaging apoptosis with positron emission tomography." *Nuclear Medicine and Biology* **2009**, *36*, 651-658.

71) Smith, G.; Glaser, M.; Perumal, M.; Nguyen, Q.D.; Shan, B.; Arstad, E.; Aboagye, E.O. "Design, synthesis, and biological characterization of a caspase

3/7 selective isatin labeled with 2-[^{18}F]fluoroethylazide." *J. Med. Chem.* **2008**, *51*, 8057-8067.

72) Kuijpers, B. H. M.; Groothuys, S.; Soede, A.; Laverman, P.; Boerman, O. C.; van Delft, F. L.; Rutjes, F. P. J. T. "Preparation and evaluation of glycosylated arginine-glycine-aspartate (RGD) derivatives for integrin targeting." *Bioconjugate Chem.* **2007**, *18*, 1847–1854.

73) Li, Z.; Wu, Z.; Chen, K.; Chin, F. T.; Chen, X. "Click chemistry for 18F-labeling of RGD peptides and microPET imaging of tumor integrin α_v β_3 expression." *Bioconjugate Chem.* **2007**, *18*, 1987–1994.

74) Glaser, M.; Goggi, J.; Smith, G.; Morrison, M.; Luthra, S.K.; Robins, E.; Aboagye, E.O. "Improved radiosynthesis of the apoptosis marker ^{18}F-ICMT including biological evaluation." *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6945-6949.

75) Nguyen, Q.D.; Challapalli, A.; Smith, G.; Fortt, R.; Aboagye, E.O. "Imaging apoptosis with positron emission tomography: 'Bench to bedside' development of the caaspase-3/7 specific radiotracer [^{18}F]ICMT-11." *European Journal of Cancer* **2012**, *48*, 432-440.

76) Nguyen, Q.D.; Smith, G.; Glaser, M.; Perumal, M.; Arstad, E.; Aboagye, E.O. "Positron, emission tomography imaging of drug-induced tumor apoptosis with a caspase-3/7 specific [^{18}F]-labeled isatin sulfonamide." *Proceedings of the National Academy of Sciences* **2009**, *106*, 16375-16380.

77) Kopka, K.; Faust, A.; Keul, P.; Wagner, S.; Breyholz, H.J.; Holtke, C.; Schober, O.; Schafers, M.; Levkau, B. "5-Pyrrolidinylsulfonyl isatins as a potential tool for

the molecular imaging of caspases in apoptosis." *J. Med. Chem.* **2006**, *49*, 6704-6715.

78) Podichetty, A.K.; Faust, A.; Kopka, K.; Wagner, S.; Schober, O.; Schafers, M.; Haufe G.; "Fluorinated isatin derivatives. Part 1. Synthesis of new N-substituted (*S*)-5[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins as potent caspase-3 and -7 inhibitors." *Bioorg. Med. Chem.* **2009**, *17*, 2680-2688.

79) Podichetty, A.K.; Wagner, S.; Schroer, S.; Faust, A.; Schafers, M.; Schober, O.; Kopka, K.; Haufe, G. "Fluorinated isatin derivatives. Part 2. New *N*-substituted 5-pyrrolidinylsulfonyl isatins as potential tools for molecular imaging of caspases in apoptosis." *J. Med. Chem.* **2009**, *52*, 3484-3495.

80) Alvernhe, G.; Laurent, A.; Haufe, G. *Synthesis* **1987**, 562–565.

81) Haufe, G.; Alvernhe, G.; Laurent, A.; Ernet, T.; Goj, O.; Kroger, S.; Sattler, A. "Bromofluorination of Alkenes." *Org. Synth.* **1998**, *76*, 159–168.

82) Choe, Y. S.; Lidstro¨m, P. J.; Chi, D. Y.; Bonasera, T. A.; Welch, M. J.; Katzenellenbogen, J. A. Synthesis of 11_-[18F]Fluoro-5α-dihydrotestosterone and 11_-[18F]Fluoro-19-nor-5R-dihydrotestosterone: Preparation via Halofluorination-Reduction, Receptor Binding, and Tissue Distribution. *J. Med. Chem.* **1995**, *38*, 816–825.

83) Shendage, D. M.; Fro¨hlich, R.; Bergander, K.; Haufe, G. Asymmetric Synthesis of γ-Fluorinated R-Amino Acids. *Eur. J. Org. Chem.* **2005**, *71*, 9–727.

84) Luae, K. W.; Haufe, G. “2-Fluoroallyl bromide – A versatile fluorinated building block. Alkylation of glycine and alanine ester imines.” *Synthesis* **1998**, 1453-1456.

85) Rohn, T.T. “The role of caspases in Alzheimer’s disease; potential novel therapeutic oppurtunities.” *Apoptosis* **2010**, *15*, 1403-1409.

86) Honson, N.S.; Johnson, R.L.; Huang, W.; Inglese, J.; Austin, C.P.; Kuret, J. “Differentiating Alzheimer disease-associated aggregates with small molecules.” *Neurobiology of Disease* **2007**, *28*, 251-260.

87) Braak, H.; Braak, E.; “Neuropathological stageing of Alzheimer-related changes.” *Acta Neuropathol* **1991**, *82*, 239-259.

88) Petkova, A.T. “Experimental constraints on quaternary structure in Alzheimer’s beta-amyloid fibrils.” *Biochemistry* **2006**, *45*, 498-512.

89) Krebs, M.R. “The binding of thioflavin-T to amyloid fibrils: localization and implications.” *J. Struct. Biol.* **2005**, *49*, 30-37.

90) Grazit, E. “A possible role for pi-stacking in the self-assembly of amyloid fibrils.” *FASEB J.* **2002**, *16*, 77-83.

91) Klunk, W.E. “Imaging brain amyloid in Alzheimer’s disease with Pittsburgh Compound-B.” *Ann. Neurol.* **2004**, *55*, 306-319.

92) Guillozet-Bongaarts, A.L. “Tau truncation during neurofibrillary tangle evolution in Alzheimer’s disease.”*Neurobiol. Aging* **2005**, *26*, 1025-1022.

93) Chirita, C.N. “Triggers of ful-length tau aggregation: a role for partially folded intermediates. *Biochemistry* **2005**, *44*, 5862-5872.

94) Necula, M.; Kuret, J. "Electron microscopy as a quantitative method for investigating tau fibrillization." *Anal. Biochem.* **2004**, *329*, 238-246.

95) Chirita, C.N. "Anionic micelles and vesicles induce tau fibrillization *in vitro*." *J. Biol. Chem.* **2003**, *278*, 25644-25650.

96) Mena, R. "Monitoring pathological assembly of tau and beta amyloid proteins in Alzheimer's disease." *Acta Neuropathol.* **1995**, *89*, 50-56.

97) Caprathe, B.W. "Method of imaging amyloid deposits." *U.S. Patent 6,001,331.* **1999**

98) Cohen, T. "Inhibition of amyloid fibril formation and cytotoxicity by hydroxyindole derivatives." *Biochemistry* **2006**, *45*, 4727-4735.

99) Jensen, J.R.; Cisek, K.; Honson, N.S.; Kuret, J. "Ligand polarizability contributes to tau fibril binding affinity." *Bioorg. Med. Chem.* **2011**, *19*, 5147-5154.

100) Eisenburg, D.; Nelson, R.; Sawaya, M.R.; Balbirnie, M.; Madsen, A.; Riekel, C.; Sambashivan, S.; Liu, Y.; Gingery, M.; Grothe, R. *FEBS J.* **2005**, *272*, 78.

101) Jensen, J. R.; Cisek, K.; Funk, K.E.; Nphade, S.; Schafer, K.N.; Kuret, J. "Research towards tau imaging." *Journal of Alzheimer's Disease* **2011**, *26*, 147-157.

102) Klunk, W.E.; Wang, Y.; HCuang, G.F.; Debnath, M.L.; Holt, D.P.; Mathis, C.A. "Uncharged thioflavin-T derivatives bind to amyloid-beta protein with high affinity and readily enter the brain." *Life Sci.* **2001**, *69*, 1471-1484.

103) Okamura, N.; Suemoto, T.; Shimadzu, H.; Suzuki, M.; Shiomitsu, T.; Akatsu, H.; C.; Yamamoto, T.; Staufenbiel M.; Yanai, K.; Arai, H.; Kudo, Y.; Sawada, T.

"Styrylbenzoxazole derivatives for in vivo imaging of amyloid plaques in the brain." *J. Neurosci*. **2004**, *24*, 2535-2541.

104) Ikonomovic, M.D.; Abrahamson, E.E.; Isanski, B.A.; Debnath, M.L.; Mathis, C.A.; Dekosky, S.T.; Klunk, W.E. "X-34 labeling of abnormal protein aggregates during the progression of Alzheimer's disease." *Methods Enzymol*. **2006**, *412*, 123-144.

105) Styren S.D.; Hamilton, R.L.; Styren, G.C.; Klunk, W.E. "X-34, a fluorescent derivative of Congo red: a novel histochemical stain for Alzheimer's disease pathology." *J. Histochem Cytochem.* **2000**, *48*, 1223-1232.

106) Yang, L.; Sugama, S.; Mischak, R.P.; Kiaei, M.; Bizat, N.; Brouillet, E.; Joh, T.H.; Beal, M.F. "A novel systemically active caspase inhibitor attenuates the toxicities of MPTP, malonate, and 3NP in vivo" *Neurobiol. Dis*. **2004**, *17*, 250-259.

107) Southerland, B.; Kulkami-Datar, K.; Keoni, C.; Bricker, R.; Grunwald, Jr., W.C.; Ketcha, D.M.; Hern, E.; Cool D.R.; Brown, T.L. "Q-VD-OPh, a negative control for o-phenoxy-conjugated caspase inhibitors" *J. Cell Death* **2010**, *3*, 33-40.

108) Loeser, R.; Abbenante, G.; Madala, P.K.; Halili, M.; Le, G.T.; Fairlie, D.P. "Noncovalent tripeptidyl benzyl- and cyclohexyl-amine inhibitors of the cysteine protease caspase-1" *J. Med. Chem*. **2010**, *53*, 2651-2655.

109) Chen, L.R.; Wang, Y.C.; Lin, Y.W.; Chou, S.Y.; Chen, S.F.; Liu, L.T.; Wu, Y.T.; Kuo, C.J.; Chen, T.S.; Juang, S.H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3058-3062.

110) Garden, S.J.; Torres, J.C.; Da Silva, L.E.; Pinto, S.C. “A convenient methodology for the N-alkylation of isatin compounds” *Synth. Commun*. **1998**, *28*, 1679-1689.

111) Kadam, A.; Zhang, Z.; Zhang, W. “Microwave-assisted fluorous multicomponent reactions. A combinatorial chemistry approach for green organic synthesis” *Curr. Org. Synth.* **2011**, *8*, 295-309.

112) Alves, R.B.; Freitas, R.P.; Ventura, C.P.; Santos, L.J. “Microwave-assisted classic reactions in organic chemistry” *Rev. Virt. Quim.* **2010**, *2*, 214-224.

113) Bariwal J.B.; Trivedi, J.C.; Van der Eycken, E.V.; “Microwave irradiation and multicomponent reactions” *Top. Heterocycl. Chem.* **2010**, *25*, 169-230.

114) Schmidt, M.S.; Reverdito, A.M.; Kremenchuzky, L.; Perillo, I.A.; Blanco, M.M. “Simple and efficient microwave assisted *N*-alkylation of isatin” *Molecules*. **2008**, *13*, 831-840.

115) Wee, X. K.; Yeo, W. K.; Zhang, B.; Tan. V. B. C.; Lim, K. M.; Tay, T. E.; Go, M.-L. *Bioorg. Med. Chem*. **2009**, *17*, 7562-7571.

116) Shuttleworth, S. J.; Nasturica, D.; Gervais, C.; Siddiqui, M. A.; Rando, R. F.; Lee, N. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2501-2504.

117) For a review of KF/alumina see: Yamawaki, J.; Ando, T. “Potassium fluoride on inorganic solid supports. A search for further efficient reagents promoting hydrogen-bond-assisted alkylations” *Chem. Lett.* **1979**, *7*, 755-758.

118) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3527-3530.

119) Clay, C. M., Abdallah, H. M.; Jordan, C.; Knisley, K.; Ketcha, D. M. *ARKIVOC* **2012** (vi) 317-325.

120) Charles M. Clay, "Synthesis of Isatin Derivatives Used for the Inhibition of Pro-Apoptotic Jurkat T Cells", WSU Master's Thesis 2011

121) L. Wolff, *Ann.*, **1912**, *86*, 394.

122) Todd, D. The Wolff-Kishner Reduction, in "Org. Reactions"; John Wiley and sons, inc., New York, 1948; 378-422.

123) Soriano, D. S. *J. Chem. Educ.* **1993**, *70*, 332.

124) Parquet, E.; Lin, Q. *J. Chem. Educ.* **1997**, *10*, 1225.

125) Crestini, C.; Saladino, R.; *Synthetic Communications* **1994**, *24*, 2835.

126) Paul J. Repasky, "Novel Trisubstituted Arylidene Oxindoles with Potent Anti-Apoptotic Properties", WSU Master's Thesis 2011.

127) Villemin, D.; Martin, B. *Synthetic Communications* **1998**, *28*, 3201.

128) Huang, A.; Kodanko, J.J.; Overman, L.E. *J.Am.Chem. Soc.* **2004**, *126*, 14043-14053.

129) Trost, B.M.; Zhang, Y. *Chem. Eur. J.* **2011**, *17*, 2916.

130) Kolb, H.C.; Finn, M. G.; Sharpless, K.B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

131) Kolb, H.C.; Sharpless, K.B. *Drug Discovery Today* **2003**, *8*, 1128.

132) Fan, W.Q.; Katritzky, A.R. *Heterocyclic Chemistry* **1996**, *4*, 101.

133) Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.

134) Appukkuttan, P.; Dehaen, W.; Fokin, V.V.; Van der Eycken, E. *Organic Letters* **2004**, *6*, 4223.

135) Jiang, Y.; Hansen, T.V. *Bioorg. & Med. Chem. Lett.* **2011**, *21*, 1626-1629.

136) Coda, A.C.; Invernizzi, A.G.; Righetti, P.P.; Tacconi, G.; Gatti, G. "(Z)- and (E)-Arylidene-1,3-dihydroindol-2-ones: configuration, conformation, and infrared carbonyl stretching frequencies" *J. Chem. Soc. Perkin Trans. II* **1984**, *4*, 615-619.

137) Singh, R.J.; Majumder,U.; Shreeve, J. M. J. Org. Chem. **2001**, 6263-6267.

138) Elsinghorst P.W.; Gutschow, M.; "Synthesis of ^{2}H and ^{13}C-labelled sunitinib and its primary metabolite." *J. Label Compd. Radiopharm* **2009**, *52*, 360–365.

139) Zhang, W.; Gox, M. L.; "Functionalized 3-benzylidene-indolin-2-ones: Inducers of NAD(P)H-quinoneoxidoreductase 1 (NQO1) with antiproliferative activity" *Bioorg. & Med. Chem. Lett.* **2009**, *17*, 2077-2090.

140) May, E.L.; Mosettig, E.; "Attempts to find new antimalarials. XVI.'.2 Amino Alcohols of the type -CHOHC&NR_2 derived from 3-Chloro-9-Acetylphenanthrene." *Journal of Organic Chemistry* **1946**, *11*, 435-440.

141) Hu, Y. "Facile synthesis of 3-arylidene-1,3-dihydroindol-2-ones catalyzed by Bronsted acidic ionic liquids." *Heterocyclic Communications*, **2008**, *14*, 263.

142) Zhang, W. "Functionalized 3-benzylidene-indolin-2-ones: Inducers of NAD(P)H-quinone oxidoreductase 1 (NQO1) with antiproliferative activity." *Bioorg. & Med.Chem.* **2009**, *17*, 2077.

143) Hagar M. Abdullah, "Small Molecule Caspase Inhibitors Using Isatin and Oxindole Scaffolds and a Combinatorial Approach", WSU Master's Thesis 2010.

144) Vine, K.L.; Locke, J.M.; Ranson, M.; Pyne, S. G.; Bremner, J.B. "An Investigation into the Cytotoxicity and Mode of Action of Some Novel *N*-Alkyl-Substituted Isatins." *J. Med. Chem.* **2007**, *50*, 5109-5117.

145) Wu, W.; Zheng, T.; Cao, S.; Xiao, Z. "5-Chloro-1-(4-methoxybenzyl)indoline-2,3-dione." *Acta Crystallographica Section E.*, **2011**, *64*, 246.

146) Wu, W.; Zheng, T.; Cao, S.; Xiao, Z. "5-Fluoro-1-(4-methoxybenzyl)indoline-2,3-dione." *Acta Crystallographica Section E.*, **2011**, *67*, 1834.

147) Goehring, R. R.; Sachdeva, Y. P.; Pisipati, J. S.; Sleevi, M. C.; Wolfe, J. F. "Photoinduced cyclizations of mono- and dianions of *N*-acyl-o-chloroanilines and *N*-acyl-o-chlorobenzylamines as general methods for the synthesis of oxindoles and 1,4-dihydro-3(2H)-isoquinolinones." *Journal of the American Chemical Society*, **1985**, *107*, 435-443.

148) Du, Ding; Hu, Zhongyuan; Jin, Jianlin; Lu, Yingyan; Tang, Weifang; Wang, Bo; Lu, Tao. "N-Heterocyclic Carbene-Catalyzed Three-Component Domino Reaction of Alkynyl Aldehydes with Oxindoles." *Organic Letters* **2012**, *14(5)*, 1274-1277.

149) Kolb, H. C.; Walsh, J. C.; Zhang, W.; Gangadharmath, U. B.; Kasi, D.; Chen, K.; Sinha, A.; Wang, E.; Chen, G.; Mocharla, V. P.; PCT Int. Appl. (2010), WO 2010011964 A2 20100128.

150) Singh, P.; Sharma, P.; Amit Anand, A.; Bedi, P.M.S.; Tandeep Kaur, T.; Saxena, A.K.; Kumar, V.; *Eur. J. Med. Chem.* **2012**, *55*, 455-461.

151) Ankati, H.; Akubathini, S. K.; Kamila, S.; Mukherjee, C.; D'Mello, S. R.; Biehl, E. R. "Synthesis of 3-benzylidene, 5-substituted 3-benzylidene, 3-

heteroarylmethylene and 5-substituted heteroarylmethylene derivatives of indolin-2-ones" *Open Organic Chemistry Journal*, **2009**, *3*, 1-10.