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Synthesis of Isatin Derivatives Used for the Inhibition of Pro-Apoptotic Jurkat T Cells

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

By

**CHARLES MICHAEL CLAY
B.S., Wright State University, 2009**

2011

Wright State University

WRIGHT STATE UNIVERSITY
SCHOOL OF GRADUATE STUDIES

July 29, 2011

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Charles Michael Clay ENTITLED Synthesis of Isatin Derivatives Used for the Inhibition of Pro-Apoptotic Jurkat T Cells BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Clay, Charles Michael. M.S., Department of Chemistry, Wright State University, 2011.
Synthesis of Isatin Derivatives Used for the Inhibition of Pro-Apoptotic Jurkat T Cells.

A range of substituted 1H-indole-2,3-diones (isatins) was synthesized to assess their capability to inhibit caspases and prevent apoptosis in Jurkat T cells. The key steps in the synthesis of such molecules involved electrophilic substitution of the C-5 position of the isatin nucleus (if necessary), *N*-alkylation, Wolff-Kishner reduction of the C-3 carbonyl group and finally, Knoevenagel condensation. The design and synthesis of such potential inhibitors was guided by SAR studies of peptide based inhibitors such as “Q-VD-O-Ph”, as well as small-molecule inhibitors based upon the isatins scaffold. Previously, 3-(2,6-difluorobenzylidene)-5-nitroindolin-2-one has been shown to inhibit apoptosis in human Jurkat T cells at 5 μ M activity. Herein, it is shown that by increasing the functionality of such oxindole derived inhibitors from two points of variability (e.g., 1-(2,6-difluorobenzyl)-3-((pyridin-4-yl)methylene)indolin-2-one), to three points of variability by adding an electron-withdrawing group such as a chlorine atom at the C-5 position, the potency of the molecules against apoptosis was approximately increased up to 2-fold. Several other 3-substituted benzylidene derivatives were tested against human Jurkat T cells and were found to be active at micromolar concentrations.

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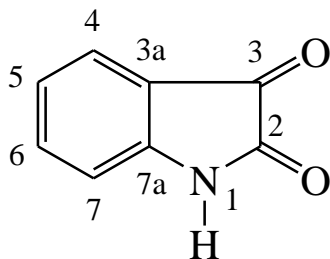
DEDICATION

I would like to dedicate this thesis to my two children Charles and Synovia, and also to my loving family, especially my mother who has worked extremely hard to make sure that all of her six kids are successful in life.

Introduction

Isatin (1-H-indole-2,3-dione, **Figure 1**) and derivatives possess a broad range of biological and pharmacological properties and are widely used as starting materials for the synthesis of a broad range of heterocyclic compounds and as substrates for drug synthesis.

Figure 1

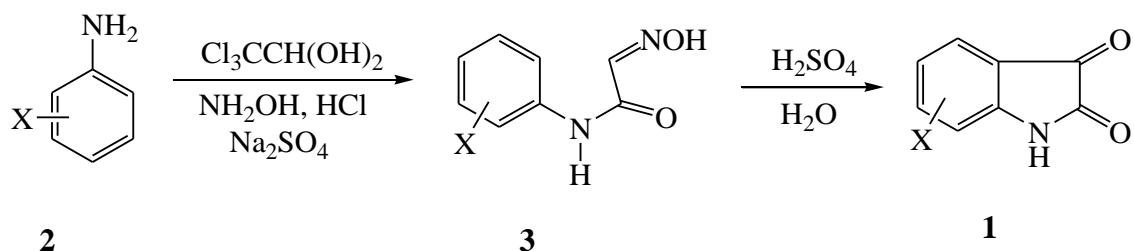


Formerly, the study of isatin derivatives was connected with dye synthesis, but more recently these heterocycles have been shown to demonstrate antiprotozoal, antibacterial, antifungal, antiviral, anti-HIV, anticonvulsant, antitumoral, anti-inflammatory, and antihelminthic activities; influence neurodegenerative diseases; participate in metabolism; acetylcholinesterase inhibitors; and stimulate the growth of plants.¹ Drugs containing the isatin skeleton are used to treat diseases such as epilepsy², tuberculosis³, and bulimia⁴. Therefore the need to create novel isatin derivatives for emerging drug targets is an active area of medicinal chemistry.

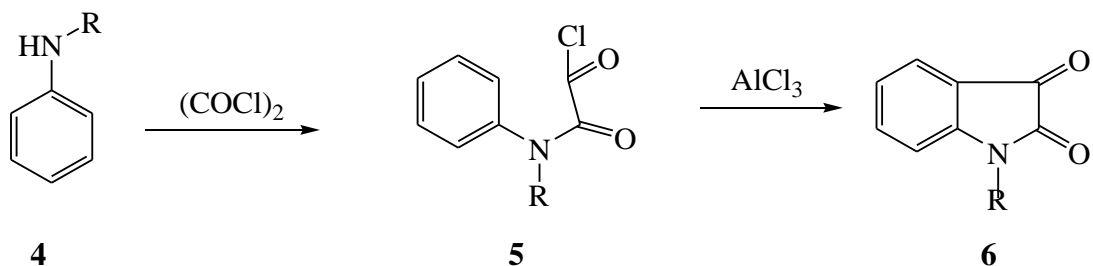
Synthesis of Isatin

The first method reported for the synthesis of isatin was developed by Sandmeyer, wherein the reaction of an aniline **2** with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate forms an isonitrosoacetanilide **3**. Subsequent cyclization to the isatin **1** is accomplished after treating **3** with concentrated sulfuric acid.⁵ The method also applies well to anilines with electron-withdrawing groups, such as 2-fluoroaniline.⁶ Despite its efficiency, the

Sandmeyer method is limited by: a) harsh conditions; b) fails if the isonitrosoacetanilides bear electron-donating groups; c) the formation of a mixture of regioisomers; and d) generally moderate yields. However, this latter shortcoming, can be improved under microwave conditions.⁷

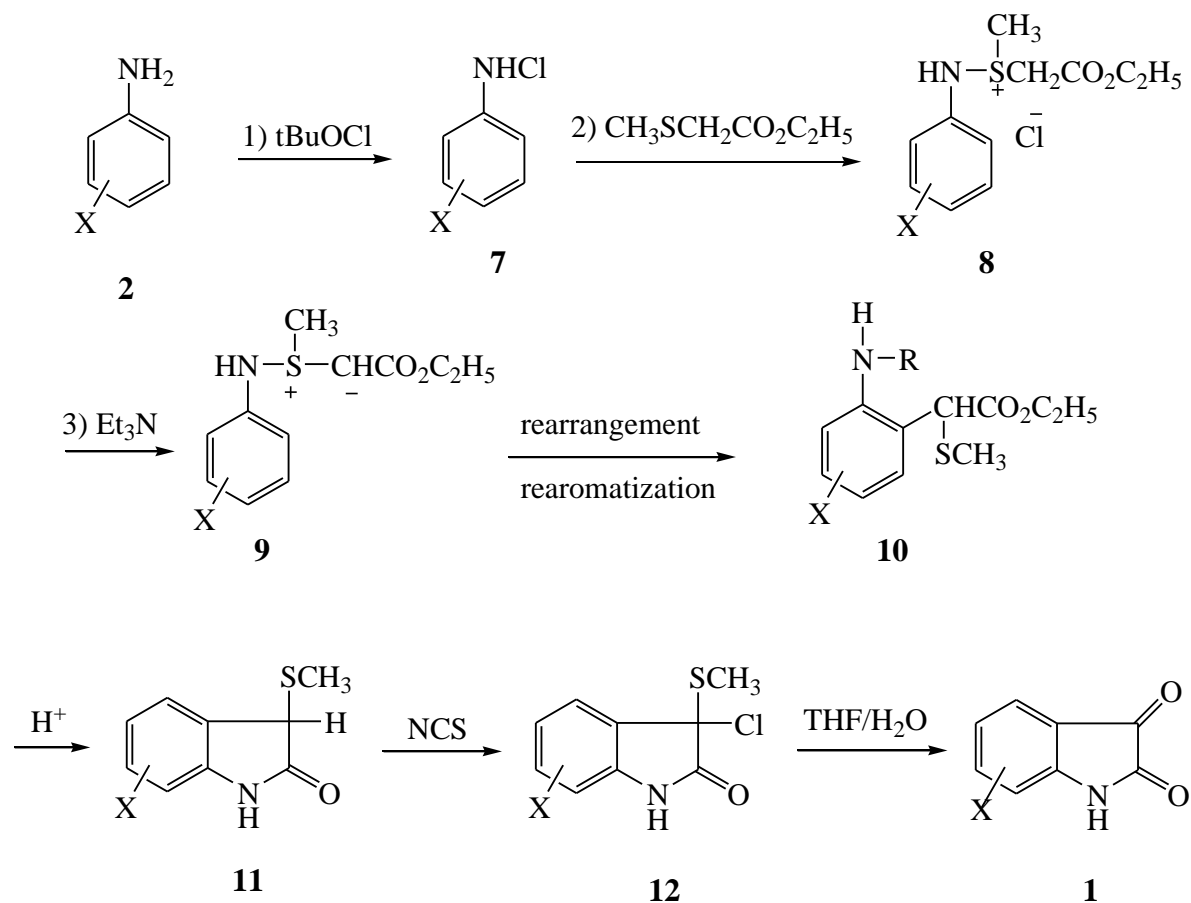


An important alternative to the Sandmeyer method is the method of Stolle. In this approach, N-substituted anilines **4** are reacted with oxalyl chloride to form an intermediate chlorooxalylanilide **5** which can be cyclized in the presence of Lewis acids such as anhydrous aluminum chloride,⁸ $\text{BF}_3 \cdot \text{Et}_2\text{O}$,⁹ or TiCl_4 ¹⁰ to form 1-aryl isatin derivatives or polycyclic isatin derivatives **6**.



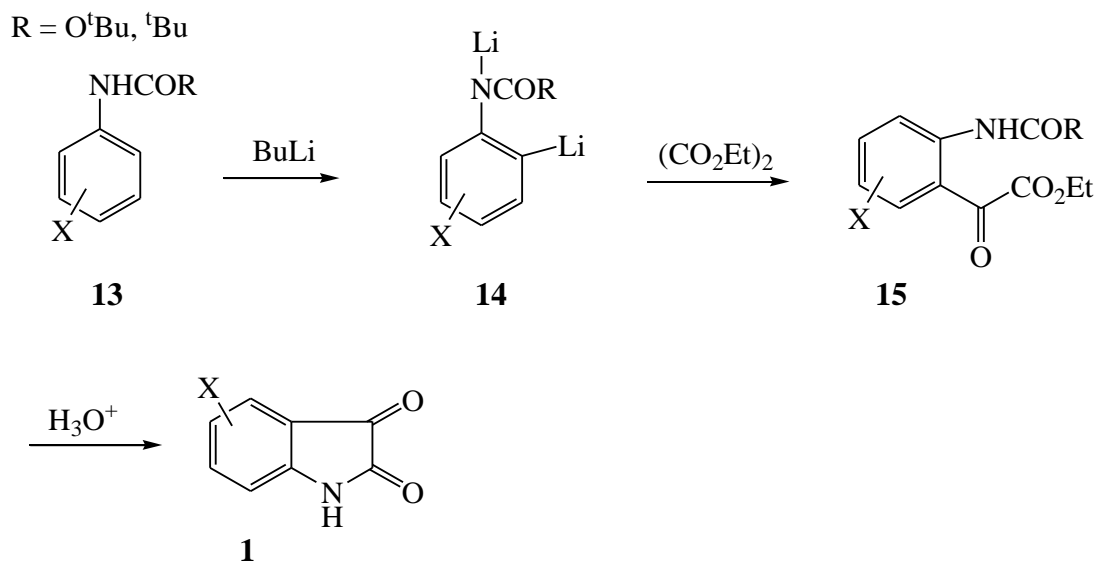
Gassman described two general methods for the synthesis of isatins involving conversion of substituted anilines **2** into 3-methylthiooxindoles **11**, which upon subsequent oxidation give the corresponding substituted isatins **1**. The choice of methodology is dictated by the nature of the electronic effects of the substituents bonded to the aromatic ring. When strong electron

withdrawing groups are present, the oxindole derivative can be synthesized via the *N*-chloroaniline intermediate **7** obtained by treating the aniline with *tert*-butyl hypochlorite at low temperatures. The resulting *N*-halo species, further reacts with a methylthioacetate ester to give the azasulfonium salts **8**.^{11,12} Treatment of **8** with base generates the ylides **9** which undergo a Sommelet-Hauser rearrangement to give **10** after rearomatization of the intermediate cyclohexadienone imines. Treatment of **10** with dilute acid rapidly gives the 3-methylthio-2-oxindole intermediates **11**. The chlorination of **11** is then accomplished by treating the 3-methylthio-2-oxindole with *N*-chlorosuccinimide (NCS) to give the 3-chloro derivatives **12**, which upon refluxing in aqueous tetrahydrofuran provide the desired isatins **1**. In the case of electron-donating groups that tend to destabilize the *N*-chloro intermediates (leading to diminished yields of the azasulfonium salt), an alternate method employs the reaction of a chlorosulfonium salt (formed by treating the methylthioacetate ester with chlorine), with an appropriate aniline to provide better yields of 3-methylthio-2-oxindoles.¹¹



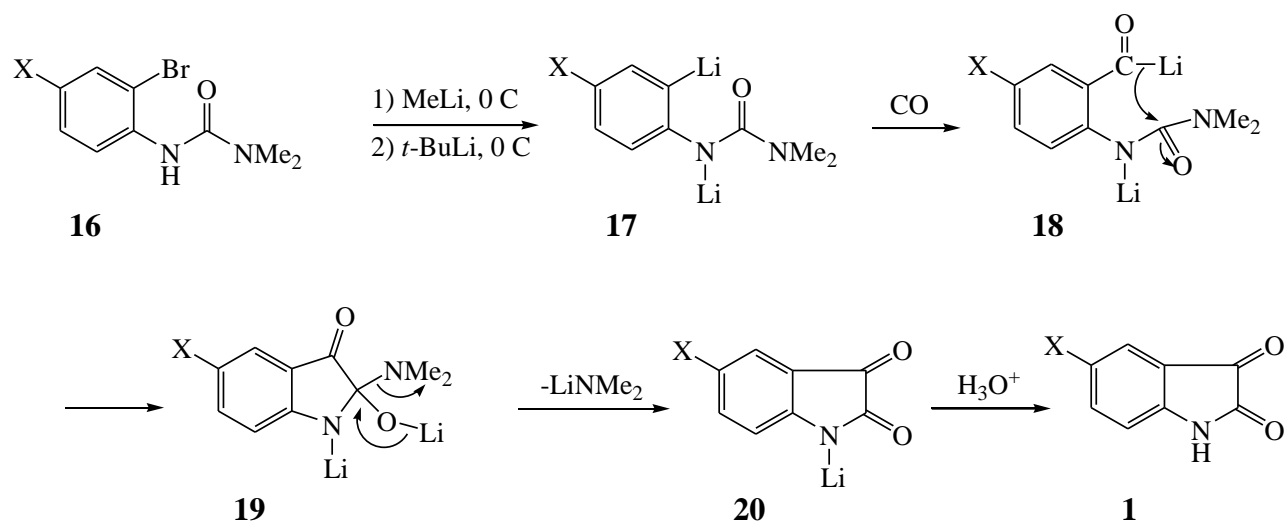
However, the Sandmeyer, Stolle, and Gassman methodologies shown above, all suffer from a lack of regioselectivity, especially in the case of *meta*-substituted anilines, wherein a mixture of 4- and 6- substituted isatins are observed. More recently, a new method for the synthesis of isatins was developed by Hewawasam and Meanwell which is insensitive to the electronic nature of substituents bound to the aromatic ring and is characterized by predictable regiochemical control.¹³ Since amino-protecting groups can direct metalation to the *ortho*-position of anilines, *N*-pivaloylanilines and *N*-(*tert*-butoxycarbonyl)anilines **13** were used to generate dianions **14** using an excess of a variety of butyllithium reagents (e.g., *n*-BuLi, *s*-BuLi, *t*-BuLi) in THF at -78°C . The resulting dianions **14** were then treated with ethyl oxalate and the isatins **1** were obtained after deprotection and cyclisation of the intermediate α -ketoesters **15**

using HCl.¹³ This method has the advantage of being regioselective for the synthesis of 4-substituted isatins from *meta*-substituted anilines where the substituent is a metalation directing group such as an amino protected group.

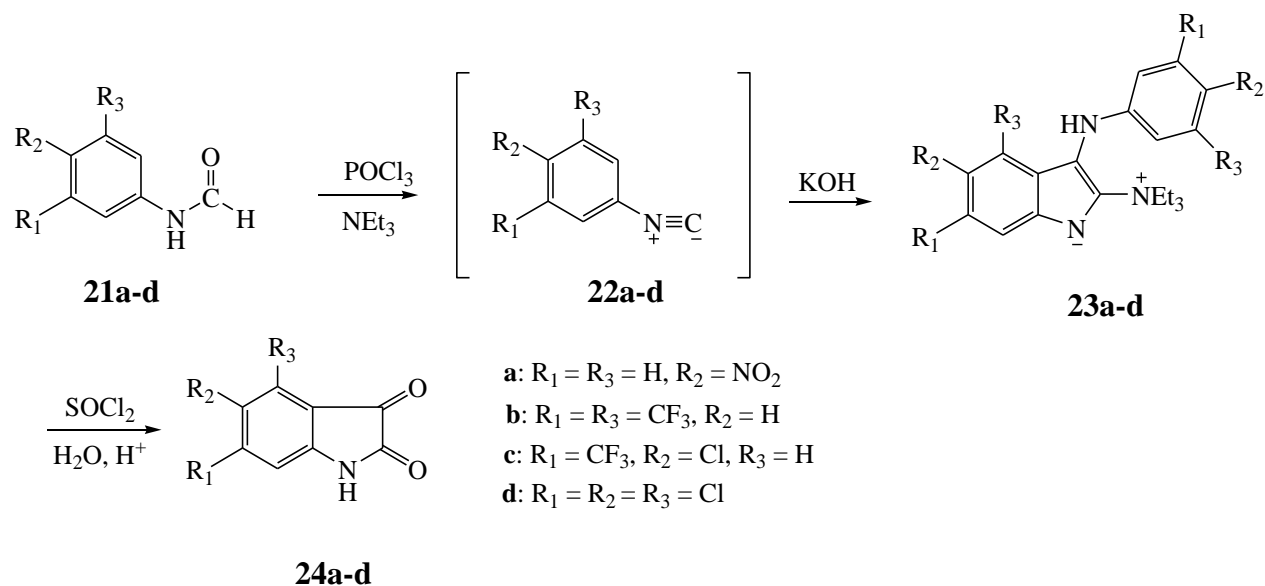


Smith at the University of Wales Swansea, used a metal-halogen exchange method for the synthesis of isatins and substituted isatins by lithiation of *ortho*-bromophenylureas, carbonylation and subsequent intramolecular cyclisation to give the desired products. The *N*⁷-2-(bromophenyl)-*N,N*-dimethylurea **16** underwent lithiation on the nitrogen to form a monolithio intermediate using MeLi, followed by bromine-lithium exchange using *t*-BuLi to give the dilithio species **17**.¹⁴ The intermediate **17** was then exposed to carbon monoxide to give **18**, which after cyclization forms the intermediate **19**, followed by loss of LiNMe₂ to give **20**, and finally after work up with dilute acid yielded the isatin product

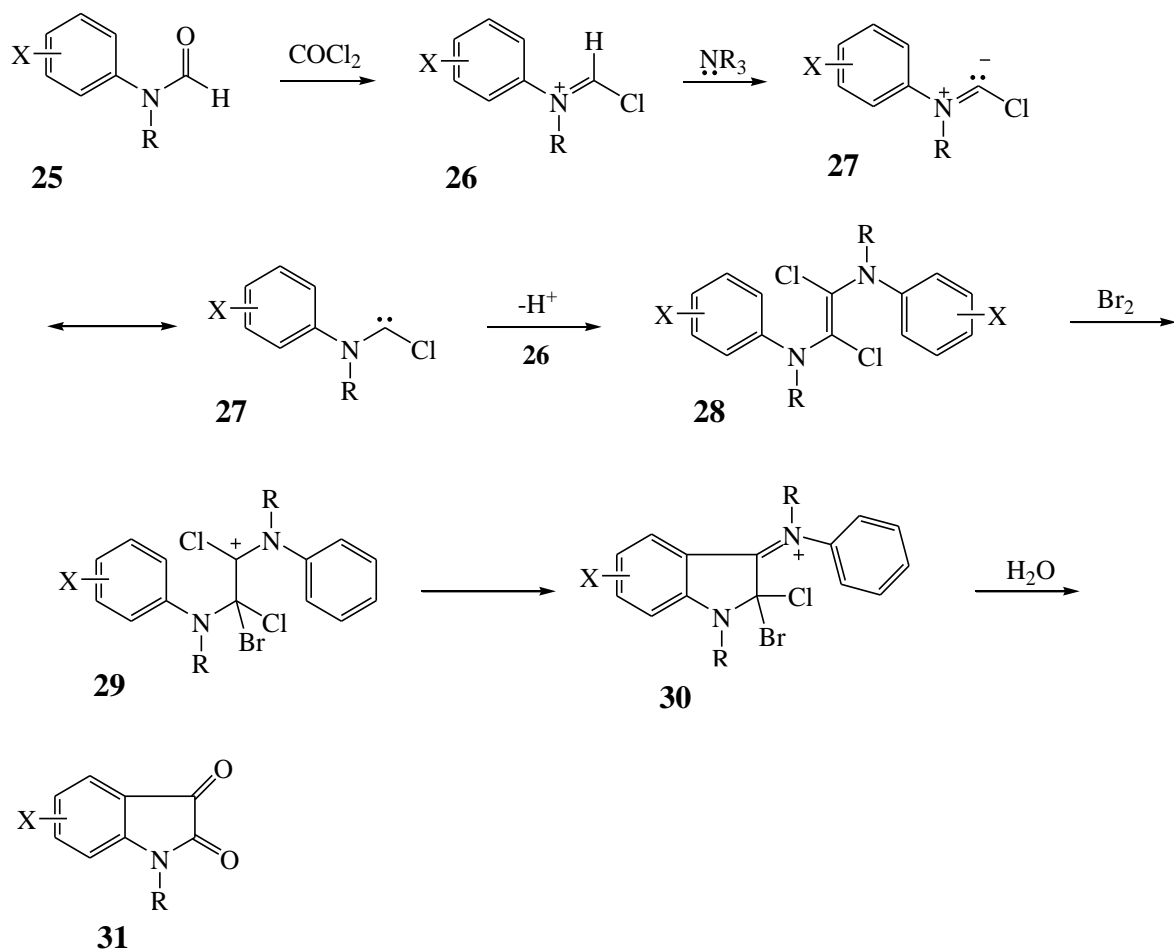
1.



A rather versatile and novel two step synthesis of isatins was presented by Mironov in 2001 and allowed for the preparation of isatins containing electron withdrawing groups such as -CF₃, -NO₂, and -Cl. The method is based on the reaction between aromatic isocyanides and tertiary amines,¹⁵ where in the first step, 2-triethylammonio-3-arylaminoindolates **23** were obtained from the corresponding aromatic formamides **21** without isolation of the intermediate isocyanides **22**.¹⁶ Heating the 2-triethylammonio-3-arylaminoindolates **23** in excess thionyl chloride followed by hydrolysis led to the target isatins **24**.¹⁶



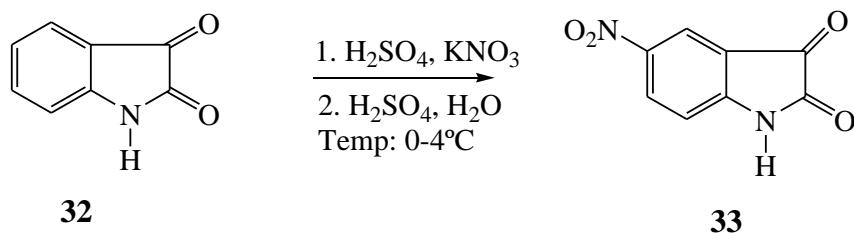
Another route to isatins which also employs *N*-substituted formanilides was developed by O. Meth-Cohn and S. Goon and involves deprotonation of a Vilsmeier reagent or substituted chloroiminium ion, followed by dimerisation of the carbene formed upon treatment with base, and finally electrophilic cyclisation of the dimer by bromonium ion action.¹⁷ This route to isatins is said to be general for various ring substituents including electron-withdrawing groups such as the nitro group. Formation of isatin in a one pot synthesis takes place by reacting the *N*-substituted formanilides **25** with oxalyl chloride in THF to give the Vilsmeier reagent **26**, followed by treatment with Hünig's base to form a nucleophilic carbene **27**. The unstable arylamino-dimers **28** have a limited shelf-life and react with electrophiles such as bromine to give intermediates such as **29**, which upon cyclisation to **30**, and hydrolysis yield the desired isatins **31**.



Electrophilic Aromatic Substitution of Isatin

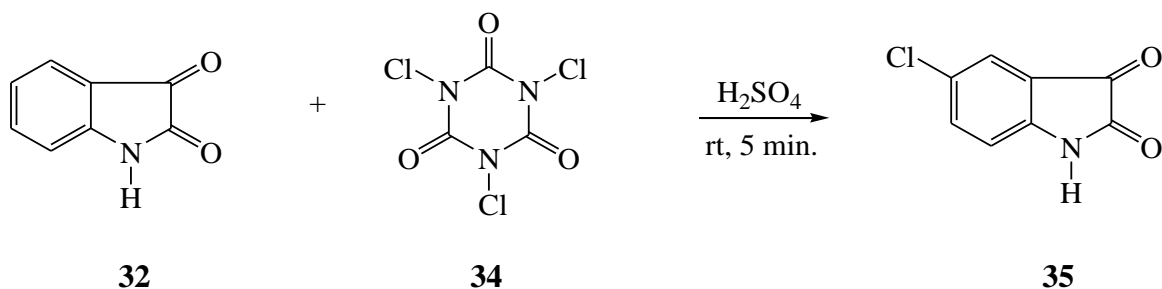
Nitration of Isatin

In 1925, Calvery reported the nitration of isatin at the C-5 position using fuming nitric acid in concentrated sulfuric acid.¹⁸ However, a more convenient method to synthesize 5-nitroisatin (**33**) involves the dropwise addition of a solution of isatin (**32**) in sulfuric acid to a solution of potassium nitrate dissolved in concentrated sulfuric acid at 0-4°C. The isatin so obtained was isolated, as bright yellow/orange crystals with a melting point of 252-254 °C.¹⁹

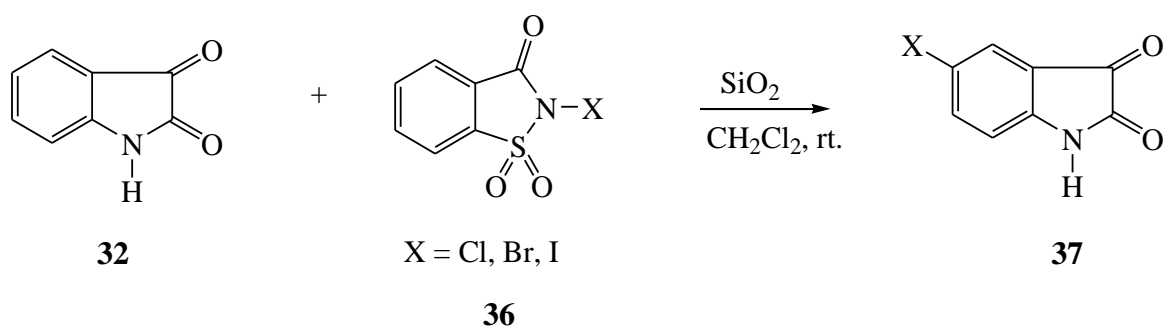


Halogenation of Isatin

The chlorination of isatin at the C-5 position has been described using *N*-chloroamides, *N*-chloroimides and also using *N*-chlorosaccharins in a heterogenous medium ($\text{SiO}_2/\text{CH}_2\text{Cl}_2$).²⁰ Furthermore G. F. Mendoca reported using the relatively stable reagent trichloroisocyanuric acid (TICA) (**34**), as an efficient new source of electrophilic chlorine and this provides a relatively inexpensive route to the chlorination of isatins (**32**).²¹ Using this protocol, the 5-chloroisatin (**35**) was isolated in 72% yield in less than 5 minutes and no *N*-chlorination of isatin or frequently formed isomeric 7-chloroisatin were detected in the crude reaction product by HRGC (high-resolution gas chromatography). It is believed that the strongly acid medium promotes the formation of a superelectrophilic species wherein TICA being either polyprotonated or protosolvated causes the “Cl⁺” transfer to isatin more efficiently due to the charge-charge repulsion relief.



Mono-halogenation (-Cl, -I, -Br) of isatin (**32**) can be achieved by reacting *N*-halosaccharins **36** with isatin in the presence of SiO₂ at r.t to specifically produce the 5-halo derivatives **37** as reported by de Silva and de Mattos.²⁰ This method is an alternative to the use of highly toxic and corrosive Cl₂ and Br₂, which can lead to other products such as 5,7-dibromo-3,3-dialkoxyoxindole when the bromination of isatin is attempted in alcoholic media.²²



***N*-Alkylation of Isatin**

Various methods have been used for the preparation of *N*-alkylated isatins which can be successfully achieved under basic conditions using alkyl chlorides, bromides, and iodides; as well as reactive allyl-, benzyl-, and propargyl halides. Conventional heating is often employed to produce the *N*-alkylated isatins from temperatures of from 40-100°C under reflux. In general, the *N*-alkylation of isatin proceeds by reacting the isatin (**32**) substrate with any variety of base and solvent combination shown in **Table 1**, to generate the salt of the isatin **38** which subsequently

reacts with an alkyl halide to produce an *N*-alkylated isatin **6**.

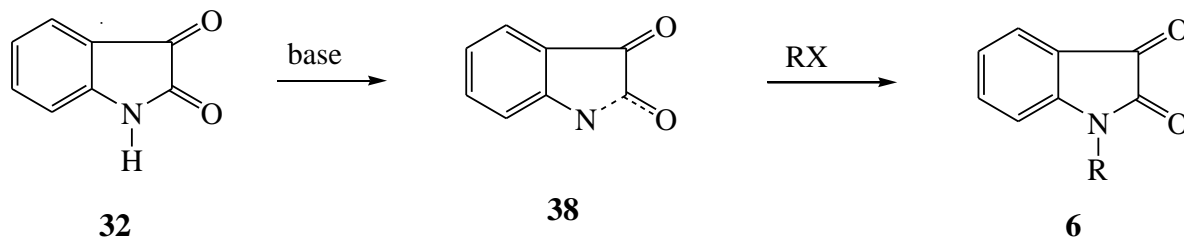
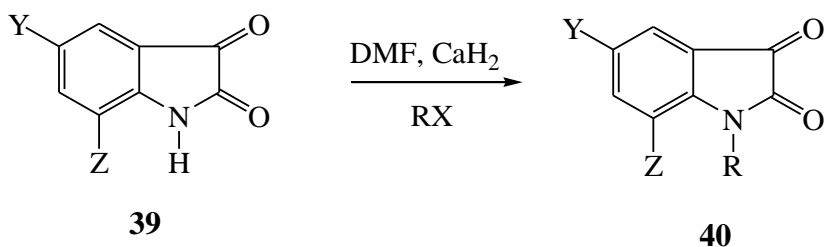


Table 1: Reported Solvents and Bases used for the N-alkylation of Isatin^{28,29,35,38,39}

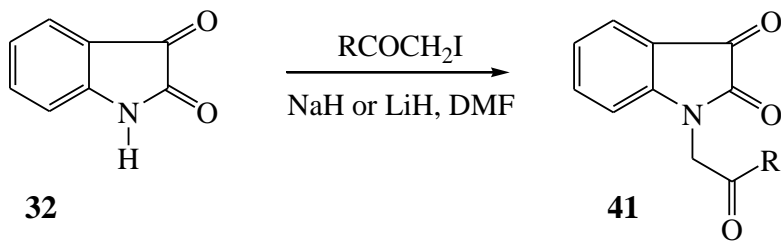
Base	Solvent
Na ₂ CO ₃ , K ₂ CO ₃ , Cs ₂ CO ₃ , LiH, NaH, CaH ₂ , TEA, LiOH, NMM, NaOEt,	DMF, DMA, HMPT, MeCN, DMSO, NMP, EtOH, MeOH, Me ₂ CO

Very few 7-substituted derivatives have been reported, however a method for the N-alkylation of isatins introduced by Garden involves the use of calcium hydride as base in *N,N*-dimethylformamide (DMF) at 40-50°C for the substituted isatin **39** to produce the 7-substituted-*N*-alkylisatins **40** that have electron withdrawing groups bonded to the aromatic nucleus. The isatin derivatives with electron-withdrawing groups bonded to the aromatic nucleus, at the C-5 and C-7 positions, reacted more readily with CaH₂ at room temperature than did isatin, revealing the increased acidity of the amide proton. The use of CaH₂ is beneficial because of the ease with

which it can be handled and exposed to the atmosphere, especially in humid climates, in comparison to NaH.²³



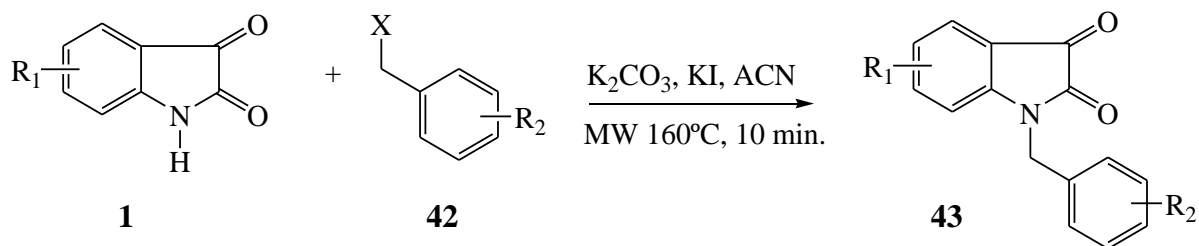
Sodium hydride is a very strong base which is most often used to perform the *N*-alkylation of isatins using alkyl halides.^{24,25,26,27} Additionally, it has been reported that the direct alkylation of isatin (**32**) by halomethyl ketones was achieved using sodium hydride or lithium hydride in anhydrous DMF at temperatures of from -15°C to ambient conditions to form the *N*-acetylisatins (R= Me) or *N*-phenacylisatins (R= Ph) **41**.²⁸



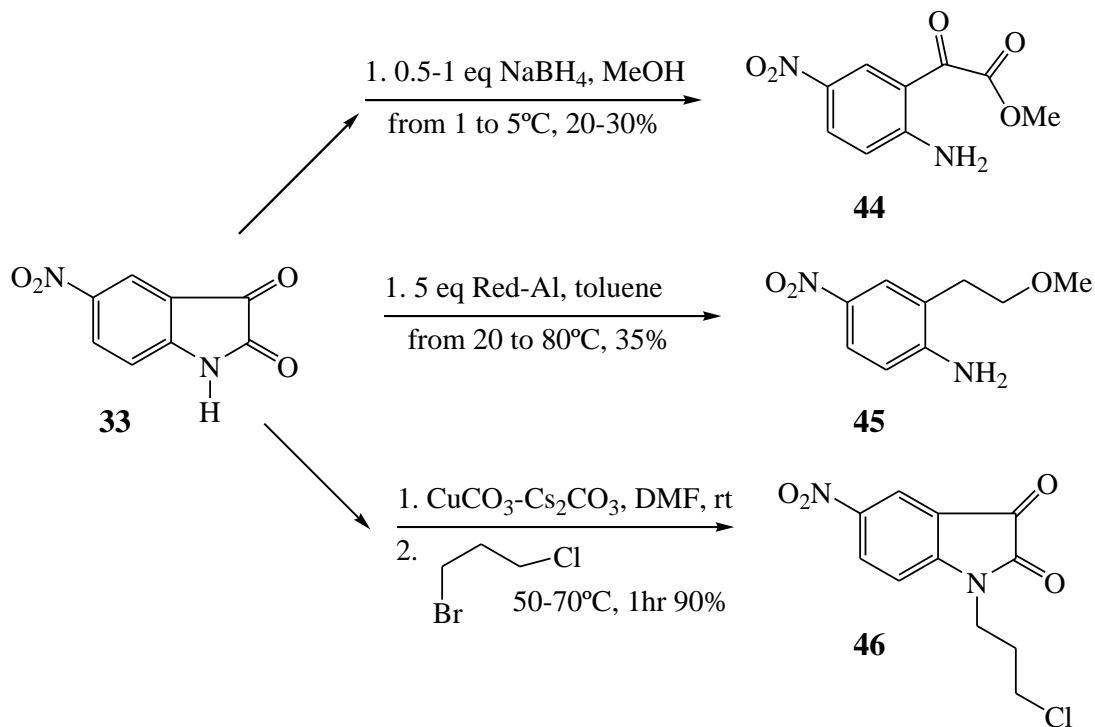
Although conventional heating is used to generate *N*-alkylated products on a large scale, it also presents some drawbacks including: the general base lability of the isatin nucleus; the use of hazardous reagents such as metal hydrides requiring anhydrous solvents; the use of high boiling aprotic organic solvents leading to complex workups; the use of carcinogenic solvents in some cases, and side reactions due to the presence of keto-carbonyls (i.e., reductions when metallic

hydrides are used, aldolisation when K_2CO_3 in acetone is employed).²⁹ In addition some conventional reactions can be lengthy, involving reaction times of hours or days with consequential formation of unwanted by-products. Thus, microwave (MW) irradiation has gained popularity in the last few decades as an alternative energy source, and it is especially utilized because it accelerates a wide variety of reactions and minimizes thermal decomposition of the products. Several reviews on the advantages of applying MW irradiation to organic synthesis have been published which involve multi-component reactions,³⁰ classic organic reactions,³¹ and green organic synthesis.³² More recently, the microwave promoted *N*-alkylation of isatins was done using various reaction conditions, however the best results were obtained using K_2CO_3 or Cs_2CO_3 and a few drops of DMF or *N*-methyl-2-pyrrolidinone (NMP).²⁹

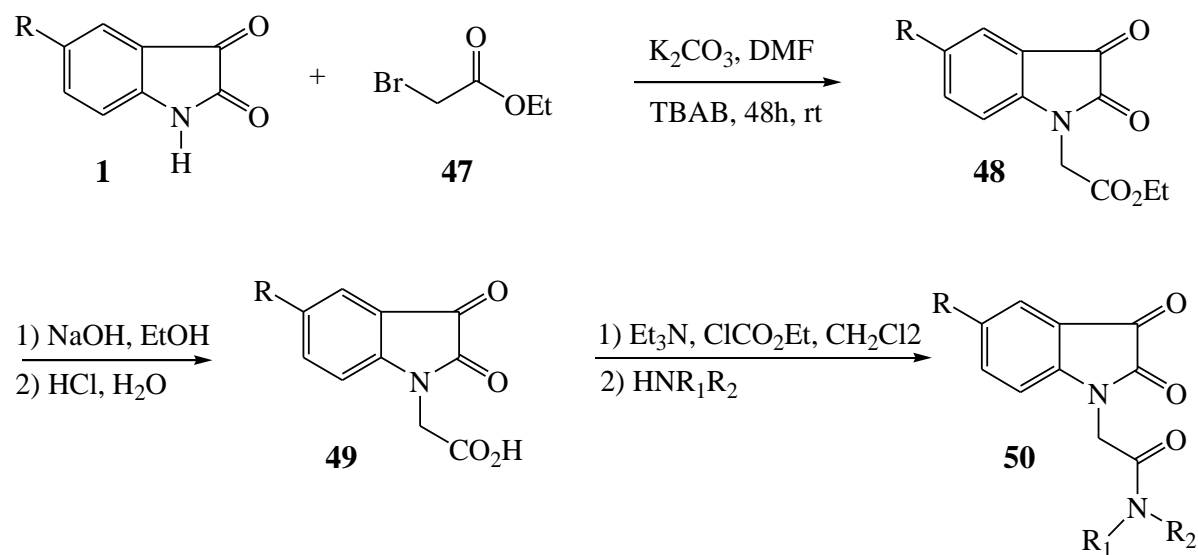
The use KI as a catalyst for the *N*-alkylation of isatins was first reported by Torres³³ followed by Vine.³⁴ In a study describing the first positive allosteric modulators of muscarinic acetylcholine receptor subtype 5, Lindsey³⁵ developed a parallel microwave procedure for the preparation of a variety of *N*-benzyl isatins **43** from substituted isatins **1** and a variety of benzyl halides **42** (2.5 equiv.) by employing K_2CO_3 (2.0 equiv.) and KI (0.1 equiv.) as a catalyst in acetonitrile (ACN) (3 mL). Next, the reactions were partitioned into CH_2Cl_2 and H_2O and then passed through disposable phase separator columns (Biotage Isolute) after which the organics were concentrated on a heated air-drying block and then analyzed by LCMS.



Interestingly, Torisawa found that attempts at reducing 5-nitro isatin (**33**) by Red-Al (5 equiv) or $NaBH_4$ provided ring-opened products **44** and **45**.³⁶ Given the susceptibility of 5-nitroisatin to undergo nucleophilic cleavage of the N1-CO bond under basic conditions, a novel modified protocol was developed for *N*-alkylation of such derivatives employing a mild base combination of $CuCO_3/Cs_2CO_3$ (1:2) in anhydrous DMF to afford **46**. This reagent combination showed increased nucleophilicity at nitrogen by the complexation between Cu and amide nitrogen.³⁶

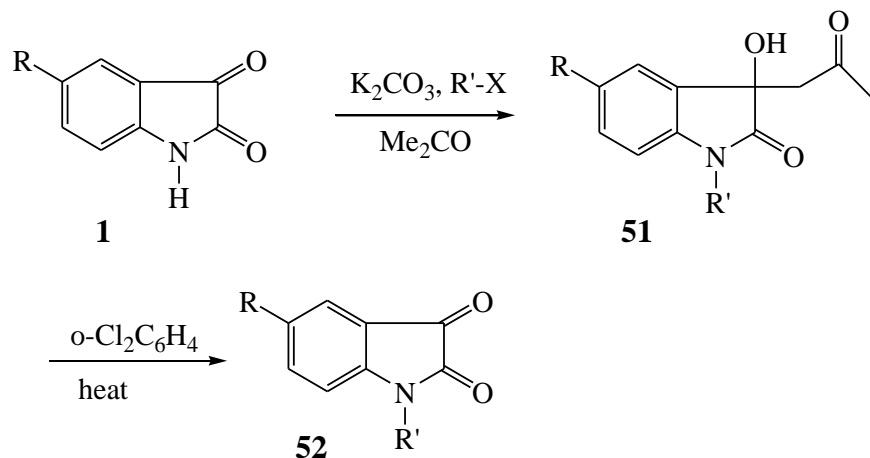


The *N*-carboxymethylation of isatin was performed under basic conditions (1.5 equiv. of K_2CO_3 in DMF), allowing the formation of the conjugate base of isatin followed by alkylation (48h) at room temperature using ethyl bromoacetate **47** (1.5 equiv.) and tetrabutylammonium bromide (TBAB, 0.1 equiv) as the phase transfer catalyst. The ester function of **48** was saponified to the corresponding carboxylic acid **49** using sodium hydroxide (2 equiv.) for 4 h at room temperature in ethanol/water (1/1) followed by neutralization with aqueous HCl. The amides **50** were then prepared by treatment with ethyl chloroformate (1.2 equiv) and triethylamine (2 equiv.) for 30 min. at $-10^\circ C$ followed by reaction with the desired amines (2 equiv.) for 2h.³⁷

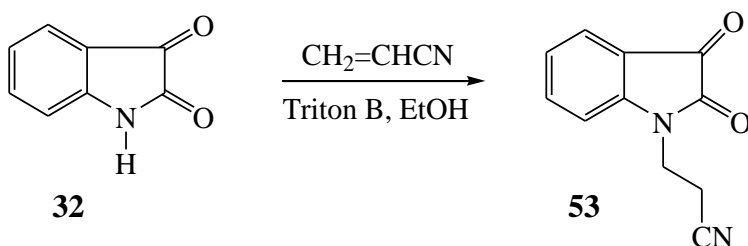


Other novel *N*-alkylation reactions involve the formation of 1-alkylisatins via aldol-retro-aldol condensation reaction. Thus, 5-substituted isatin **1** was refluxed in acetone with alkyl halides in the presence of anhydrous potassium carbonate to give *N*-alkylated aldol product **51**. The *N*-alkylated aldol product was then heated in *o*-dichlorobenzene at $160-165^\circ C$ for dealdolisation to occur, giving the 1-alkylated isatins **52**.³⁸ Thus, this methodology was developed primarily for employing *N*-alkylation reactions using acetone as the solvent and

anhydrous potassium carbonate which consequently forms the undesired aldol product **51**, which can be converted to the isatin derivative by the dealdolisation procedure.

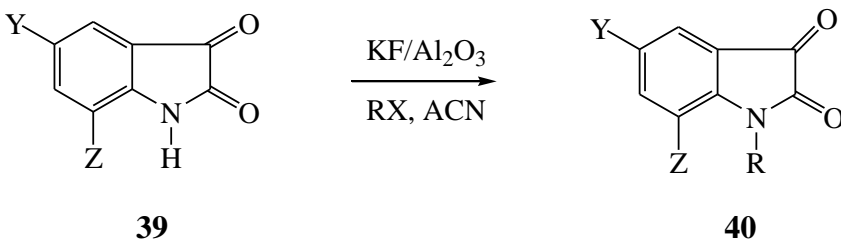


Di Carlo and Lindwall showed the *N*-alkylation of isatin via a “Michael addition” involving the reaction of acrylonitrile with isatin (**32**) in ethanol, in the presence of a catalytic amount of trimethylbenzylammonium hydroxide to give 1-cyanoethylisatin (**53**).³⁹



One of the more novel bases for *N*-alkylation reactions is the solid supported reagent potassium fluoride on alumina ($\text{KF}/\text{Al}_2\text{O}_3$).⁴⁰ $\text{KF}/\text{Al}_2\text{O}_3$ was originally introduced in 1979 by Ando et al.⁴¹ as a useful agent for inducing alkylation reactions in general. It possesses advantages for both solution phase and solid phase chemistry and the excess support bound reagent can be removed by filtration, avoiding tedious work-ups. The strong basic nature of $\text{KF}/\text{Al}_2\text{O}_3$ has allowed it to replace organic bases in a number of reactions including selective *N*-

alkylation of amides,⁴² epoxidations,⁴³ diazotizations,⁴⁴ Sonogashira couplings,⁴⁵ Suzuki couplings,⁴⁶ Knoevenagel reaction,⁴⁷ and Horner-Emmons chemistry.⁴⁸ Furthermore, Ando et al. have argued that KF/Al₂O₃ derives its basicity from the presence of three basic species thought to be present in KF/Al₂O₃: (1) the presence of active fluoride, (2) the presence of [Al-O⁻] ion which generates OH⁻ when water is added, and (3) the cooperation of F⁻ and [Al-OH].³⁸ Chiyanzu reported using the KF/Al₂O₃ to perform high throughput parallel solution phase synthesis of *N*-alkylated isatins **40** in acetonitrile starting from the substituted isatin **39**.⁴⁰



Biological Background

Caspases or cysteine aspartyl-specific proteases have been known to play a key role in apoptosis, or programmed cell death.⁴⁹ Apoptosis, is a highly conserved process which is an energy-dependent and genetically controlled mechanism of cell disposal characterized by lack of inflammatory response that serves to maintain homeostasis in multi-cellular organisms.

Dysregulated apoptosis is believed to play a role in various serious pathological conditions such as cancer or autoimmune disorders or even ischemic injury and some neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases.²⁵ The caspase family of cysteine proteases can be subdivided into three groups; where caspases-1, -4, -5, and -13 are involved in inflammation; caspases-6, -8, and -10 are initiator caspases found at the top of the

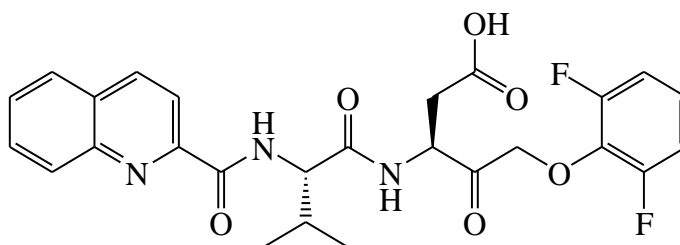
signaling cascade; and caspases-2, -3, and -7 are effector caspases which are activated further downstream.⁵⁰ The effector or executioner caspases are responsible for the physiological changes (e.g., cleavage of the DNA repair enzyme poly(ADP-ribose) polymerase-1) and morphological changes (e.g., nuclear membrane damage and membrane blebbing, which is an irregular bulge in the plasma membrane) that occur in apoptosis.

Caspase cleavage of peptide targets is based on nucleophilic cysteine residues located within the caspase which attack the carbonyl group of an aspartic acid residue within a specific tetrapeptide sequence (e.g., ~P₄-P₃-P₂-P₁~) of a large peptide molecule.⁵¹ The standard nomenclature for protease substrate cleavage, P_n, P₃, P₂, P₁, P₁' , P₂' , P₃' , P_n' , etc designates amino acid side chains of a peptide substrate, whereas, the corresponding bonding sites in the protease active site are designated as the S_n, S₃, S₂, S₁, S₁' , S₂' , S₃' , S_n' , etc. subsites.⁵² Caspases have a near absolute requirement for aspartic acid (P₁) in the S₁ subsite of the enzyme, such that substitution for another amino acid results in >100 fold reduction in catalytic efficiency.⁵⁰ Cleavage at the aspartic acid residue occurs by hydrolysis of the amide bond between the P₁ residue and the adjacent "C-terminal" amino acid residue.

When designing peptide based caspase inhibitors, the inhibitor is comprised of a peptide sequence (up to a tetrapeptide) possessing an electrophilic functionality (termed the "warhead") at the C-terminus of the peptide which may function as a reversible (e.g., ketones, aldehydes, and nitriles) or irreversible (e.g., acyloxymethylketones and halomethylketones) moiety in order to react with the sulfur atom of cysteine.⁵⁰ In addition, the P₄ amino acid can be capped with a non-specific neutral group such as an acyl moiety (i.e., Ac-P₄-P₃-P₂-P₁-W, where "Ac" is an acyl group and "W" is a 'warhead'). In previous years, several peptide based caspase inhibitors such as Z-VAD-fmk⁵³ and Boc-D-fmk were found to prevent apoptosis,⁵⁴ unfortunately when used at

high doses these inhibitors appear to have non-specific effects and/or become cytotoxic. Moreover, their size and polarity render them inadequate to pass through the blood brain barrier (BBB) for uses in diseases affecting the central nervous system. More recently, the broad-spectrum apoptosis inhibitor, quinolyl-valyl-O-methylaspartyl-[2,6-difluorophenoxy]-methyl ketone Q-VD-OPh,⁵⁵ (seen in **Figure 2**) has been shown to prevent all major apoptotic pathways, be non-toxic even at high concentrations, and be capable of crossing the blood brain barrier. Q-VD-OPh has proven to inhibit apoptosis at concentrations as low as 5 μM against human Jurkat T lymphoma cells.⁵⁶ Although useful tools *in vitro*, there are several drawbacks of peptide-based inhibitors as potential drugs such as indiscriminate competing reactions *in vivo* with other nucleophiles, rapid physiological clearing times, and poor metabolic stability.

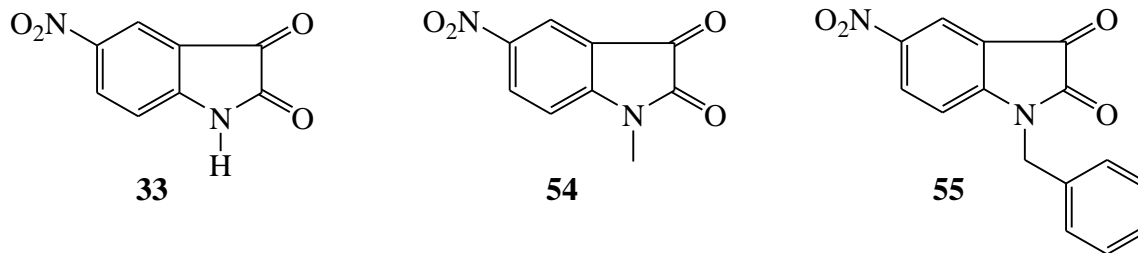
Figure 2



Small Molecule Approach

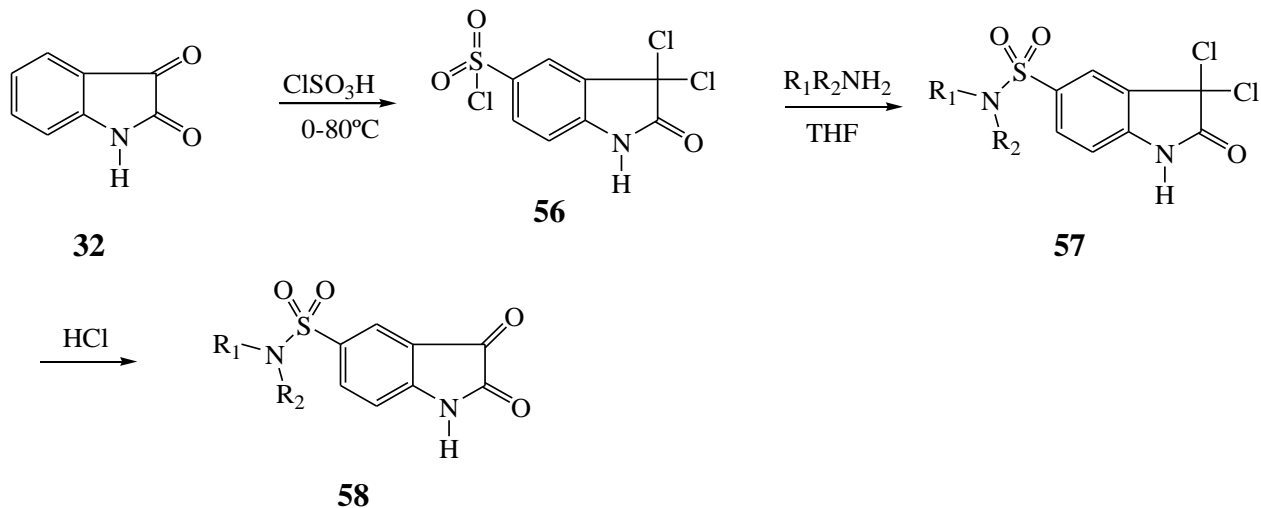
The limitations of peptide based caspase inhibitors led SmithKline Beecham to conduct a high-throughput screening of their compound library for small-molecule inhibitors against caspase-3. This investigation led to the identification of the 5-nitroisatins below which possessed IC_{50} 's of 3

μM (**33**), $1 \mu\text{M}$ (**54**), and $0.25 \mu\text{M}$ (**55**) respectively.⁵⁷

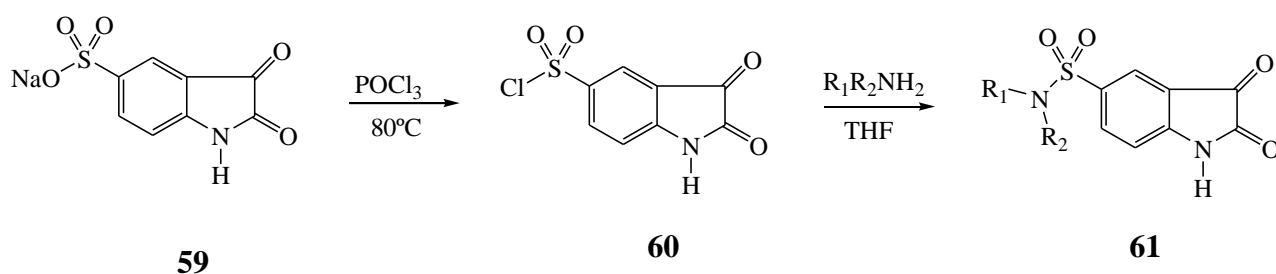


The importance of an electron-withdrawing group at the C-5 position was established by screening several 5-substituted isatins and observing a correlation between the electron-withdrawing ability and effectiveness of the molecules to inhibit caspase-3.⁵⁷ Their results suggested that the electrophilicity of the isatin carbonyl was important for activity, even though none of the electron-withdrawing substituents are in resonance with the C-3 carbonyl. The critical electron-withdrawing effects are presumably attributable to simple inductance of electron density from the aromatic nucleus of the isatin core to the nitro-group. In addition, the SmithKline Beecham group provided binding models based on X-ray structures demonstrating the formation of a tetrahedral intermediate between the cysteine thiol of caspase-3 and the isatin carbonyl group. It was found that the positioning of the inhibitor suggested that access to the extended binding regions of the active site ($\text{S}_2\text{-S}_4$) could possibly be attainable by extending groups off the C-5 position of isatin.⁵⁷ Thus, a sulfonamide group was introduced as the replacement for the nitro group at C-5 position due to the fact that the nitro group is susceptible to metabolic reduction. Furthermore, the sulfonamide retains the desired electronic properties, is metabolically stable, and allows for the incorporation of molecular diversity.⁵⁸

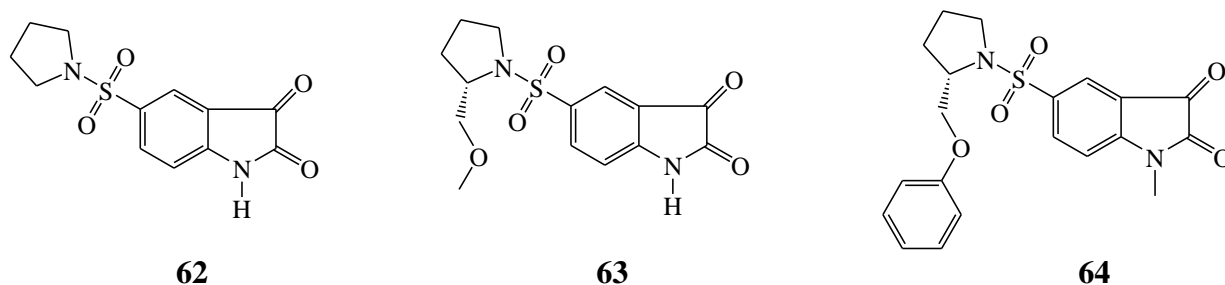
The first attempt at the chlorosulphonation of isatin involved the addition of chlorosulphonic acid in an ice bath followed by heating at 70 °C done by Somasekhara et al.⁵⁹ This procedure was reported to afford the desired derivative **60** as a yellow powder which was recrystallized from acetone-benzene and had a melting point of 150-152 °C. SmithKline Beecham's first attempt at preparing the desired 5-*N,N*-dialkylisatin sulfonamides was done initially by following the Somasekhara method in hopes of securing the 5-chlorosulfonylisatin as an intermediate. Thus, the SmithKline Beecham group found that upon heating isatin (**32**) in chlorosulfonic acid, they actually formed the gem-dichloro derivative **56**. In any event, the 5-isatin sulfonamide was ultimately obtained by reaction of the resulting gem-dichloro species **56** with an amine to give **57**, followed by hydrolysis to yield **58**. Later, SmithKline Beecham recognized that the confusion arose from the fact that Somasekhar's combustion analysis results for the suspected sulfonyl chloride **60** were erroneous based on the fact that their characterization relied on only N and Cl analysis (N = 5.0, Cl = 24.70),⁵⁹ for which the gem-dichloro derivative prepared by SmithKline Beecham also fits the combustion analysis data reported by Somasekhara.



Lee et al. also showed that a more straightforward route to obtaining the 5-*N,N*-dialkylisatin sulfonamides **61** involved the preparation of 5-chlorosulfonylisatin **60** by treating the then commercially available sodium 5-isatin sulfonate **59** with phosphorus oxychloride in tetramethylene sulfolane at 80°C.⁶⁰ In addition, the melting point of the 5-chlorosulfonylisatin (188-190°C)²⁶ varies greatly compared to the mp reported by Somasekhara.

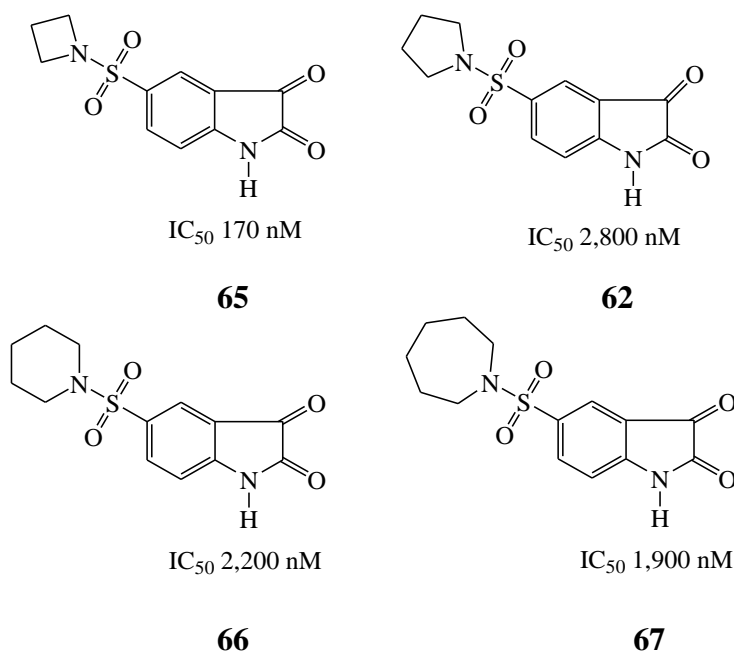


Evaluation of a series of amine inputs led to the identification of isatin sulfonamide **62**, which retained significant activity against caspase-3 ($K_{i(\text{app})} = 1.4 \mu\text{M}$), compared to the lead **33** ($K_{i(\text{app})} = 0.5 \mu\text{M}$), and furthermore, the isatin sulfonamide **62** possessed much improved selectivity for caspases-1, 3, and 7.⁵⁸

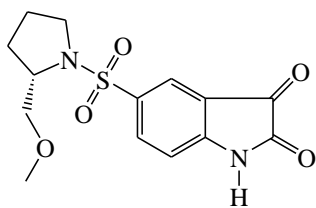


The extension of groups off the pyrrolidine ring of the sulfonamides led to the identification of isatin sulfonamides **63** and **64** with activities of 60 and 15 nM ($K_{i(\text{app})}$) respectively as inhibitors of caspase-3. It was found that these inhibitors exhibited 100-fold or greater selectivity for caspase-3 and 7 versus all other family members with the exception of caspase-9 showing selectivity from 10- to 50 fold.⁵⁷ Lee et al. believe that the observed selectivity for the isatin sulfonamides was likely due to three hydrophobic residues in the S_2 pocket (Tyr²⁰⁴, Trp²⁰⁶, and Phe²⁵⁶) that are unique to caspase-3 and 7, by examining protein residues proximal to the catalytic cysteine active site. A 2.8-Å resolution X-ray co-crystal structure of caspase-3 and the inhibitor **64** was obtained and reveals that the S_2 pocket is involved in extensive hydrophobic contacts with the pyrrolidine ring, conferring specificity to the isatin sulfonamides.⁵⁸ As mentioned previously, the S_1 subsite of caspases confers critical selectivity for cleavage of substrates possessing a P_1 aspartic acid residue in peptide based inhibitors, however, in the co-crystal structure shown by Lee et. al. the S_1 subsite is occupied only by a water molecule.⁵⁸ Thus, previous inhibitor studies suggested the S_1 , S_3 , and S_4 subsites are critical for potent and selective inhibition of caspases, given the S_1 subsite confers selectivity for caspase, while the S_3 and S_4 subsites are believed to be critical for selectivity amongst caspases. However, using the sulfonamide co-crystal structure model it was shown 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. It is also speculated that possible elaboration with chemical groups which can bind in the extended binding sites (S_3 and S_4) where caspase-3 and 7 diverge may enhance selectivity and affinity for one caspase over the other.⁵⁷

The importance of ring size of the isatin sulfonamides on activity was investigated and evaluated for compounds **62** and **65-67**. In general, there was no great effect on activity based on the ring size from five to seven carbons, yet the azetidine ring for **65** resulted in a 10-fold increase of potency compared to the pyrrolidine moiety **62**. Nonetheless, structural activity relationships (SAR) were thus further pursued using the pyrrolidine derivatives due to the commercial availability of substituted pyrrolidines.⁵⁷

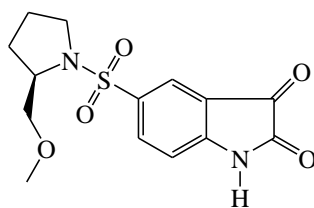


Using compound **62** and extending groups from the C-2 position of the pyrrolidine led to another pivotal discovery involving the importance of chirality of the extended functionalities. Using both *R* and *S* configurations of the methoxymethyl groups for **63** and **68**, the (*S*)-configuration was found to be significantly more active providing >100 fold activity over the *R*-stereochemistry.⁵⁷



IC₅₀ 120 nM

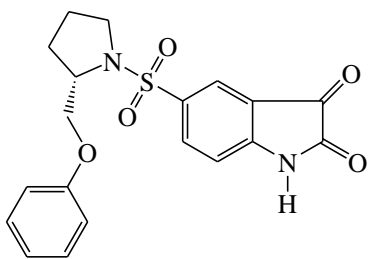
63 (S)-configuration



IC₅₀ 18,000 nM

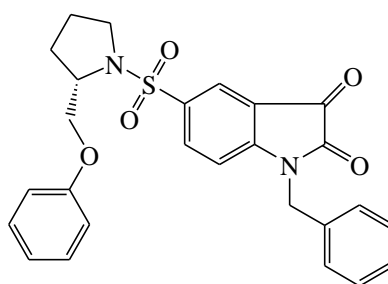
68 (R)-configuration

Furthermore the SmithKline Beecham group increased the potency of their compounds by 2-3-fold by replacing the methoxymethyl group attached to the pyrrolidine ring of **63** with a more hydrophobic moiety such as a phenoxy group, as seen in **69**.⁵⁷ The same strategy used to increase the potency of their 5-nitroisatins by way of N-alkylation was then applied to the isatin sulfonamide compounds. Similarly, a direct correlation was noticed when alkylating with larger hydrophobic groups attached to the nitrogen and an increase in potency was displayed by **70** over its N-H analog **69**.



IC₅₀ 44 nM

69

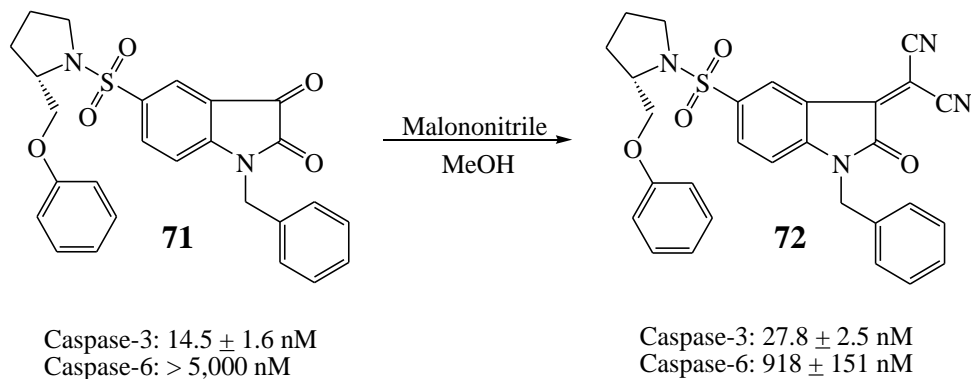


IC₅₀ 2.5 nM

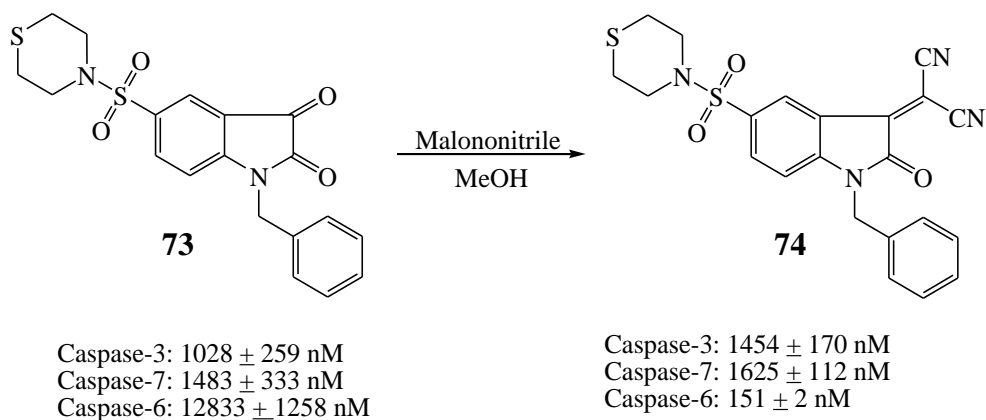
70

W. Chu et al. at the Washington University School of Medicine further applied the use of the potent isatin sulfonamides derived by the SmithKline Beecham corporation and suggested another mechanistic mode of attack of the isatin sulfonamide scaffold by introducing an isatin Michael acceptor (IMA) such as a vinyl dicyano group at the C-3 position. The Michael addition

reaction generally occurs by the cysteine thiol “soft” nucleophile attacking an α,β -unsaturated carbonyl compound. By condensing the isatin sulfonamide **71** with malononitrile in methanol, the IMA analog **72** showed an increased potency of roughly 10-fold for caspase-6 when compared to its complementary precursors, while still retaining the high selectivity for caspases-3/7 as well, as a reversible inhibitor.⁶¹



In order to find caspase 6 inhibitors, Chu et al. sought to replace the phenoxyethylpyrrolidine ring in **72** with other nitrogen heterocycles to create the thiomorpholine analogue **74** by condensing **73** with malononitrile in methanol, which further exhibited a reduction in selectivity for caspase-3 and increased caspase-6 selectivity by 85-fold.⁶²



Other Applications of Isatin Sulfonamides

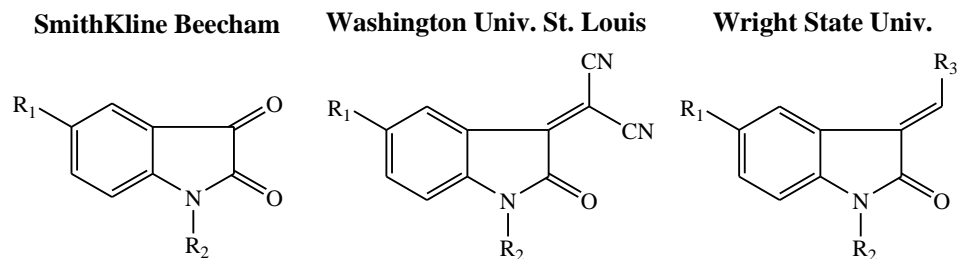
More recently, isatin sulfonamides have been utilized extensively as biomarkers for noninvasive positron emission tomography (PET) imaging of apoptosis *in vivo* which can permit the detection of tumor biological changes.⁶³ This process is initiated by labeling the isatin group with an ^{18}F radio isotope using novel “click” reactions. Given that the half-life of the radiotracer is $t_{1/2} = 110$ minutes, the ^{18}F labeled isatin sulfonamide can be administered to a living organism using PET imaging which allows for high resolution quantitative imaging of biochemical processes by detecting the distribution pattern of the biomarker over time. The development of a noninvasive imaging procedure that can study the process of apoptosis in a variety of disease states and monitor the ability of a drug or other treatment is a great value to the on-going research for drug discovery and the community.

Results and Discussion

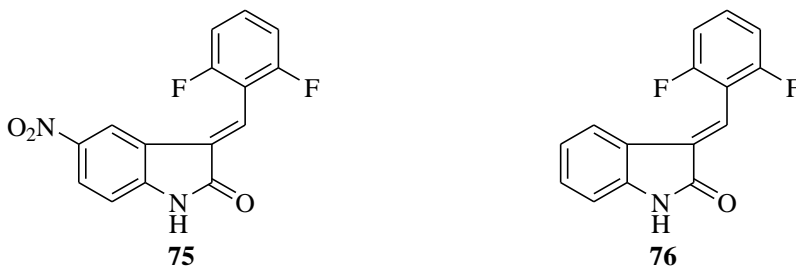
WSU Approach

The goal of this project was to design and synthesize potential cell death inhibitors based on the isatin scaffold while incorporating structural features gleaned from previous SAR studies of peptide based as well as small molecule inhibitors. To this end, both isatins and oxindoles were used as starting materials and further modified to ultimately synthesize a series of benzylidene oxindole derivatives which provide three points of variability, and function as caspase inhibitors likely via a Michael type addition reaction. This strategy also employed the “libraries from libraries”⁶⁴ concept using a “toolbox” of various chemical transformations to complete a library of isatin and oxindole derivatives to assess biological properties and make use of such information in the design of more potent inhibitors. The idea of employing three points of variability is a novel concept given that the SmithKline Beecham lead (e.g., the 5-nitroisatins), possessed only two points of variability at the C-5 and N-1 positions. The group at Washington University St. Louis developed their lead isatin sulfonamide molecules using just two points of variability, eventually incorporating the vinyl dicyano group at the C-3 position which displayed selectivity for caspase-6 over caspase-3, however such derivatives still allow for only two points of diversity. The WSU approach entails the generation of a library of oxindole molecules derived from isatins which takes advantage of the potential for exchangeable chemical transformation at the C-3 position of the oxindole substrate shown in **Figure 3**.

Figure 3



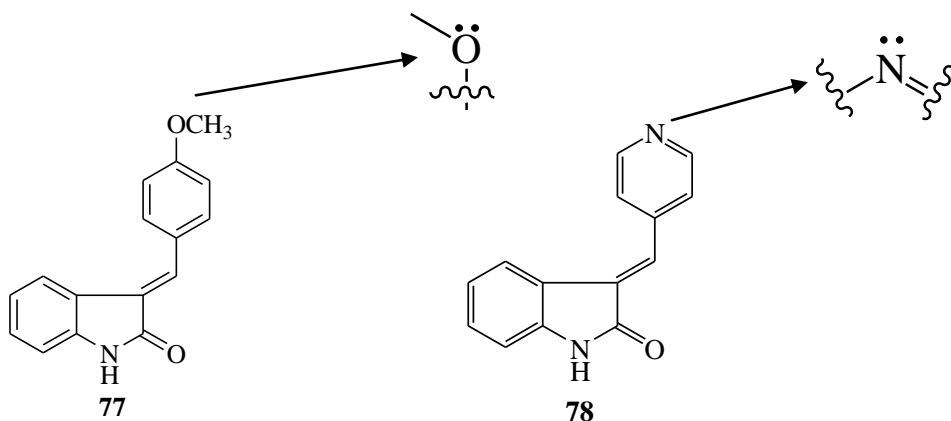
Given that Q-VD-O-Ph proved to inhibit apoptosis against Jurkat T cells at micromolar concentrations, it was deemed important to employ some of the functionalities of this peptide inhibitor to the design of our isatin library. One of the main points of interest was the 2,6-difluorophenoxy “warhead”, which is believed to play an important role as an electrophilic moiety that may act as a stable leaving group post cysteine nucleophilic attack. In addition Hagar Abdallah found that 5-nitrooxindole bearing a 2’6’-difluoro moiety at C-3 (i.e., **75**) was also active, matching the 5 μ M activity seen by Q-VD-O-Ph.⁵⁵



Further investigations showed that the 2,6 difluorophenyl moiety on the oxindole scaffold alone was not active (i.e., **76**), and the electron withdrawing properties of the nitro group at the C-5 position, similar to SmithKline Beecham molecule **33**, were critical for biological activity. Thus, it was deemed important to introduce an electron withdrawing group at the C-5 position. Therefore, one of the goals of this project was to design molecules which incorporate non-

reducible electron withdrawing groups in place of the nitro and sulfonamide groups employed previously, and to that end the chlorine atom was chosen, given its metabolic stability.

In addition, various substitution patterns were examined on the benzylidene moiety also based upon previous findings. For instance, the *para*-methoxy benzylidene group in particular was found by H. Abdallah to inhibit apoptosis against Jurkat T cells in vitro at approximately 50 μM , in contrast to the former preconception that a strong electron-withdrawing group was necessary to promote nucleophilic attack at the beta-carbon of the α,β -unsaturated amide moiety. This finding suggested that there was a role played by the lone pair electrons on a group attached to the benzylidene group, possibly exerting its effect by way of hydrogen bonding (*vide infra*, **77**). This latter rationalization is supported by the research of Loser et al. who propose that the hydrogen binding between the inhibitor and the caspase greatly influences the inhibition capabilities of the inhibitor. Accordingly, substituent groups with available electrons such as hydroxy and methoxy groups could be used as effective groups to inhibit caspases.⁶⁵ Adding support to this hypothesis was the finding that an oxindole bearing a 4-pyridyl substituent (e.g., compound **78**) was found to inhibit apoptosis in Jurkat T cells at 50 μM , similar to the methoxy moiety, given that pyridine nitrogen also possesses a pair of electrons which is capable of hydrogen bonding.



Following up on that finding and described herein (*vide infra*), a *para*-methoxy benzylidene oxindole analogue was prepared and was *N*-alkylated with the 2,6-difluorobenzyl moiety in hopes of increasing the potency of the previous lead. In addition, several pyridine benzylidene derivatives were prepared and tested based on the fact that they possess lone pairs of electrons, as does the methoxy functional group.

Thus, the starting point for this work involved the fact that the 5-nitro-2,6-difluoro analog **75**, the *para*-methoxy derivative **77**, and 4-pyridyl substituted **78** benzylidene compounds were tested previously by H. Abdallah and found to be active against Jurkat T cells. It was envisioned that by incorporating a non-reducible, electron-withdrawing functionality at the C-5 position, along with groups on the benzylidene moiety capable of accepting hydrogen bonds would likely lead to positive results. Moreover, given that previously only *N*-unsubstituted benzylidene compounds were tested against Jurkat T cells by H. Abdallah, it was deemed important to perform the *N*-alkylation of these active compounds by analogy to the improvement in caspase inhibition found by *N*-alkylation of isatin sulfonamides described earlier. In addition to the synthesis of the desired benzylidene oxindole compounds mentioned, a small library of *N*-

alkylated isatins was created along with their 5-chloro derivatives as possible caspase inhibitors which might be biologically tested if time permitted.

The *N*-alkylations of the isatins were conducted using a range of alkylating groups including hydrophobic, disubstituted and unsubstituted benzylic groups based upon the findings by SmithKline Beecham which showed that the *N*-alkylated hydrophobic modifications possibly extend to the hydrophobic S₂-S₄ regions of a caspase enzyme, and significantly increase inhibition for caspases 3/7.⁵⁶ Based upon these findings, isatin was *N*-alkylated with a simple benzyl group which would provide the relevant hydrophobic characteristics and allow possible access to the S₂-S₄ subsites of an enzyme. Also, a 2,6-difluorobenzyl group was added onto the nitrogen atom since this particular substitution was thought to play a key role in the inhibitory effects demonstrated by the Q-VD-OPh molecule and the Abdallah compound **75**. As an alternative to the 2,6-difluoro moiety, a 2,6-dichloro analog was also created. Furthermore, two specific outlier *N*-alkylations were performed, one by way of a nucleophilic substitution reaction to make a carboxy ester using ethyl bromoacetate and the other by way of Michael addition at the isatin nitrogen to make a *tert*-butyl carboxy ester using *tert*-butyl acrylate. The carboxy ester moieties could potentially be hydrolyzed into carboxylic acid groups and were chosen due to this potential and consequential resemblance to an aspartic acid and glutamic acid residue, respectively. In the event, the *tert*-butyl group, in which the carbon backbone extended from the isatin nitrogen is one carbon longer than the carboxy ester moiety obtained using ethyl bromoacetate, was indeed spontaneously hydrolyzed into a carboxylic acid group and not only resembles a glutamic acid residue in a peptoid sense but also acts as a hydrophilic moiety. While such a hydrophilic group was not found effective in the corresponding isatin sulfonamides, it is possible that such an analogue could serve as an antagonist to the known inhibitory effects

created when using hydrophobic groups off the isatin nitrogen. Moreover, it was envisioned that these substrates could be further modified to add an amide group at the carboxylic acid end, allowing another point of variability.

In summary, in order to synthesize a compound that might have potential to inhibit caspases *in vivo*, it appeared ideal to incorporate electron withdrawing groups at the C-5 position; extend groups from the C-3 position that may act as “Michael acceptors” such as a benzylidene moiety or that may hydrogen bond, and finally extend possible hydrophobic groups off the nitrogen atom of the isatin scaffold to access the S₂-S₄ subsites of the targeted enzyme. Thus, **Figure 4** below shows one route to achieve the desired tri-substituted isatin targets.

Figure 4

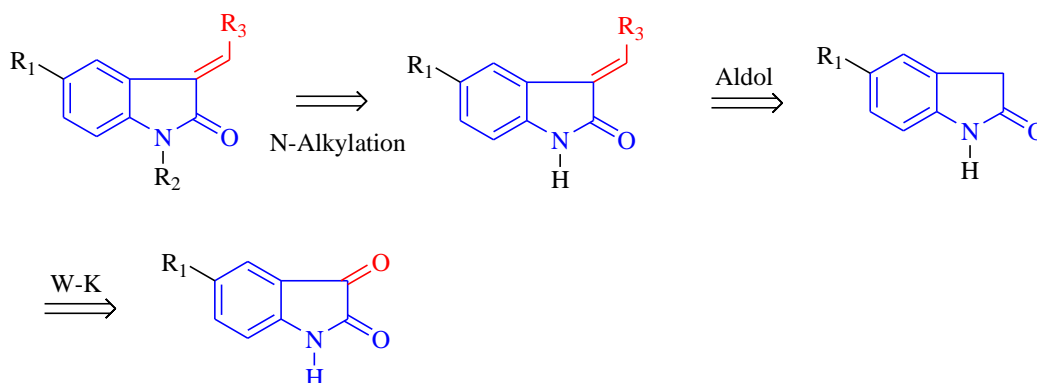
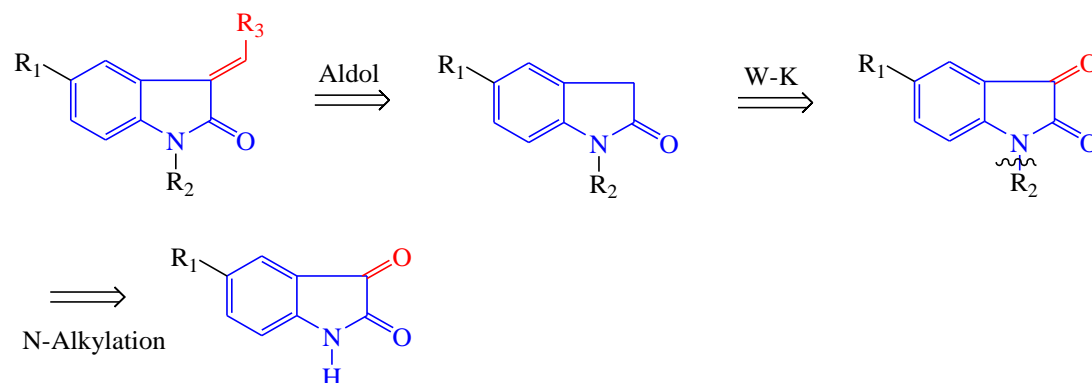


Figure 4 shows a retro-synthetic analysis in which an *N*-alkylated benzylidene oxindole is seen as arising from *N*-alkylation of the C-3 substituted oxindole in a final step. The benzylidene oxindole, in turn, is visualized as arising from the aldol condensation of an oxindole. The oxindole can then be seen as arising from a Wolff-Kishner reduction on a 5-substituted isatin. However, it can be demonstrated that the desired end product can also be achieved by

performing the *N*-alkylation as the first step as opposed to the last step, followed by the Wolff-Kishner reduction and condensation reaction as shown in **Figure 5** below.

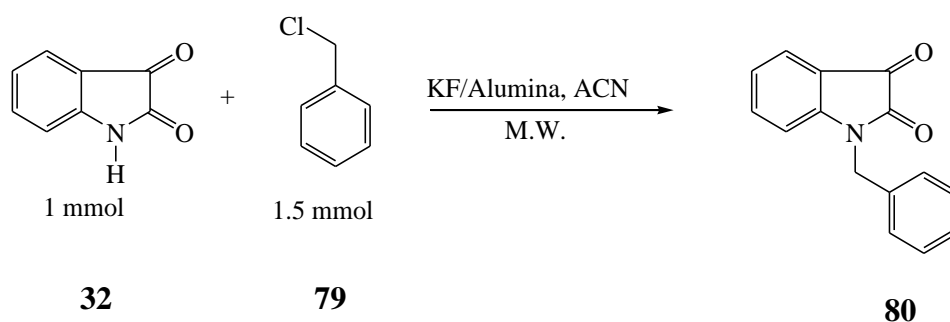
Figure 5



***N*-Alkylation of Isatin via Microwave Irradiation**

The first phase of this project involved an examination of the *N*-alkylation of isatins using the solid base KF/alumina and was initially carried out using the CEM Discover microwave so as to rapidly probe the optimal parameters before upscaling the process by means of conventional heating. Microwaves heat an organic reaction sample more evenly (since heating is generated from within the sample) than the conventional heating mantle or immersion bath where the walls of the vessels are warmer than the cores causing hot spots which might trigger the formation of side-products.⁶⁶ The use of microwave accelerated synthesis is also beneficial in that it allows for homogeneous heating which causes the reaction temperatures to rise quickly (up to 10°C per/sec), a feature which also minimizes the generation of side products. Eventually, however, once the optimal conditions for these reactions were ascertained, the *N*-alkylations could be scaled up in order to produce a greater quantity of product to be used in subsequent reaction

steps. Since it appeared that the *N*-alkylation of isatin had yet to be conducted using KF/alumina as a base, it was decided to examine the use of this basic catalyst in acetonitrile (ACN) under microwave irradiation. The microwave conditions initially varied temperatures and reaction times at 300 watts for the model reaction of isatin (**32**) and 1.5 equivalents of benzyl chloride (**79**) to yield 1-benzylindoline-2,3-dione (**80**).



The base KF/alumina was chosen because it is relatively easy to prepare, inexpensive, and a relatively strong base. The procedure utilized for the preparation of this solid base involved adding approximately 58 g of potassium fluoride to a 1 L round bottom flask and dissolving in 100 mL of deionized water.⁶⁷ Next ~100 g of aluminum oxide was added to the flask with continued stirring for 30 min. The water was then evaporated from the KF/alumina combination by rotary evaporation at 80°C. The cement-like product was then scraped out of the round bottom flask and pulverized into a fine powder using a mortar and pestle. Finally, the product was transferred to a vacuum oven at 70-80°C for 72 h to afford a fine white powder at approximately 38 %/wt KF/alumina. The 38 %/wt calculation compares to the 40 %/wt KF/alumina sold by Sigma-Aldrich for \$1.92 per gram (\$48 for 25 g), whereas the 38 %/wt KF/alumina prepared from KF and aluminum oxide both provided by Sigma-Aldrich only totals \$0.24 per gram. Given that the results of these *N*-alkylation reactions using either sample of base are almost

identical (inferred by TLC), the prepared sample of KF/alumina was chosen to initiate the *N*-alkylation process study.

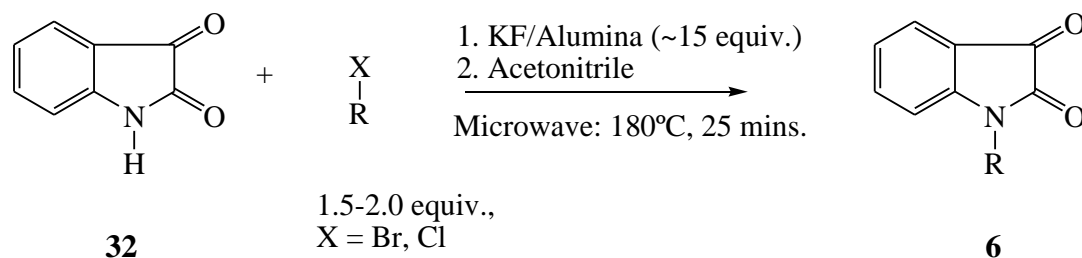
As shown in **Table 2** below, the parameters for the microwave assisted *N*-alkylation of isatin using benzyl chloride and KF/Al₂O₃ in ACN were progressively modified in order to achieve 100% completion. In each scenario, approximately 0.3398 mmol of isatin (0.050 g) was added to a 10 mL microwave vial along with 15 equivalents of KF/Al₂O₃ (0.779 g), 1.5 equivalents of benzyl chloride and 5 mL of acetonitrile. The relative equivalency of KF/Al₂O₃ is based upon the fact that only the KF is reactive and the Al₂O₃ is a solid support. Thus the amount of KF for any given reaction is determined by the percent by weight of KF/Al₂O₃ which was calculated to be approximately 38%. To calculate the total grams of KF/Al₂O₃ required for ~15 equiv. of the active reagent KF, first, the moles of KF were determined to be 5.1 mmol by multiplying 15 times the molarity of isatin which is 0.3398 mmol. Further using the molecular weight of potassium fluoride (58.1 g/mol), the number of grams of KF alone needed for 15 equiv. was figured to be approximately 0.296 g. However, because the KF and alumina are temporarily fused together, the total amount of KF/Al₂O₃ solid needed to include 0.296 g of KF could be determined by allowing $0.296/X = 0.38$. Finally, the total amount of KF/Al₂O₃ was calculated to be $X = 0.779$ g based on the 38 %/wt. ratio of KF to KF/Al₂O₃. Initially it was not known if the KF/Al₂O₃ base could be used stoichiometrically or catalytically, and it was initially assumed that it might be capable of acting catalytically. However, it was eventually established that 15 equiv. of the base was optimal for achieving complete conversion of isatin into the corresponding *N*-benzylisatin derivative at 180°C for 25 min under microwave irradiation. Going from the lowest temperature and time at 120°C for 10 min to the highest temperature and time at 180°C for 25 min, the ratio of starting material to product was determined as the temperature was

increased by 20°C increments and the time of reaction was varied between 10 and 25 min for each temperature interval. TLC analyses of the reaction mixtures were conducted as well as GC/MS analysis for the ratio of starting material (isatin) versus product (N-benzyl isatin) determined directly from aliquots of the crude reaction mixtures. **Table 2** summarizes a direct correlation between temperature and time to the complete conversion into the desired *N*-substituted product. Thus, as the time was increased from 10 to 25 min in each temperature setting, the turnover of product was increased. In addition, as the temperature was gradually increased there was noted a significant increase in product formation as well. In trial 1, the least amount of product conversion was noted at 120°C, wherein the temperature was inadequate to overcome the activation energy barrier despite allowing further reaction time. In trial 2, at 140°C there was a 9% increase in product generation due to the 15 min increase in reaction time. Likewise, in trial 3, at 160°C the greatest product conversion was achieved providing only 3% starting material impurity at 25 min reaction with a 14% increase in product generation overall. Finally, 99% conversion was achieved at 180°C for 25 min in trial 4. Once a time and temperature was found for optimal reaction conditions, the amount of base was also assessed for product generation by using 5 equiv. (trial 5) and 10 equiv. (trial 6) of KF/Al₂O₃ at 180°C for 25 min and GC/MS analysis was taken on the crude reaction mixtures showing 84:16 and 63:37 ratios of isatin versus N-benzyl isatin for each reaction, respectively. This indicated that a seemingly excessive, 15 equiv. of KF/Al₂O₃ base was needed for the microwave reactions to go to completion at 180°C and 25 min. Even though an optimal temperature and time was finally found, the “upper end” 180°C temperature and relatively long reaction time of 25 minutes are certainly not ideal for a facile, microwave-accelerated reaction, and leaves room for improvement. One further improvement might entail the use of potassium iodide (KI) as a

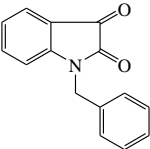
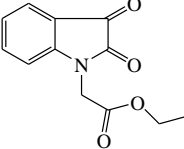
catalyst, and performing the reaction in other polar aprotic solvents such as dimethylformamide (DMF) to facilitate this nucleophilic substitution onto a benzylic chloride.

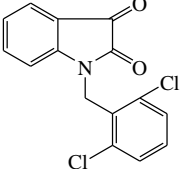
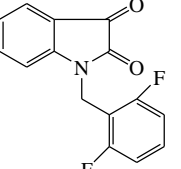
Table 2: Ratio of Isatin versus N-Benzyl Isatin via Microwave Irradiation			
		Time (minutes)	
		10	25
	Temperature (°C)	Ratio (Isatin:N-benzyl isatin)	
Trial 1	120	34:66	33:67
Trial 2	140	28:72	19:81
Trial 3	160	17:83	3:97
Trial 4	180	6:94	1:99
Trial 5 (5 equiv. KF/Al₂O₃)	180	X	
Trial 6 (10 equiv. KF/Al₂O₃)	180	X	

After ascertaining “optimal” conditions for effecting the *N*-benzylation of isatin under microwave irradiation, these conditions were employed to conduct other *N*-alkylation reactions using the isatin (**32**) scaffold and solid supported KF/alumina base to produce the desired *N*-alkylated isatins **6**.



As concluded from the microwave *N*-benzylation trials, approximately 15 molar equivalents of KF/alumina were needed in order to drive the *N*-alkylation of isatin to completion at 180°C and 25 minutes reaction time. The reactions were prepared using approximately 0.3398 mmol of isatin (0.050 g) in a 10 mL microwave vial along with 15 equivalents of KF/Al₂O₃ (0.773 g) and 1.5 equivalents of each alkyl halide in 5 mL acetonitrile. The crude products were separated from the solid support by vacuum filtration and then washed with cold solvent and the purity confirmed by TLC analysis and melting point comparison with known values if available. The percent yield results and melting points for these reactions are reported in **Table 3** below along with the structure of the product of interest.

Table 3: N-Alkylation of Isatin Via Microwave Irradiation			
Final Product	Melting Point	Literature Melting Point	Percent yield
 80	126-131°C	126-127°C ⁶⁸	91%
 81	122-126°C	132-133°C ⁶⁸	94%

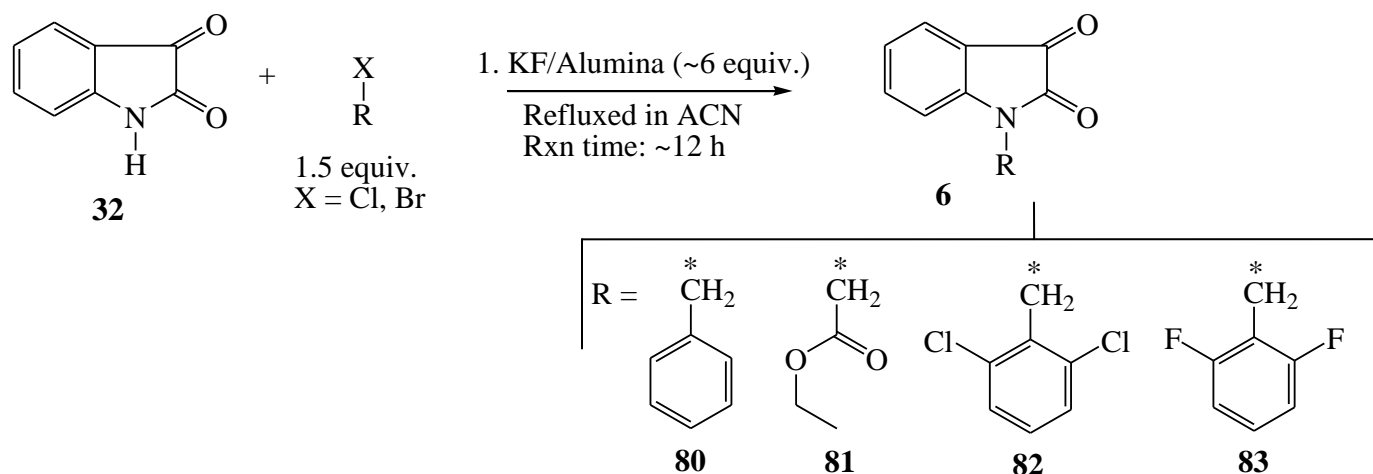
 82	184-189°C	N/S	97%
 83	147-151°C	N/S	94%

*N/S = product has not been synthesized before in literature

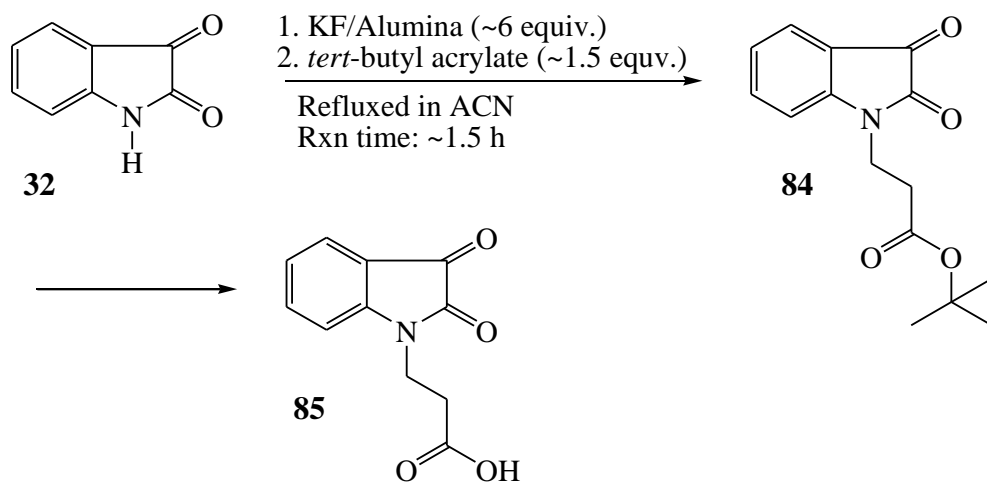
It is again emphasized that the products from the table above are the results from microwave synthesis on a small scale (due to the large amount of KF/alumina required) and these reactions could later be scaled up using conventional heating so as to provide sufficient amounts of product for further synthetic steps and/or biological screening. However, it is noteworthy to mention that the microwave synthesis reactions produced high yields and the melting points were very close to those reported in the literature.

***N*-Alkylation of Isatin via Conventional Heating**

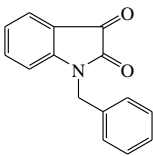
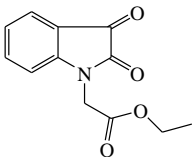
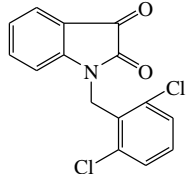
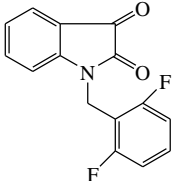
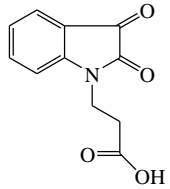
The following reaction scheme shows the method for *N*-alkylation of isatin (**32**) on a larger scale employing conventional heating to afford the desired *N*-alkylated isatins **6**. Interestingly, when conducting the reactions on a larger scale, it was found that much less base was needed in order to ensure that each reaction went to completion in a reasonable time frame. Under these conditions, isatin was refluxed in acetonitrile using 6 equivalents of KF/Al₂O₃ for an average of 10 hours for 4 of the 5 products shown in **Table 4**.



As a point of information, it is noteworthy to mention that the formation of compound **85** occurs by way of Michael reaction and not a simple $\text{S}_{\text{N}}2$ reaction. In this case, refluxing isatin (**32**) with $\text{KF}/\text{Al}_2\text{O}_3$ and *tert*-butyl acrylate in acetonitrile afforded the ester **84** as an oily product, which was confirmed by GC/MS analysis. However, during the course of work-up and recrystallization from 2-propanol the ester **84** underwent adventitious hydrolysis to form the acid **85** shown.



In **Table 4** below, the products along with their melting point and percent yields are presented and compared to literature melting points where available.

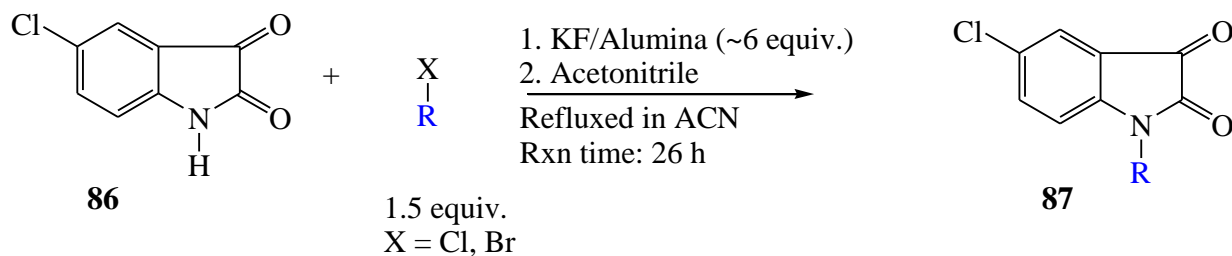
Table 4: N-Alkylation of Isatin Via Conventional Heating			
Final Product	Melting Point	Literature Melting Point	Percent yield
 80	128-133°C	126-127°C ⁶⁸	88%
 81	124-129°C	132-133°C ⁶⁸	97%
 82	175-180°C	N/S	66%
 83	154-156°C	N/S	84%
 85	144-149°C	N/S	18%

*N/S = product has not been synthesized before in literature

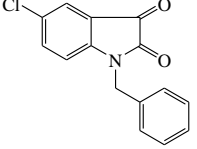
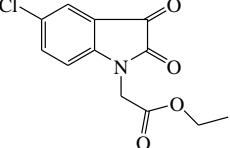
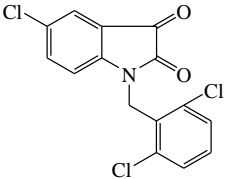
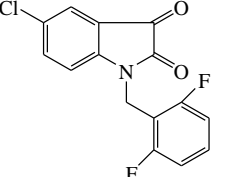
All of the compounds shown in **Table 4** and **Table 5** below were either sufficiently pure from work-up for complete characterization or were further purified by recrystallization if

necessary. Ultimately, all compounds were shown to be pure by GC/MS measurements as well as by TLC analysis and their identities secured by ^1H and ^{13}C NMR analyses (*vide infra*).

In addition to the *N*-alkylation of isatin itself, a similar series of reactions was then conducted on 5-chloroisatin (**86**), purchased from Sigma-Aldrich, to produce the corresponding 5-chloro-*N*-alkylated isatins **87**.



The results are summarized in **Table 5** wherein the reactions were refluxed for approximately 26 hours in the presence of 6 equivalents of $\text{KF}/\text{alumina}$. The extended reaction times are presumably attributable to the electron withdrawing effect of the chlorine atom, which weakens the nucleophilicity of the isatin salt. To date, none of the compounds listed in **Table 5** have known melting points or have been previously synthesized in literature.

Table 5: N-Alkylation of 5-Chloroisatin Via Conventional Heating			
Final Product	Melting Point	Literature Melting Point	Percent yield
 88	134°C	N/S	65%
 89	130-135°C	N/S	87%
 90	233-235°C	N/S	53%
 91	172-177°C	N/S	89%

*N/S = product has not been synthesized before in literature

NMR Analysis of N-Substituted Isatins

All of the compounds made by conventional heating listed in Tables 4 and 5, were achieved in moderate to high yields with relative ease. The *N*-alkylated isatins and *N*-alkylated 5-chloroisatin molecules prepared by conventional heating were further characterized by ¹H and ¹³C NMR spectroscopy using the Bruker Avance 300 MHz NMR. The samples prepared using conventional heating were primarily chosen for NMR analysis over the samples prepared by microwave irradiation simply due to the abundance of product generated. Prior to the analysis of the substituted molecules, a thorough analysis of the chemical shifts of isatin 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 ^1H NMR spectrum of isatin (Sigma-Aldrich) is presented below in **Figure 1**. There are clearly five signals which represent the five protons found on the isatin molecule.

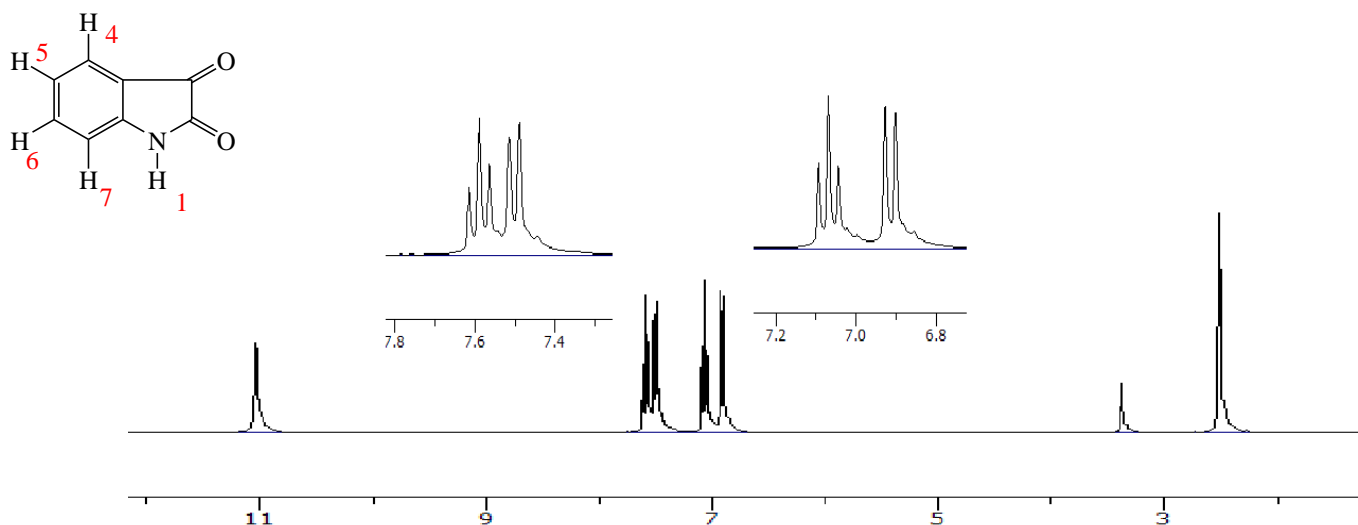


Figure 6

The signal furthest downfield is a singlet peak at 11.04 ppm and its downfield shift is due to a strong deshielding caused by the effect of the nitrogen atom bonded to the proton. There is a triplet at 7.59 ppm ($J = 7.71\text{Hz}$) and a doublet at 7.50 ppm ($J = 7.59\text{Hz}$) which are assigned to the protons labeled 5 and 4 in **Figure 6** respectively. Additionally, there is another triplet signal at 7.07 ppm ($J = 7.55\text{Hz}$) and doublet at 6.91 ppm ($J = 7.83\text{Hz}$) which correspond to the protons 6 and 7 above. The signals from protons 4 and 5 appear further downfield due to the proximity to the carbonyl group being in resonance to the carbons of the corresponding protons 4 and 5. For reasons which are not clear at this point none of the signals exhibit secondary splitting or show any signs of meta coupling. Finally there are two singlets upfield which are both due to the solvent DMSO- d_6 which appear at 3.38 ppm and 2.52 ppm.

The ^{13}C NMR spectrum of isatin (**32**) is presented in **Figure 7** below and shows eight signals which correspond to the eight carbons in the isatin molecule. The peak furthest downfield at 184.4 ppm correlates to the C-3 ketone carbonyl group of the isatin molecule, while the amide carbonyl appears at 159.3 ppm. Carbons 3a (117.8 ppm) and 7a (150.7 ppm), like carbons 2 and 3 are quaternary carbons and consequently do not appear in the ^{13}C DEPT spectrum. The remaining signals for carbons 4-7 fall within the aromatic region of the spectrum and appear at 138.3, 124.6, 122.7, and 112.8 respectively. For reference, the solvent signal appears at 39.6 ppm for DMSO- d_6 . The ^{13}C data above are consistent with the shifts reported in the literature by Gassman completed in dimethyl-sulfoxide- d_6 solvent, wherein the values in descending order are 184.6, 159.5, 150.9, 138.5, 124.8, 122.9, 117.9, and 112.4 ppm.¹²

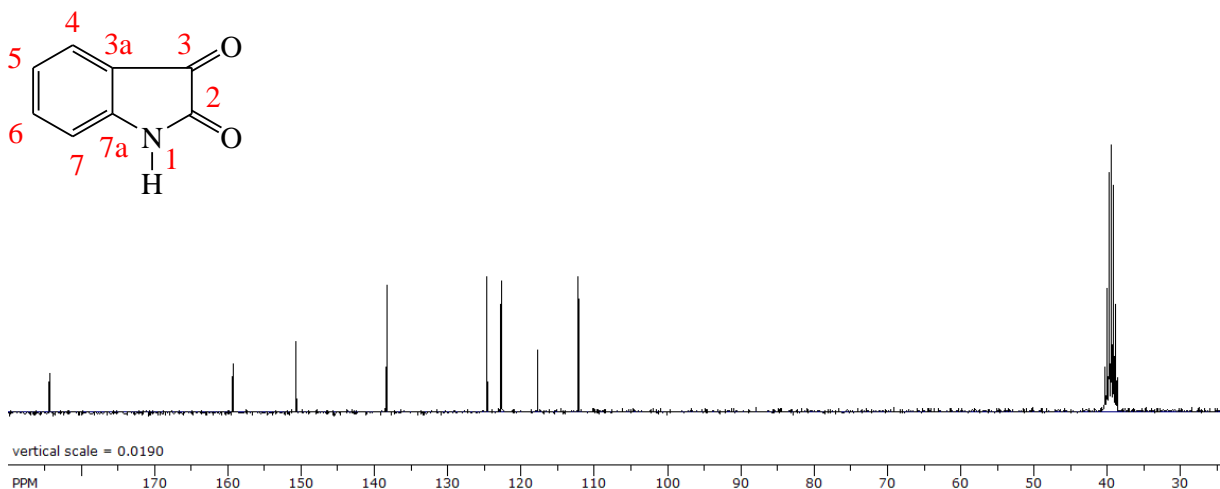
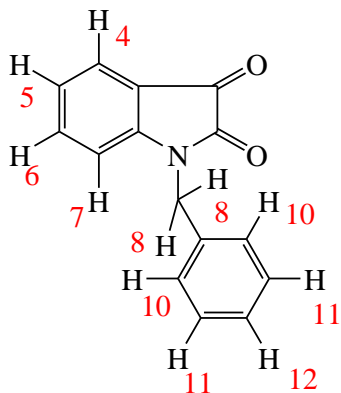
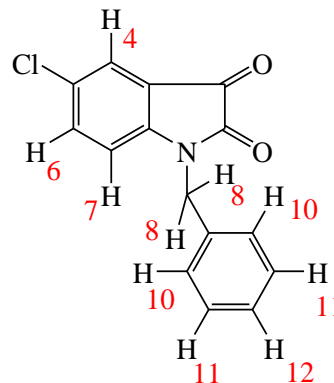


Figure 7

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 benzyl group at the nitrogen atom, along with of course the appearance of new proton and carbon signals.



1-benzylindoline-2,3-dione (80)



1-benzyl-5-chloroindoline-2,3-dione (88)

Compound **80** was prepared by refluxing isatin, KF/alumina base, and benzyl chloride in acetonitrile for 2 h until confirmation of product completion by TLC. The product above was then recrystallized from hexanes and dichloromethane and isolated as bright orange crystals. This compound was of interest to our research due to the potential of the benzyl ring to extend and possibly form hydrophobic contact with the S_2 subsite of a caspase binding site. In the ^1H NMR spectrum of **80** the solvent used was CDCl_3 which appears as a singlet at 1.67 ppm. There is a doublet of doublets which appears downfield at 7.62 ppm ($J = 7.50\text{Hz}, 0.75\text{Hz}$) and is likely due to proton 4. Furthermore, there is a triplet of doublets at 7.5 ppm ($J = 7.83\text{Hz}, 1.32\text{Hz}$) representing proton 5. Proton 7 experiences meta splitting with proton 5 just as proton 6 experiences meta splitting with proton 4. This is supported by the fact that secondary splitting for protons 4 and 6 are both 0.75Hz, while there is a secondary splitting of 1.32Hz for proton 5, however there is no secondary splitting for proton 7, for reasons not known. Thus, proton 6 appears as a triplet of doublets signal at 7.10 ppm ($J = 7.53\text{Hz}, 0.75\text{Hz}$) and proton 7 appears as a doublet at 6.8 ppm ($J = 7.92\text{Hz}$). Finally, there is a multiplet due to the five protons on the benzyl ring which appears at 7.41-7.30 ppm while a singlet signal appears at 4.95 ppm due to the two equivalent benzylic protons 8.

Compound **88** was prepared by refluxing 5-chloroisatin, KF/alumina, and benzyl chloride in acetonitrile for 47 h, after which the final product was then recrystallized from ethanol. When comparing the ^1H and ^{13}C NMR spectra of **80** to its 5-chloro derivative **88** there are several differences worth mentioning. In the proton NMR spectrum which was also done in CDCl_3 , there is a strong doublet at 7.57 ppm ($J = 2.22\text{Hz}$) which is attributable to proton 4 which is *ortho* to the 5-chloro position and experiences meta splitting due to its hydrogen-hydrogen interaction with proton 6. Furthermore, a doublet of doublets' signal can be seen at 7.45 ppm ($J = 8.43\text{Hz}$, 2.21Hz), which is due to proton 6 coupling with proton 7 and meta splitting with proton 4, while proton 7 appears as a doublet signal at 6.75 ppm ($J = 8.34\text{Hz}$). Similarly to the proton NMR spectrum for **80** a multiplet is visible at 7.37-7.31 ppm due to the aromatic hydrogens from the benzyl ring and a singlet signal is observed at 4.94 ppm due to the methylene protons 8.

The ^{13}C NMR spectrum of **80** is shown below which gives 13 signals.

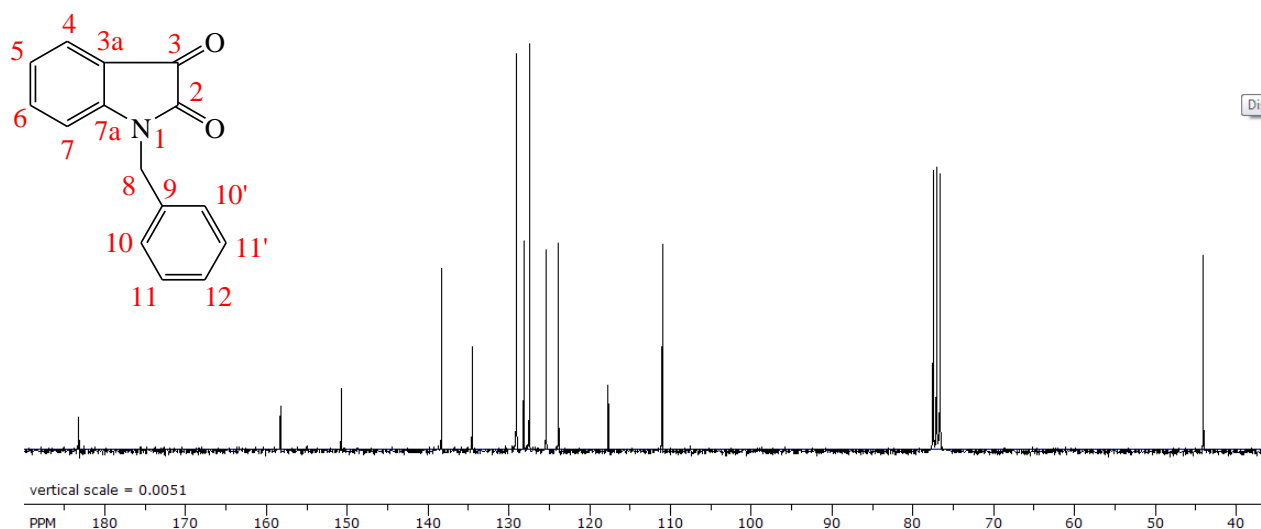
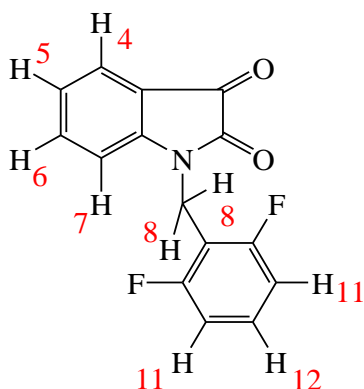
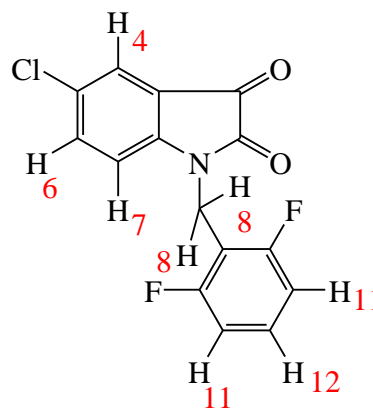


Figure 8

In **Figure 8** the CDCl_3 signal appears at 77.04 ppm. There are two carbonyl peaks which are located at 183.2 ppm for carbon 3 and 158.3 ppm for carbon 2. In addition the remaining aromatic peaks for carbons 3a-7a are consistent with those of the isatin molecule carbon shifts and only change slightly, appearing at 117.7, 138.3, 125.4, 123.84, 111.0, and 150.74 ppm respectively. In addition the spectrum includes an aliphatic peak due to carbon 8, which appears at 44.1 ppm. Likewise there are an additional four signals which correspond to the benzyl phenyl ring. The signal for the quaternary carbon 9 appears at 134.54 ppm and does not appear in the ^{13}C DEPT spectrum. The carbons 10 and 10' are symmetric and are shown as a large intense peak at 127.4 ppm, as does the signal for carbons 11 and 11' which appears at 129.1 ppm. Carbon 12 appears at 128.2 ppm in the spectrum.



1-(2,6-difluorobenzyl)indoline-2,3-dione (83)



1-(2,6-difluorobenzyl)-5-chloroindoline-2,3-dione (91)

Compound **83** was prepared by refluxing isatin, $\text{KF}/\text{alumina}$ and 2,6-difluorobenzyl bromide in acetonitrile for approximately 20.5 h after which time the product was isolated and recrystallized from hexanes and dichloromethane to form miniscule brown crystals. The idea of incorporating the “2,6-difluorobenzyl” moiety was derived from the protease inhibitor Q-VD-

OPh wherein the difluorophenoxy group serves as a terminal “war-head” and is thought to covalently react with the active-site cysteine residue of the enzyme due to its electrophilic nature. The ^1H NMR spectrum of **83**, was collected using CDCl_3 as the solvent. There is a doublet of doublets signal at 7.60 ppm ($J = 7.47\text{Hz}, 0.75\text{Hz}$) which is due to the proton 4 labeled which undergoes *meta* splitting with proton 6. Similarly to the ^1H spectrum for 1-benzylindoline-2,3-dione **80**, the next peak is a triplet of doublets which occurs at 7.53 ppm ($J = 7.83\text{Hz}, 1.32\text{Hz}$) due to proton 5 which also undergoes *meta* splitting with proton 7. A set of multiplet peaks can be seen from 7.37-7.26 ppm in the spectrum which is a result of hydrogen coupling from the equivalent protons 11 and proton 12 within the benzyl ring. A suspected doublet signal for proton 7 is not distinctive in the spectrum and could possibly be hidden in the multiplet signal or abnormally large triplet signal seen at 6.94 ppm. Following suit from the isatin molecule and **80**, a triplet of doublets’ signal appears at 7.10 ppm ($J = 7.52\text{Hz}, 0.57\text{Hz}$) due to proton 6, which experiences *meta* splitting with proton 4. Dissimilar from the former compounds, another triplet signal appears at 6.94 ppm ($J = 8.22\text{Hz}$) which is a result from proton 12 coupling with protons 11. Lastly, a singlet from the pair of methylene protons 8 appears at 5.03 ppm which is again consistent with the ^1H NMR spectrum for compound **80**.

Compound **91** was prepared by refluxing the 5-chloroisatin in acetonitrile with $\text{KF}/\text{alumina}$ base and 2,6-difluorobenzyl bromide for 24 h. The product was then recrystallized from dichloromethane and hexanes to afford ruby red crystals. The ^1H NMR spectrum of the 5-chloro derivative **91**, shows a doublet at 7.57 ppm ($J = 7.07\text{Hz}$) due to proton 4, which is isolated between the chlorine atom and a quaternary carbon. Similar to compound **88**, there is a doublet of doublets signal at 7.50 ppm ($J = 2.27\text{Hz}$) which is due to proton 6 and its *meta* splitting with proton 4. Proton 7 is shown at 6.75 ppm ($J = 8.55\text{Hz}$) in the spectrum as a doublet. A triplet

signal appears at 6.96 ppm ($J = 8.15\text{Hz}$) due to proton 12 coupling with protons 11, and a multiplet is seen at 7.39-7.28 ppm which is due to coupling between protons 11 and 12 on the benzyl phenyl ring.

Figure 9 below shows the ^{13}C spectrum for 1-(2,6-difluorobenzyl)indoline-2,3-dione (**83**), and displays 14 signals using CDCl_3 as the solvent.

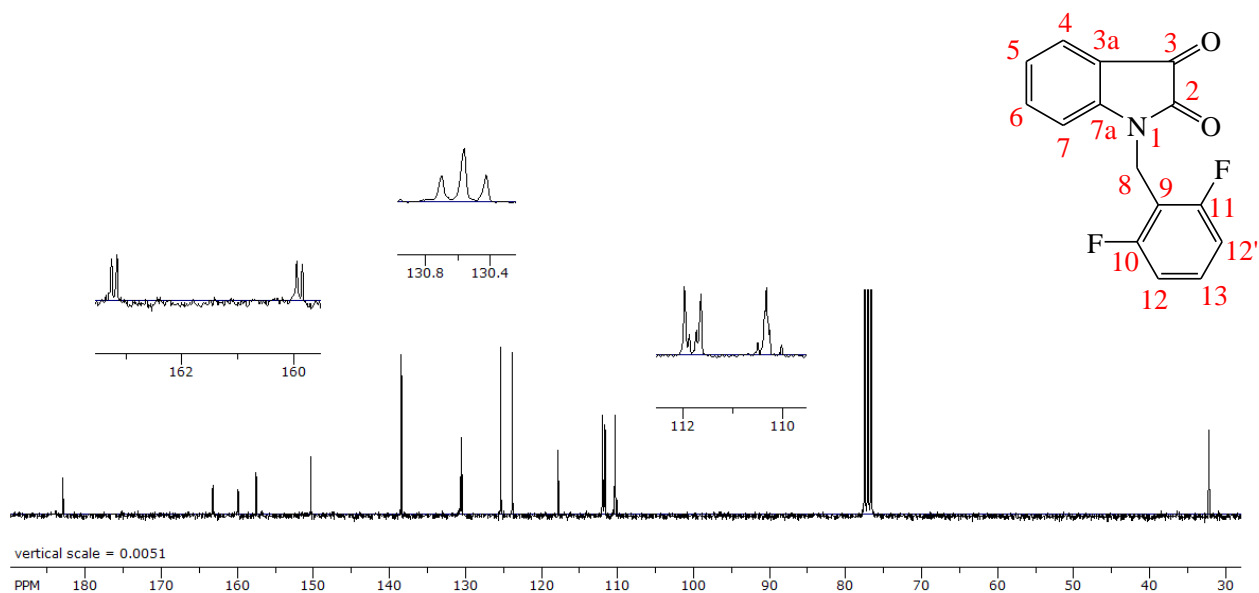
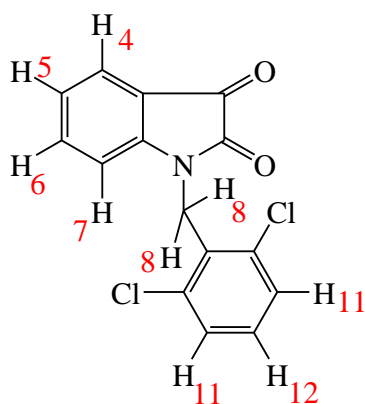


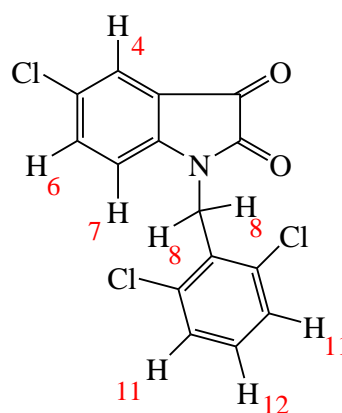
Figure 9

In the ^{13}C spectrum for compound **83** there are seven quaternary signals, as evidenced by their absence in the ^{13}C DEPT spectrum. The two carbonyl signals from the isatin core appear at 157.5 ppm for carbon 2 and 182.9 ppm for carbon 3 above. Carbons 3a and 7a appear at 117.74 and 150.4 ppm, respectively, which is consistent with the isatin carbon shifts for those positions. Carbons 10 and 11 experience strong electron-withdrawing effects from the two attached fluorine atoms and unique fluorine-carbon splitting which results in a broad set of doublet of doublets at 159.9 ppm and 163.2 ppm ($J = 29.06\text{Hz}$). Carbon 9 appears as a triplet signal at

110.25 ppm ($J = 66.09\text{Hz}$) due to it being split by the two fluorine atoms attached to carbons 10 and 11 and it is also overlapped by carbon 7 at 110.3 ppm in the spectrum which appears as a singlet. Carbons 12 and 12' appear as a more narrow set of doublets of doublets centered at 111.8 ppm ($J = 29.58\text{Hz}$) which is also due to splitting from nearby fluorine atoms. Another triplet signal can be seen at 130.52 ppm ($J = 40.59\text{Hz}$) for carbon 13 which is a direct results of splitting by the fluorine atoms again located on carbons 10 and 11 which are *meta* to carbon 13. Carbons 4-6 fall into the expected range for the isatin and which are at 138.4, 125.3, and 123.8 ppm respectively. The methylene carbon 8 is found within the aliphatic region of the spectrum at 32.2 ppm.



**1-(2,6-dichlorobenzyl)
indoline-2,3-dione (82)**



**1-(2,6-dichlorobenzyl)-5-
chloroindoline-2,3-dione (90)**

Compound **82** shown above was recrystallized from hexanes and chloroform as bright orange miniscule crystals. This molecule is similar to the difluoro derivative **83**, but instead incorporates slightly larger chlorines to resemble the electrophilic moiety present in Q-VD-OPh which might also be able to interact with the active-site of a targeted enzyme. The ^1H NMR spectrum of **82** was done using CDCl_3 as the solvent. The proton spectrum of compound **82**

appears to be similar to that of 1-(2,6-difluorobenzyl)indoline-2,3-dione (**83**) however there are some slight differences. Similar to the spectrum of the previous compound there is a doublet of doublets at 7.61 ppm ($J = 7.47$ Hz, 0.75Hz) which is due to proton 4 and its *meta* splitting to proton 6, and soon after there is a triplet of doublets signal at 7.46 ppm ($J = 7.83$ Hz, 1.35Hz) due to proton 5 with *meta* splitting to proton 7. However, unlike the previous compound, instead of a multiplet, two doublets appear at 7.39 ppm ($J = 7.92$ Hz) and also at 7.28 ppm ($J = 7.23$ Hz) (which are slightly overlapping with the following triplet signal) due to protons 11 on the benzyl phenyl ring. Likewise, the triplet signal which corresponds to the benzyl phenyl ring for proton 12 appears at 7.26 ppm ($J = 6.51$ Hz). Proton 7 appears as a doublet at 6.75 ppm ($J = 7.89$ Hz) and proton 6 appears as a triplet of doublets signal at 7.08 ppm ($J = 7.56$ Hz, 0.57Hz). Lastly, as expected, protons 8 appear as an intense singlet at 5.26 ppm.

Compound **90** was prepared by refluxing 5-chloroisatin in acetonitrile with the KF/alumina base and 2,6-dichlorobenzyl bromide for 24 h to yield reddish-orange crystals. The ^1H NMR spectrum of **90** was obtained using DMSO- d_6 as the solvent. There is a doublet of doublets signal at 7.71 ppm ($J = 8.49$ Hz, 2.30Hz) due to proton 5 which experiences *meta* splitting with proton 4. Likewise proton 4 appears as a singlet signal which also shows *meta* splitting at 7.66 ppm ($J = 2.32$ Hz). Unlike the proton spectrum for compound **82**, instead of two doublets for protons 11, there exists only one large and intense doublet signal at 7.54 ppm ($J = 8.01$ Hz) due to the symmetrical protons 11' coupling with proton 12. Proton 12 appears as a triplet signal at 7.42 ppm ($J = 7.92$ Hz) and finally there is an intense singlet signal at 5.13 ppm due to the pair of protons 8.

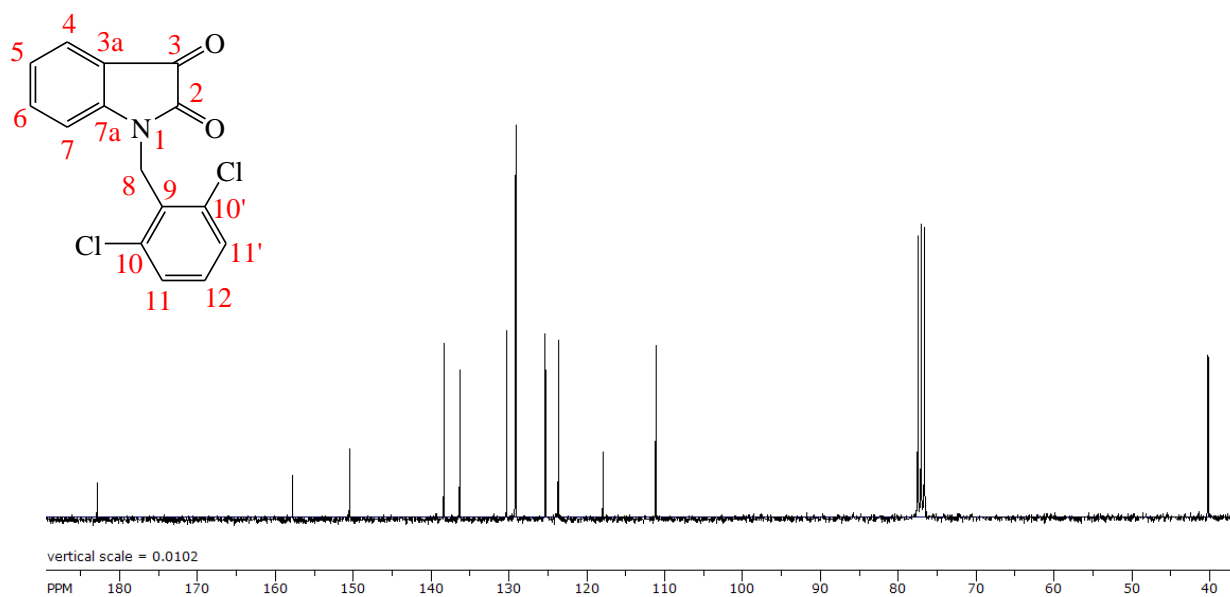
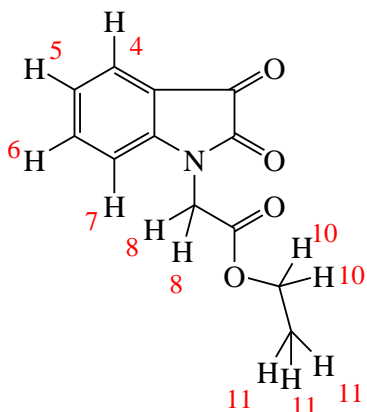
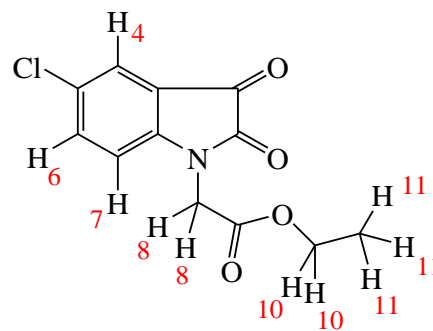


Figure 10

The ^{13}C spectrum of 1-(2,6-dichlorobenzyl)indoline-2,3-dione **82** was obtained in CDCl_3 and shows 13 signals in **Figure 10**. The carbons labeled 2-7a on the isatin core were observed to be very similar to the values reported in previous work done by Gassman.¹² The dichloro-substitution on the benzyl ring had contrasting effects when compared to the carbon shifts for the difluoro-moiety. The quaternary carbon 9 which appears at 129.2 ppm is overlapped by the symmetrical carbons 11 and 11' which appear as an intense singlet peak at 129.1 ppm, and expectedly disappears in the ^{13}C DEPT spectrum of the molecule. Carbons 10 and 10' are also quaternary carbons which disappear in the DEPT spectrum and both appear as a singlet peak around 136.4 ppm. Carbon 12 exists around 130.3 ppm in the aromatic region of the spectrum, while carbon 8 is shown at 40.3 ppm in the aliphatic area.



**Ethyl 2-(2,3-dioxindolin-1-yl)
acetate (81)**



**Ethyl 2-(5-chloro-2,3-dioxindolin-
1-yl)acetate (89)**

Compound **81** was obtained upon refluxing isatin in acetonitrile with 6 equiv. of KF/alumina base and 1.5 equiv. of ethyl bromoacetate for approximately 21 h until complete formation of product was achieved. After simple filtration and washing, the identity of the product was verified by TLC and melting point analysis and no other purification method was employed. The acetate group on our isatin scaffold was introduced due to its similarity to an aspartic acid residue which is a key recognition motif for protein cleavage by way of presumed effector capsases and due to the susceptibility of the carbonyl to be attacked by a cysteine nucleophile. The ^1H NMR spectrum of compound **81** was obtained using CDCl_3 as the solvent. There is a doublet of doublets signal at 7.64 ppm ($J = 7.56\text{Hz}, 0.75\text{Hz}$) which is due to the aromatic proton 4 *meta* splitting with proton 6. At 7.6 ppm ($J = 7.83\text{Hz}, 1.35\text{Hz}$) a triplet of doublets signal appears which is also due to an aromatic proton 5 *meta* splitting with proton 7. In addition protons 7 and 6 appear as a doublet at 6.81 ppm ($J = 7.98\text{Hz}$) with no secondary splitting observed, and a triplet of doublets at 7.16 ppm ($J = 7.54\text{ Hz}, 0.72\text{Hz}$), in that order. The symmetrical protons 8 appear as an intense singlet peak at 4.49 ppm, while the aliphatic protons

10 is shown as a quartet at 4.25 ppm ($J = 7.15\text{Hz}$). The three equal protons 11 appear furthest upfield as a triplet signal at 1.29 ppm ($J = 7.08\text{Hz}$).

Compound **89** was prepared by refluxing 5-chloroisatin in acetonitrile with KF/alumina base and ethyl bromoacetate. The ^1H NMR spectrum of **89** was obtained using DMSO- d_6 as the solvent. There is a doublet signal at 7.75 ppm ($J = 8.55\text{Hz}, 2.22\text{Hz}$) due to proton 6, which experiences *meta* splitting with proton 4. The latter appears a singlet signal at 7.67 ppm ($J = 2.01\text{Hz}$). Another doublet signal is seen at 7.25 ppm ($J = 8.46\text{Hz}$) due to proton 7 coupling with proton 6. The protons 8 are seen as an intense singlet peak in the aliphatic region of the spectrum at 4.63 ppm. In the same region, protons 10 couple with protons 11 and the signal for the former appears as a quartet at 4.17 ppm. Lastly, furthest upfield exists a triplet signal due the protons 11 at 1.22 ppm. Not surprisingly the shifts for **89** compare very closely to those of compound **81**, with the exception of the absence of the proton substituted by the chlorine atom.

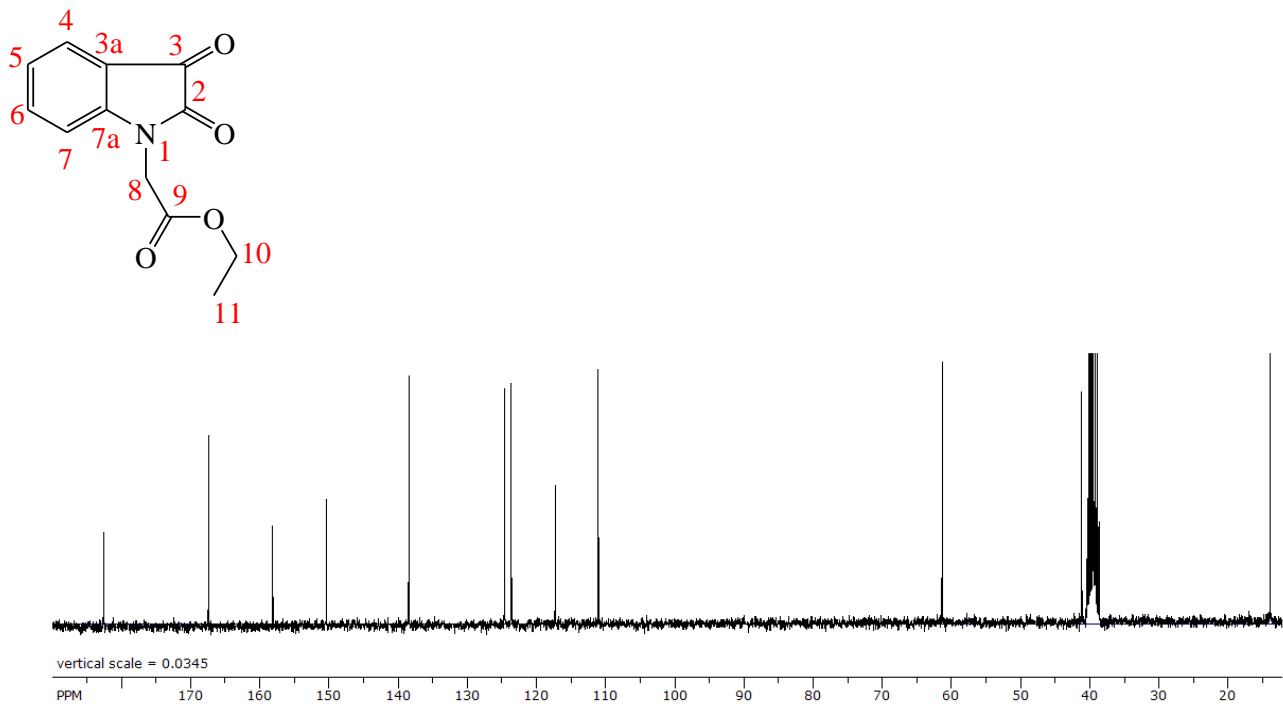
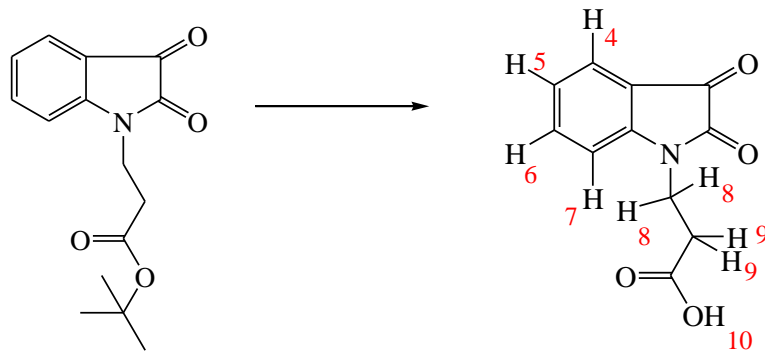


Figure 11

The ^{13}C spectrum of compound **81** (shown in **Figure 11** above) was obtained in $\text{DMSO-}d_6$ and showed 12 signals for 12 carbons in the molecule. Not surprisingly the carbons labeled 2-7a are all very similar to literature values based on the isatin core as reported in previous work by Gassman et al.¹² Moreover, carbons 8, 10, and 11 all appear in the aliphatic region of the ^{13}C spectrum at 41.2, 61.3, and 14.0 ppm, respectively. Carbon 9 appears much further downfield at 167.34 ppm due to the strong electronegative pi cloud from the oxygen atom which is double bonded to the carbon causing strong electron withdrawing effects.



84

tert-butyl 3-(2,3-dioxindolin-1-yl)propanoate (**85**)

Compound **85** was prepared by refluxing isatin acetonitrile with KF/alumina base and *tert*-butyl acrylate for approximately 1.5 h to give a reddish-brown oil which was purified by column chromatography using silica and identified as the *tert*-butyl compound **84** by GC/MS analysis. After standing for some time, the oil was recrystallized from 2-propanol to give only 18% yield of what was later determined to be compound **85**. At this point it is not clear at which point the whether the *tert*-butyl group of compound **84** was hydrolyzed into the carboxylic, assuming both compounds were not initially produced from this reaction. Unlike the previous S_N2 reactions, the mechanism for this specific molecule occurs by way of a Michael addition, in which the nitrogen nucleophile attacks the β-carbon of the α,β-unsaturated carbonyl of the acrylate. Furthermore, this compound may have potential as a caspase inhibitor due to its susceptibility to be attacked by a cysteine nucleophile at the carbonyl. The ¹H NMR spectrum of compound **85** was obtained using DMSO-*d*₆ as the solvent. There is a singlet signal at 12.41 ppm which is due to the hydroxyl proton 10 of the carboxylic acid. At 7.66 ppm (*J* = 7.76Hz) a triplet signal appears which is due to proton 5 which couples with proton 4 seen as a doublet signal at 7.54 ppm (*J* = 7.32Hz). At 7.12 ppm (*J* = 7.47Hz) a triplet signal appears due to proton 6 which couples with protons 5 and 7, in which proton 7 appears as a doublet signal at 7.24 ppm

($J = 7.89\text{Hz}$). Protons 8 and 9 appear in the aliphatic region of the spectrum both as triplet signals at 2.62 ppm ($J = 7.19\text{Hz}$) and 3.89 ppm ($J = 7.28\text{ Hz}$) respectively. The ^{13}C spectrum of compound **85** (shown in **Figure 12** below) was obtained in $\text{DMSO-}d_6$ and showed 11 signals.

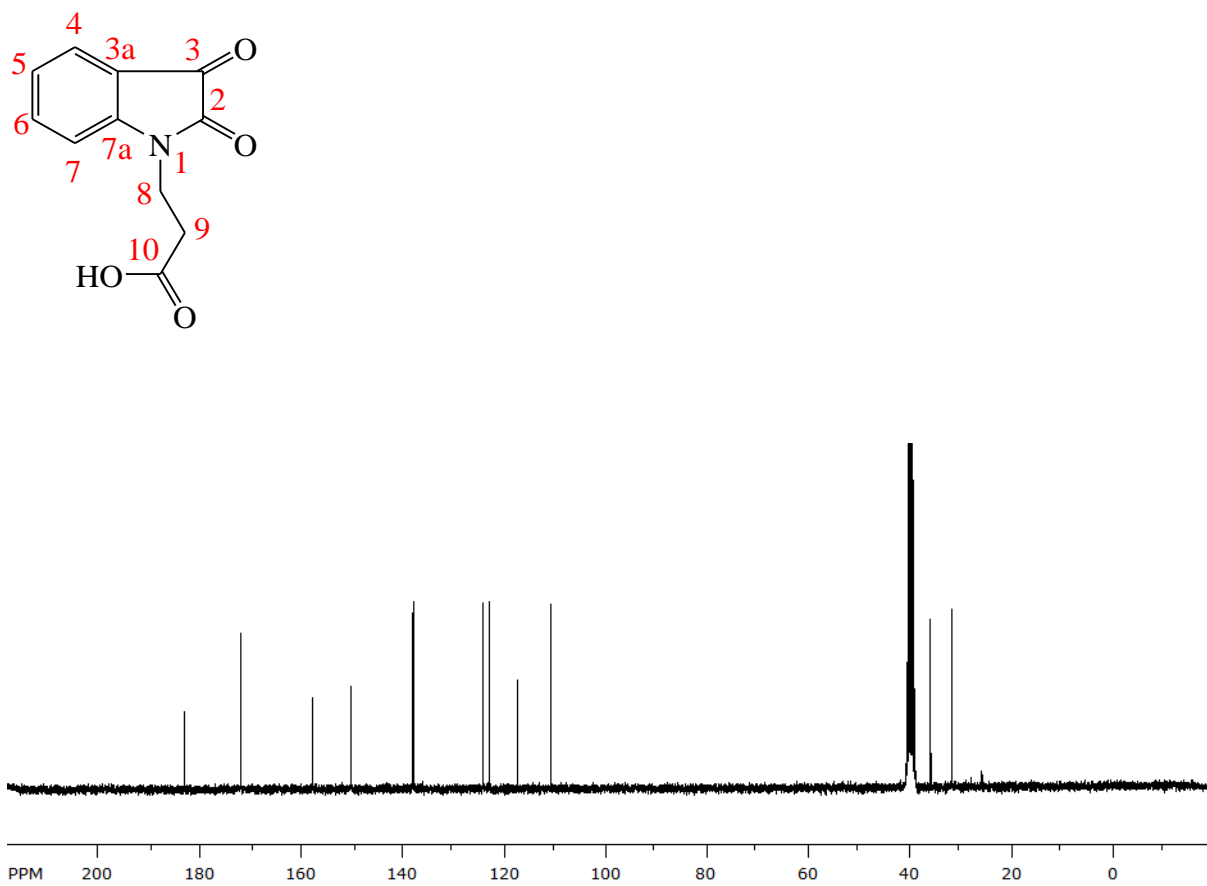
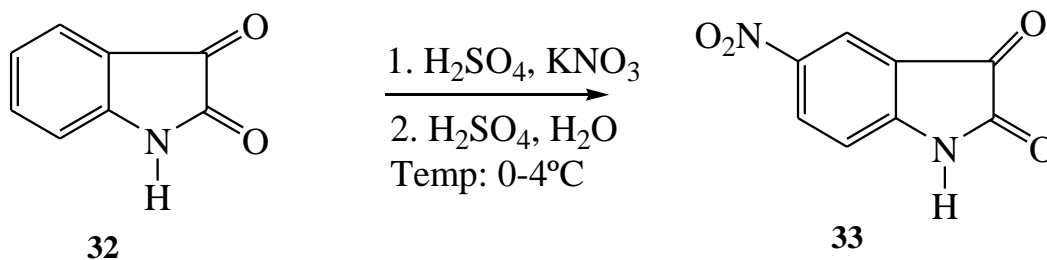


Figure 12

The carbons labeled 2-7a on the isatin core were observed to be very similar to the values reported in previous work done by Gassman et al. and are given in descending order as 183.4, 158.0, 150.4, 138.1, 124.4, 123.1, 117.5, and 110.9 ppm.¹² Additionally, the carbons labeled 8 and 9 appear in the aliphatic region of the carbon spectrum at 31.44 and 35.6 ppm, respectively. The carbonyl carbon of the carboxylic acid appears at 172.21 ppm.

EAS Reactions for Isatins and Oxindole

As mentioned previously, the nitro-group of the “5-nitro-2,6-difluoro” moiety of compound **75** prepared by H. Abdallah (specifically showing up to 5 μM activity, preventing cell death in Jurkat T cells), as well as the efficacy of the 5-nitroisatin lead described by SmithKline Beecham, is thought to play a vital role in the inhibition of caspases. It is not known exactly how or if the nitro-group binds within a specific subsite of a caspase enzyme, however, the nitro-group as well as other strong electron withdrawing groups were desired at the C-5 position of isatin and oxindole derivatives in order to acquire low micromolar and even possibly nanomolar inhibitory concentrations against apoptotic cells. In this research, electrophilic aromatic substitution reactions upon isatin were performed to incorporate a nitro ($-\text{NO}_2$) group at the C-5 position. The reaction proceeded by adding a solution of isatin (**32**) in concentrated sulfuric acid (H_2SO_4) dropwise to a solution of potassium nitrate (KNO_3) in concentrated sulfuric acid at 0°C , over a time period of 1 h.¹⁹ The reaction produced 5-nitro-indoline-2,3-dione (**33**) as an orange solid with a yield of 81% and mp of $249\text{-}254^\circ\text{C}$ which compares exactly to the literature mp of $249\text{-}254^\circ\text{C}$ ⁶⁹.



The 5-nitroisatin was confirmed by ^{13}C NMR analysis in $\text{DMSO-}d_6$ solvent and was compared to the ^{13}C NMR spectra for 5-nitroisatin obtained by Gassman,¹² using $\text{DMSO-}d_6$ solvent, and furthermore both of these spectra were compared to the ^{13}C NMR spectra of isatin (Sigma-Aldrich) (**Table 6** below) to compare chemical shifts created by the addition of the nitro-

group. It was observed that the replacement of the C-5 proton with the nitro group resulted in significant shifts at C-4, C-5, C-6, and C-7 with a difference in electron density of -5.2, +18.0, -3.2, and +4.5, respectively. Furthermore, when comparing the ^{13}C NMR of compound **33** to the ^{13}C of 5-nitroisatin reported by Gassman, each carbon shift matches almost identically with a difference in shift ≤ 0.2 ppm as shown in **Table 6** below.

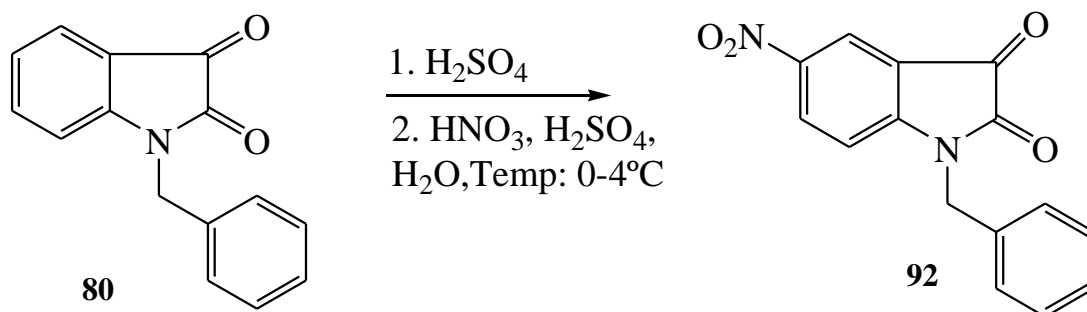
Table 6: Chemical Shifts for Isatin and 5-nitroisatin

	C-2	C-3	C-3a	C-4	C-5	C-6	C-7	C-7a
Isatin	159.3	184.4	117.8	138.3	124.6	122.7	112.2	150.7
5-nitroisatin ¹²	159.9	182.4	118.2	133.2	142.7	119.6	112.6	155.3
Compound 33	159.8	182.4	118.0	133.1	142.6	119.5	112.5	155.2

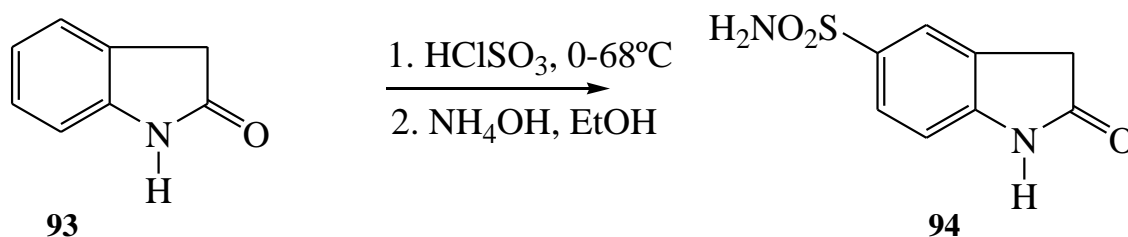
Since a 5-nitro-N-benzylisatin molecule had never been successfully synthesized or tested by the Ketcha research group, the desire to synthesize this molecule was fueled by the promising results obtained by SmithKline Beecham of their 5-nitro-N-benzylisatin compound which provided 0.25 μM activity against caspase-3. In addition, if successfully accomplished, the anticipated compound could be further functionalized at the C-3 position by adding the 2,6-difluorophenyl group via Wolff-Kishner reduction of the C-3 carbonyl, followed by aldol condensation to create the benzylidene oxindole. This molecule could then be tested against Jurkat T cells and compared to compound **75**, which lacks the N-benzyl group. Therefore, the N-alkylation was attempted on 5-nitroisatin (**33**) in order to add functionality upon the core structure. However, after several attempts at N-alkylation of the former molecule using KF/alumina base and benzyl chloride as the alkylating reagent, the reaction was unsuccessful. A

possible explanation could be that the nitroisatin skeleton is susceptible to ring opening at the amide bond in a basic environment, as reported by Y. Torisawa et al.³⁶ The latter group found that attempts at reducing 5-nitroisatin using Red-Al (5 equiv) or NaBH₄ provided ring-opened products.³⁶ However, using a milder base combination such as CuCO₃/Cs₂CO₃ in anhydrous DMF they were indeed successful at alkylating 5-nitroisatin. Thus it can be speculated that the addition of the nitro group compromises the stability of isatin skeleton making it vulnerable to opening, and for future work, a weaker base may be necessary in order to perform *N*-alkylation reactions on 5-nitroisatins.

Due to the failed attempt at alkylating 5-nitroisatin, an alternative route was investigated where the pre-alkylated *N*-benzylisatin was nitrated in order to make the same molecule. The nitration of the *N*-benzylisatin (**80**) was attempted repeatedly using the method prescribed by K. Vine et al.,¹⁹ and furthermore attempted by way of the addition of concentrated nitric acid to a solution of the *N*-benzylisatin (**80**) in sulfuric acid, a method previously employed by Sumpter et al. on oxindole to afford 5-nitrooxindole.⁷⁰ The efforts at isolating 5-nitro-*N*-benzylisatin (**92**) were finally abandoned after numerous repeats of Sumpter's nitration method and since the desired product could not be confirmed by GC/MS or melting point analysis.



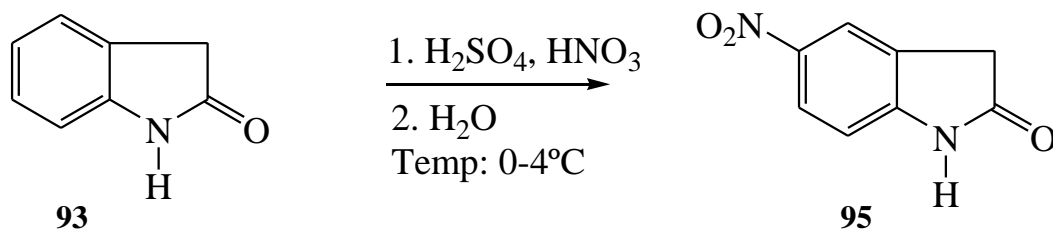
An alternative means of creating an electron withdrawing group at the C-5 position of the isatin/oxindole core can be achieved by synthesizing the oxindole sulfonamide via chlorosulfonation of oxindole as seen below. The multistep process of introducing this functionality on an isatin described earlier suggested the analogous reaction on an isatin would be preferable. If successful, the oxindole sulfonamide provides additional benefits compared to the 5-nitro derivative, given that it is not reducible *in vivo*, and can be further functionalized via a variety of amine groups to afford the corresponding sulfonamides which could possibly allow access to other subsites of a caspase enzyme.



For these reasons, the oxindole sulfonamide was synthesized as opposed to the isatin sulfonamide due to fact that the isatin derivative would eventually be reduced to the oxindole derivative. To this end, oxindole (**93**) was slowly added to highly corrosive chlorosulfonic acid while maintaining the temperature below 30°C for 1.5 h. Subsequently, the solution was heated, then allowed to cool, and finally added to an ammonium hydroxide and ethanol solution and allowed to stir overnight to afford **94**. Unfortunately, the insoluble solid collected by vacuum filtration could not be identified by GC/MS, and furthermore the ^{13}C NMR analysis showed an unidentified compound given by the addition of several peaks which are not consistent to the spectra analysis done by L. Sun et al.⁷¹

Nonetheless, further attempts at incorporating a relatively strong electron withdrawing group onto the isatin/oxindole benzene ring, led now to the direct nitration of oxindole (**93**).

The 5-nitro-oxindole (**95**) was prepared and isolated by way of the Sumpter et al. method⁷⁰ as mentioned above and provided a 23% yield of the desired nitrated product with a mp of 240-243°C, which compares to the literature mp of 240-241°C⁶⁸.



Wolff-Kishner Reduction Results

Following the pathway shown in **Figure 5**, the Wolff-Kishner reaction was performed on selected *N*-alkylated products previously described. Thus, using compounds **80**, **83**, and **88**, the C-3 carbonyl of the isatin scaffold was reduced into a methylene group via the formation of a hydrazone intermediate to give *N*-substituted-indolin-2-ones **96**. The Wolff-Kishner reaction proceeds by refluxing the isatin derivative **6** in 80 % hydrazine-hydrate for 3-5 hours followed by work up in 6N HCl and water to give the oxindole derivatives **96** presented in **Table 7**.

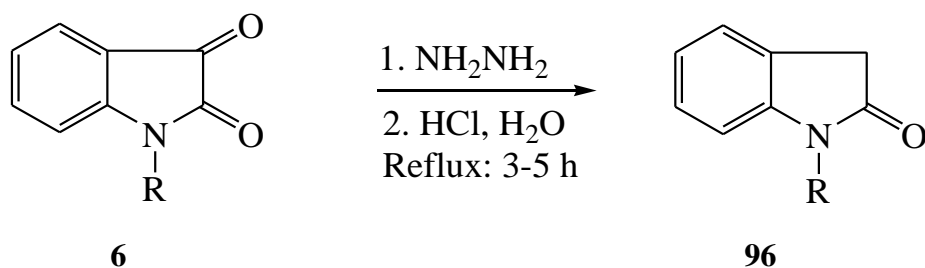
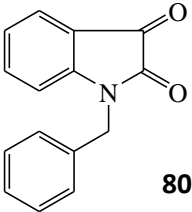
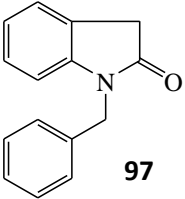
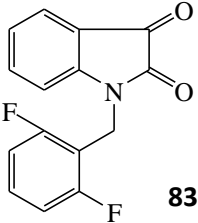
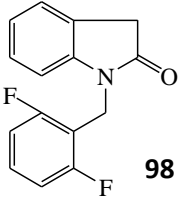
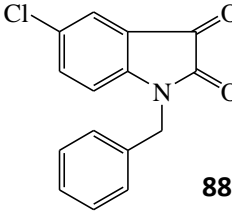
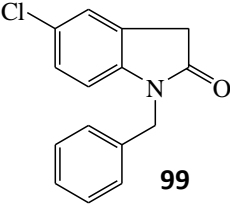


Table 7: N-Substituted-Indolin-2-ones from Wolff-Kishner Reduction

Starting Material	Percent Yield	Product mp	Literature mp	Product
 80	N/A	oil	80-81°C ⁷²	 97
 83	95	157-162°C	N/S	 98
 88	76	103-105°C	103-105°C ⁷²	 99

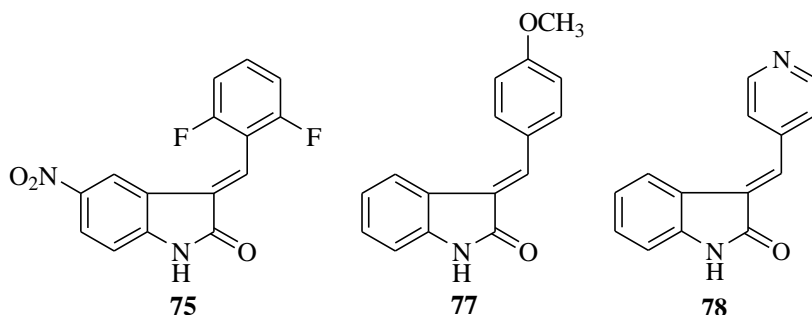
*N/S = product has not been synthesized before in literature

Compound **97** was isolated as an oil and could not be solidified even after several attempts at recrystallization. Therefore, the resultant oil obtained was used to perform the Knoevenagel condensation with 4-pyridincarboxaldehyde in ethanol and a catalytic equivalent of piperidine base. The reaction produced a black “tar-like” oil which was shown to be a mixture of the desired product and starting material by GC/MS analysis. To separate the compounds, a silica gel column was applied using 70% hexanes and 30% ethyl acetate as the mobile phase solvent. Unfortunately, the separation technique proved to be inadequate for the two compounds based on their R_f values.

Aldol Condensation Results

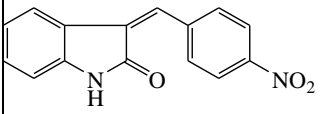
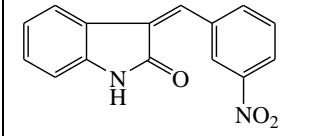
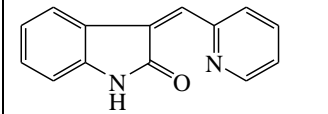
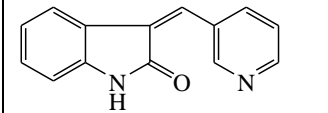
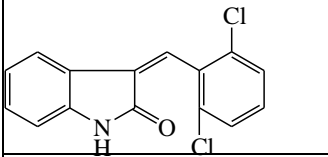
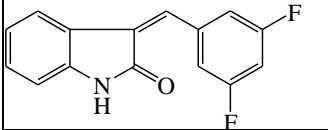
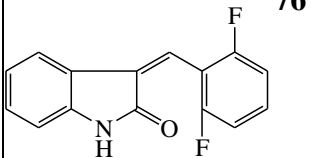
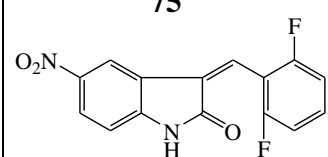
The last synthesis step required to create a library of desired trisubstituted benzylidene compounds involves an aldol or Knoevenagel condensation reaction. Oxindole, N-substituted oxindole derivatives (shown in **Table 7**), as well as 5-nitro-indolin-2-one (**95**) were used to perform the Knoevenagel condensation reaction at the C-3 methylene group, so as to allow for an additional site of diversity which would facilitate regulation of properties such as selectivity amongst caspases. The design of these compounds was also based upon the previously discussed SAR studies of Q-VD-Oph and the micromolar activity demonstrated against Jurkat T cells using compounds **75**, **77**, and **78** (shown below) presented by Hagar Abdallah in previous years. Given the speculation that 5-nitro-2,6-difluoro-indolin-2-one (**75**) was active at 5 μ M due to the electrophilicity created by the strong electron withdrawing effects of the two fluorine atoms extended from the C-3 position, combined with the electron withdrawing effects of the nitro-group at the C-5 position, compound **75** was recreated to determine whether the inhibition activity shown against Jurkat T cells could be repeated, while the 2,6-difluoro derivative lacking the nitro group, **76** was also synthesized as an antagonist to **75**. The 2,6-dichloro analog **107** extended from the C-3 position, held promise as a closely related alternative to the 2,6-difluoro derivative, even though it possesses slightly weaker electron withdrawing effects using similar halogen atoms. The 3,5-difluoro moiety seen in **108** was desired given that it retained the fluorine atoms but arranged at different positions in comparison to the known active compound **75** in aspiration to gain equivalent or enhanced potency against caspases. Ideally, other strong electron withdrawing groups might prove effective and help to understand the expectations needed to increase binding affinity of these small molecules to targeted enzymes and therefore

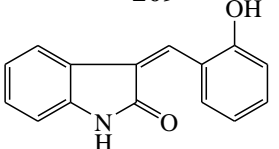
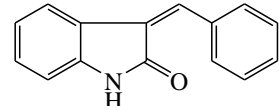
compounds **103** and **104** were of interest due to the positioning of a nitro-group about the benzylidene ring.



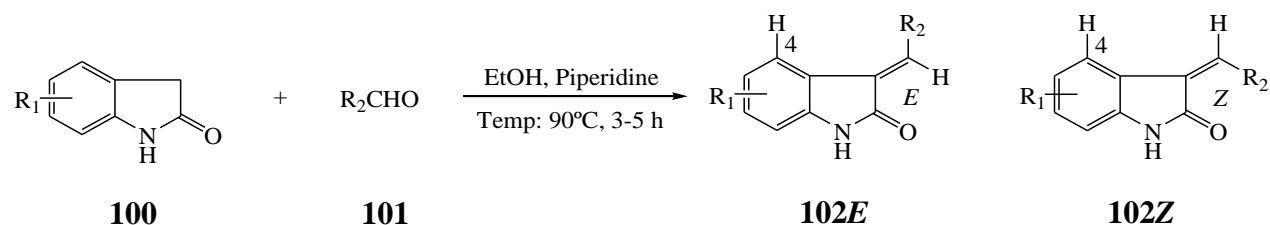
Beyond designing benzylidene oxindole analogs bearing electron-withdrawing substituents which might mimic the effects of the 2,6-difluoro moiety, another set of analogs were prepared which exhibited the hydrogen bond accepting motifs demonstrated by the para-methoxy benzylidene **77** and 4-pyridine benzylidene **78**. Thus, compound **109** was created due to the hydroxyl group which also possesses a lone pair electrons which could partake in hydrogen bonding interactions. Additionally, compounds **105** and **106** incorporated a pyridine ring in which the position of the nitrogen is displaced about the benzylidene ring compared to compound **78**, to survey the optimal location of the nitrogen atom. Finally, compound **110** does not possess any EWG or hydrogen bond accepting groups, but as such could serve as a baseline indicator of the necessity of such. The compounds in **Table 8** below were prepared using the oxindole core **100** with aromatic aldehydes **101** in ethanol with a catalytic amount of piperidine base, usually at 90°C for 3-5 hours to give the 3-substituted indolin-2-ones **75-76** and **103-110** represented by **102E** and **102Z**, depending on their configuration.

Table 8: 3-Substituted-Indolin-2-ones from Knoevenagel Condensations

Compound	Percent Yield	Melting Point	Literature Melting Point	Configuration (Z:E)
103 	82	251-252°C	245-250°C ⁷³	100:0
104 	90	244-246°C	258°C ⁷⁴	60:40
105 	75	206-209°C	202-203°C ⁷⁵	100:0
106 	36	161-165°C	N/S	60:40
107 	57	182-184°C	179-181°C ⁷⁶	100:0
108 	59	206-210°C	202-205°C ⁷⁷	100:0
76 	69	233-238°C	213-218°C ⁷⁷	100:0
75 	48	257-258°C	251-255°C ⁷⁷	100:0

<p style="text-align: center;">109</p> 	44	193-195°C	195°C ⁷⁸	0:100
<p style="text-align: center;">110</p> 	66	172-175°C	180-181°C ⁷⁹	0:100

*N/S = product has not been synthesized before in literature



The 3-substituted indolin-2-ones **102** may exist in either the *E* or *Z* isomer about the exocyclic double bond depending on the characteristics of the substituents at the C-3 position.

Identification of the *E* or *Z* isomers was determined using ¹H NMR analysis due to the chemical shifts of the aromatic protons on the benzylidene ring (H-2' and H-6') or *ortho*-arylidene protons and the vinylic proton being significantly influenced by configuration. The vinylic proton (H_{vin}) is more deshielded in the *E*-isomer due to the influence of the C-2 carbonyl just as the *ortho*-benzylidene protons (H_o) are shifted downfield due to the C-2 carbonyl as shown in **Figure 13** below. 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.⁷⁵ In some cases where the H-2' and H-6' positions were substituted as in compounds **107**, **75**, and **76**, 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.⁸⁰

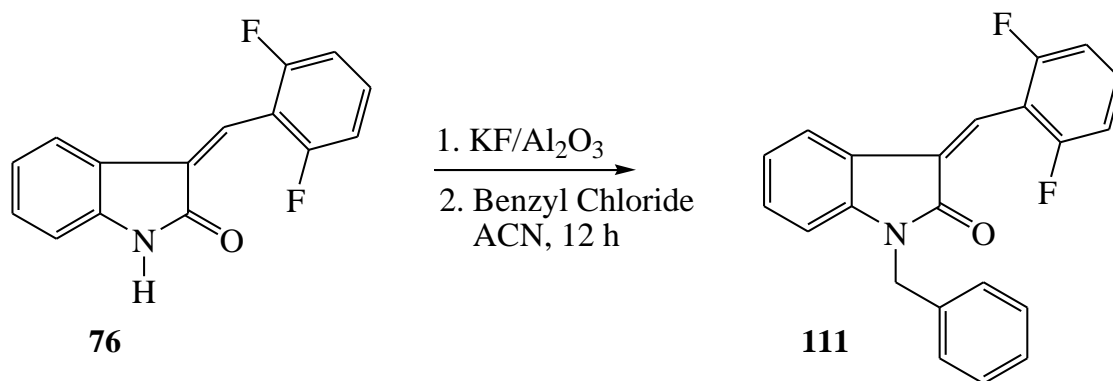
Figure 13-E/Z Configuration of 3-Substituted Benzylidenes



In addition to the deshielding effects created by the carbonyl, other factors may influence the configuration of the benzylidene compounds. For example, in the case of **110**, the *E* isomer is favored due to steric repulsion between the carbonyl group and the H-2' and H-6' protons of the phenyl ring in the *Z* form. Compound **109** tends to favor the *E* configuration as well which may be due to electrostatic repulsion between the C-2 carbonyl and the oxygen attached to the phenyl ring. However, most of the compounds observed showed 100% of the *Z* isomer which could be due 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, -Cl, and -NO₂ upon the benzylidene ring. Compounds **106** and **104** possess either a nitrogen substitution in place of the third carbon incorporated into the phenyl ring (**106**) or an addition of atoms at the third carbon of the phenyl ring (**104**), and both provide a 60:40 ratio of the *Z* to *E* isomer based on the ¹H NMR analysis which could possibly be supported by the fact that previous investigators have observed equilibrium between the *Z* and *E* isomer forms in polar solvents such as DMSO or methanol, or in the presence of light.⁸¹

The design feature of incorporating the 2,6-difluorobenzyl moiety exhibited in compounds **76** and **98** was based on a structural resemblance to the “warhead” of the protease inhibitor Q-VD-OPh⁵⁵ which possibly forms covalent interactions with the cysteine residue of a

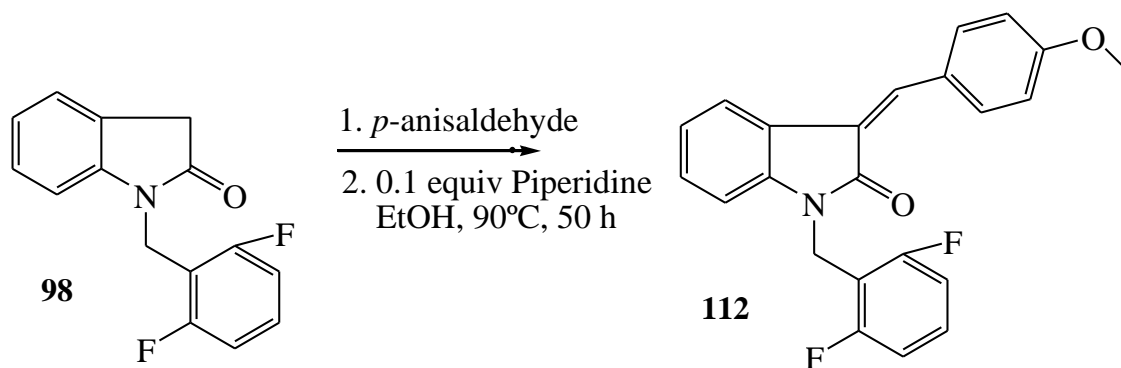
caspace enzyme due to its electrophilic nature. Although compound **76** was found ineffective as a cell death inhibitor, it was thought that the N-alkylation of this substrate using benzyl chloride might result in an enhancement in inhibitory properties since N-alkylation of isatin sulfonamides usually led to approximately ten fold increase in activity. Thus, compound **76** was refluxed in acetonitrile using KF/alumina base for 12 h. The reaction produced an orange oil which was then triturated with several milliliters of methanol to yield orange crystals of **111** in 39% yield.



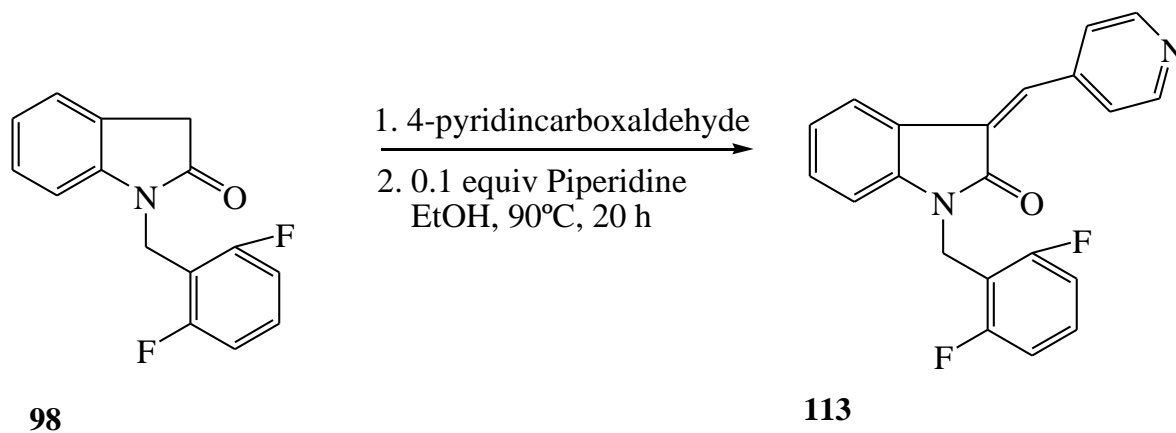
In an effort to utilize the activity of 3-((pyridine-4-yl)methylene)indolin-2-one (**78**), and its positional isomers **105** and **106**, as well as possibly increase the potency of the benzylidene derivatives, the N-alkylation of compound **78** was attained using 2,6-difluorobenzyl bromide. The reaction was allowed to reflux in acetonitrile using KF/alumina base for up to 5 days while being monitored by TLC analysis periodically. However, despite this prolonged reaction time the desired compound **113** was not isolated. The next step was to attempt the isolation of the compound where by the aldol condensation reaction would be done in the last step.

Given the demonstrated activity exhibited by 3-(4-methoxybenzylidene)indolin-2-one, it was thought likely that introduction of the difluorophenyl moiety on this substrate would result in enhanced activity. Thus, compound **98** was used to form 1-(2,6-difluorobenzyl)-3-(4-

methoxybenzylidene)indolin-2-one (**112**) by way of Knoevenagel condensation with *p*-anisaldehyde as shown below. As described earlier, this molecule incorporates seemingly important features such as a hydrogen bond acceptor motif at the C-3 position due to the *para*-methoxy group, along with the difluorophenyl pattern exhibited by Q-VD-OPh.⁶⁵



Although it was not possible to N-alkylate 3-((pyridine-4-yl)methylene)indolin-2-one (**78**) with 2,6-difluorobenzyl bromide, the synthesis of the desired target compound could be achieved by condensing **98** with 4-pyridinecarboxaldehyde, using 0.1 equiv. piperidine as a catalyst for 20 h yielding **113** in 53% yield.



Biological Activity

From the outset, the unexpected inhibitory activity exhibited against Jurkat T cells in vitro by the 3-((pyridine-4-yl)methylene)indolin-2-one (**78**)⁷⁷ wherein no electron withdrawing substituent was necessary at the C-5 position warranted an examination of the positional isomers, namely compounds **105** and **106**. The 4-pyridine compound showed complete inhibition of apoptosis at 50 μ M and only partial inhibition at 20 μ M concentrations. Compounds **105** and **106** were prepared as described and sent to Dr. Thomas L. Brown at the Wright State University Department of Neuroscience, Cell Biology, and Physiology for biological screening. The biological assay was performed to verify if indeed the compounds being tested could inhibit apoptotic cell death. The tests conducted were blind tests, meaning that the researchers doing the screening were prevented from knowing the identities of the compounds. The screening was also performed by different people at different times to give consistent results. The gels for compounds **106** and **105** are shown in **Figure 14** and **Figure 15**, respectively.

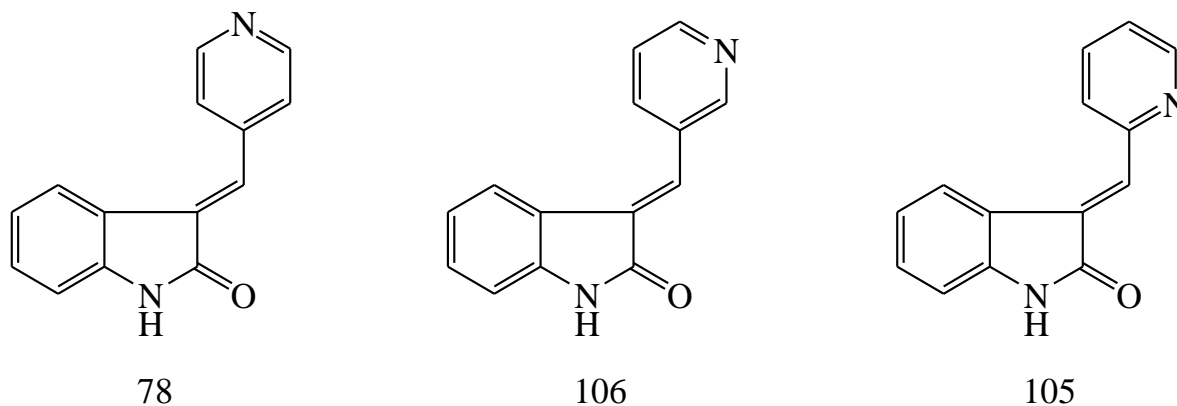
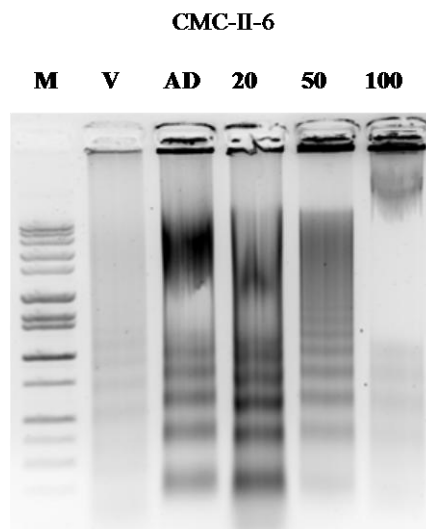


Figure 14: DNA laddering for Compound 106



6.46 x 10⁶ cells/ml of Jurkat cells

M- Marker

V-Vehicle: 20μl DMSO & 10μl Methanol

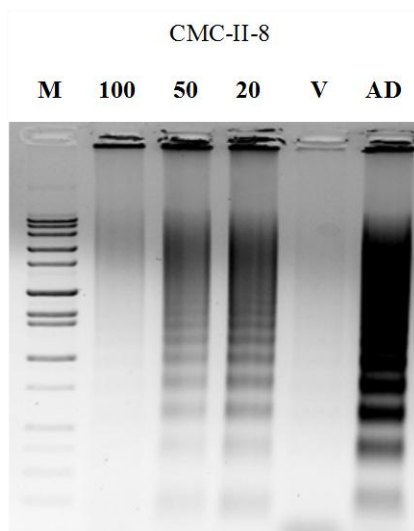
AD- 10μl AD

20μM- 4μl CMC-II-6 & 10μl AD

50μM- 10μl CMC-II-6 & 10μl AD

100μM- 20μl CMC-II-6 & 10μl AD

Figure 15: DNA laddering for Compound 105



5.5 x 10⁶ cells/ml of Jurkat cells

M- Marker

V-Vehicle: 20µl DMSO & 10µl Methanol

AD- 10µl AD

20µM- 4µl CMC-II-8 & 10µl AD

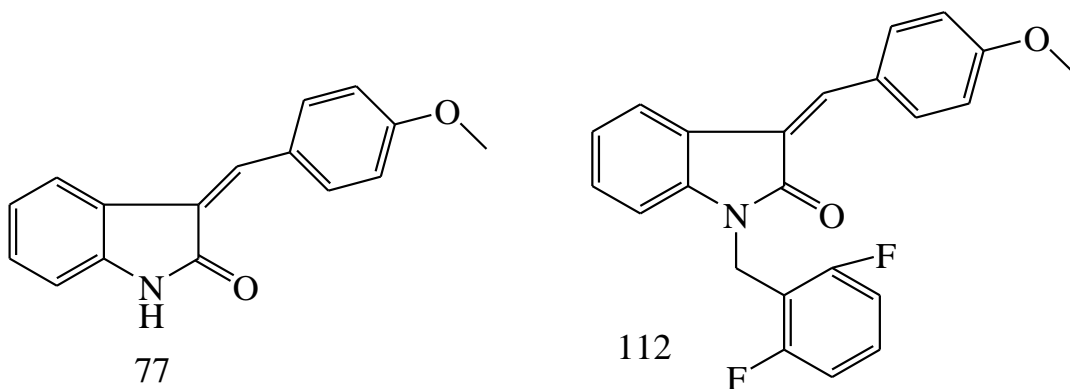
50µM- 10µl CMC-II-8 & 10µl AD

100µM- 20µl CMC-II-8 & 10µl AD

The “M” channel is the DNA marker channel, which shows the DNA laddering of 180-200 base-pair DNA fragments being tested. The “V” channel contains DMSO and methanol solvent and the cells being tested only. The “AD” channel contains cells that are treated with the Actinomycin D protein, which is a DNA dependent inhibitor of RNA synthesis and acts as a cytotoxic inducer of apoptosis. The channels that are marked with 20, 50 and 100 are the micromolar concentrations of the compound being tested and the assays were treated for four

hours. In each example the disappearance of the dark laddering at 100 μM translates to activity, which means that apoptosis was prevented in the Human Jurkat T cells (Type 2 cells) at 100 μM concentrations of the compound. Based on the results it was hypothesized that the lone pair of electrons on the nitrogen atom of the benzylidene ring is involved in hydrogen bonding with the active site of the caspase and also resembles the quinoline ring of the known peptide inhibitor Q-VD-OPh.⁵⁶

As discussed earlier, the idea for synthesizing compound **112** was based on the fact that 3-(4-methoxybenzylidene)indolin-2-one (**77**) was previously found to be active against Jurkat T cells at approximately 50 μM . It was hypothesized that incorporating the 2,6-difluoro moiety at the nitrogen would possibly increase the potency versus the Actinomycin D induced apoptotic cells. Unfortunately, **112** tested inactive against the Jurkat T cell assay, and the addition of the 2,6-difluorobenzyl group appeared to weaken the affinity of the *para*-methoxybenzylidene compound for the active site.



Likewise, given the demonstrated activity of 3-((pyridine-4-yl)methylene)indolin-2-one (**78**), it was deemed important to examine the inhibitory properties of the analog possessing the biologically relevant 2,6-difluorophenyl moiety. The appropriately designed derivative (e.g.,

113) was therefore prepared and sent to Dr. Brown for biological screening and it was found to inhibit the cell death of 100% of Jurkat T cells at 20 μ M (as shown in **Figure 16**) and was further evaluated and found to be partially active at 10 μ M (laddering analysis not shown).

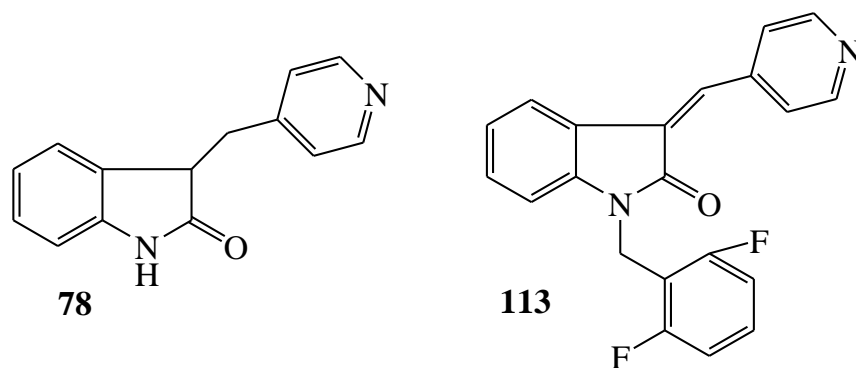
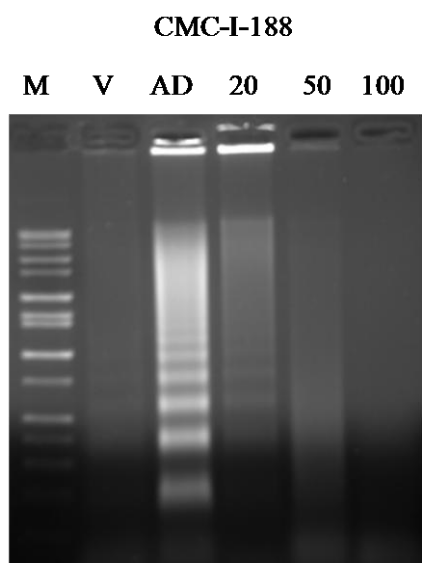


Figure 16: DNA laddering for Compound 113



6.6 x 10⁶ cells/ml of Jurkat cells

M- Marker

V-Vehicle: 20 μ l DMSO & 10 μ l Methanol

AD- 10 μ l AD

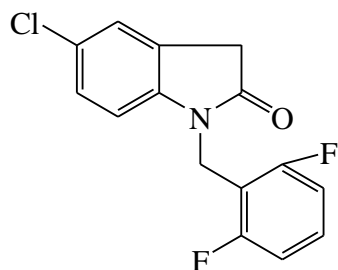
20 μ M- 4 μ l DK Chem CMC-I-188 & 10 μ l AD

50 μ M- 10 μ l CMC-I-188 & 10 μ l AD

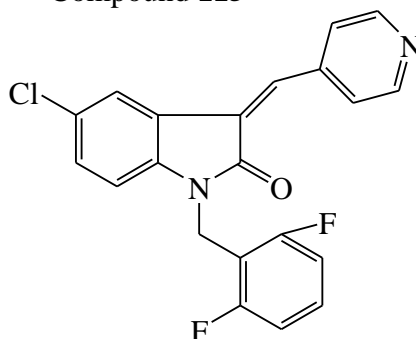
100 μ M- 20 μ l CMC-I-188 & 10 μ l AD

The cell death inhibitory properties of compound **113** as evidenced from the gel above were interesting in that this was the first oxindole derived inhibitor with two points of variability to exhibit substantial biological activity against Jurkat T cells. To compare and understand further how diverse functional groups extended from the isatin/oxindole scaffold work together to maximize binding affinity to caspases, an electron withdrawing group was needed at the C-5 position of **113**. Thus compound **115** was synthesized by Paul Repasky (colleague) by way of refluxing 1-(2,6-difluorobenzyl)-5-chloroindolin-2-one (**114**) with 4-pyridincarboxaldehyde in ethanol. Biological screening of compound **115** against Jurkat T cells showed that compound **115** possessed 90% inhibition of cell death at 10 μ M.

Compound **114**



Compound **115**



Even though the results for compound **115** only showed an approximately 70-90% increase of inhibition compared to compound **113**, this was a remarkable finding. Adding an additional point of functionality to the isatin/oxindole core provided the ability to create a larger

library of compounds that would aid in the quest to find a potent caspase inhibitor for drug discovery. Not only was this true, but the “3-points of variability” idea was first introduced by Dr. Ketcha’s group at Wright State University and seems to have led to the preparation of increased potency of a small molecule like caspase inhibitor.

Summary and Conclusion

Initially, small scale microwave irradiation reactions were performed to probe for optimum parameters in order to acquire 100% completion for the generation of *N*-alkylated isatin derivatives. Ultimately these reactions were employed via conventional heating in the first step of a sequence designed to create a diverse library of compounds possessing the characteristics of the peptide inhibitor Q-VD-Oph as well as features common to the analogous isatin sulfonamide inhibitors. Furthermore, the Wolff-Kishner and aldol condensation reactions were employed in addition to *N*-alkylation reactions to ultimately synthesize targeted tri-substituted compounds. The ultimate targets, namely oxindole benzylidene compounds possessing up to 3-points of variability were then screened for biological activity against Jurkat T cells. Thorough NMR analysis was performed on the *N*-alkylated products as well as all of the compounds presented in this study to understand the electronic effects of substituents attached to the isatin/oxindole scaffold and how the NMR analysis results of both active and non-active compounds could possibly be used to assess and direct the synthesis of targeted compounds to enhance inhibition activity.

In previous years, H. Abdallah showed that the 4-pyridyl analog **78** was active against Jurkat T cells at 50 μ M presumably via hydrogen bonding interactions within an unknown

enzyme subsite. The present study sought to enhance the inhibition activity of the 4-pyridyl benzylidene oxindoles by adding a 2,6-difluorobenzyl group at the nitrogen. In this study, further steps were taken to assess the position of the nitrogen atom about the pyridine ring which led to the 2-pyridine and 3-pyridine moieties, **105** and **106**, respectively, which were both screened against Jurkat T cells for comparison to the known inhibitory effects of the 4-pyridine lead **78**, which in the end proved to be the most potent compound amongst the three positional isomers. Due to this fact, the next important objective was to perform an N-substitution reaction on the 4-pyridine benzylidene for which the resultant product **113** was desired to possibly show enhanced inhibition concentrations against Jurkat T cells. Based on the DNA laddering gel results, the potency of the former compound was indeed increased by approximately 2.5-fold but still only exhibited 2-points of variability. A third point of functionality of the former compound was utilized at the C-5 position about the oxindole core, incorporating a chlorine atom, which is important due to its electron-withdrawing ability and anticipated metabolic stability, to ultimately generate the desired tri-substituted analog, **115**, showing a nearly 2-fold increase in potency versus the “2-point” derivative. Unfortunately, there were no mechanistic or toxicity studies performed and it is not currently known exactly how the former active compounds bind within an enzyme sub-site. However, in this report, the importance of isatin and oxindole derivatives ranging from mono-substituted (i.e., 3-substituted benzylidene compounds) to tri-substituted compounds was demonstrated to possess novel pro-apoptotic properties as potentially useful therapeutic agents. Moreover, the compounds generated in this report, along with composed analytical data, and correlating biological findings are significant to the advancement of this research and its entirety can be utilized as a “stepping stone” for the continuing efforts and discovery of small molecular based cell death inhibitors.

Future Work

In the near future a library of molecules should be created that will be further utilized and assessed for biological activity not only towards Jurkat T cells, but other lines of cells to assess the known and anticipated activity of these simple heterocyclic molecules. The next generation of these molecules should provide four or more points of variability which will allow for a larger quantitative molecular library which will provide more opportunities for fine tuning and designing molecular species to achieve their highest potential as caspase inhibitors.

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 ^1H and ^{13}C NMR data were obtained on a Bruker Avance 300 MHz NMR in CDCl_3 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".

5-Nitroindoline-2,3-dione (33)

To a 50 mL Erlenmeyer flask in an ice bath, was added a solution of isatin (6.86 mmol, 1.01 g) in concentrated H₂SO₄ (6.4 mL) dropwise to a solution of KNO₃ (6.80 mmol, 0.688 g) in concentrated H₂SO₄ (7.6 mL) over a period of 1h. The reaction mixture was allowed to stir for 30 min. and then poured over ice. An orange solid was collected by vacuum filtration. The product was then recrystallized from ethanol to give an orange solid (1.06 g, 81%); mp 249-254°C, (lit.¹²mp 252-254°C); R_f = 0.23 (EtOAc/hexanes, 1:1); ¹H NMR (300 MHz DMSO-*d*₆) δ 11.66 ppm (s, N-H), 8.44 (dd, *J* = 8.64, 1.71Hz, 1H), 8.20 (s, *J* = 2.01Hz, 1H), 7.09 (d, *J* = 8.67Hz, 1H); ¹³C NMR δ: 182.3, 159.8, 155.2, 142.6, 133.1, 119.5, 118.0, 112.5; MS (m/z): 192 (M⁺), 164 (100%).

(Z)-3-(2,6-Difluorobenzylidene)-5-nitro-indolin-2-one (75)

To a vial was added 5-nitro-indolin-2-one (1.133 mmol, 0.2018 g) with 2,6-difluorobenzaldehyde (1.2 equiv., 1.36 mmol, 0.1932 g, 0.147 mL), piperidine (0.1359 mmol, 0.0097 g, 0.0112 mL) and EtOH (12 mL). The vial was placed on a hot plate at 90°C for 2h while stirring. A light green precipitate (0.1647 g, 48%) was collected by vacuum filtration: mp 257-258°C; (lit.⁷⁷mp 251-255°C); R_f = 0.73 (EtOAc/hexanes, 1:1); ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.45 (s, N-H), 8.22 (d, *J* = 8.67Hz, 1H), 7.75-7.65 (m, 3H), 7.58 (s, H_{vinyl}), 7.37 (t, *J* = 8.31Hz, 1H), 7.08 (d, *J* = 8.58Hz, 1H); ¹³C NMR δ: 167.8, 161.3, 157.9, 148.9, 141.9, 133.0, 130.2, 127.2, 123.3, 120.9, 118.3, 112.3, 111.3, 110.3 ppm; MS (m/z): 302 (M⁺, 100%).

(Z)-3-(2,6-Difluorobenzylidene)indolin-2-one (76)

To a 50 mL erlenmeyer flask was added oxindole (7.510 mmol, 1.00 g) with 2,6-difluorobenzaldehyde (1.2 equivalents, 9.01 mmol, 0.9725 mL), piperidine (0.1 equivalents, 0.7510 mmol, 0.0743 mL) and EtOH (25 mL). The flask was placed on a hot plate at 90°C for 3 h while stirring. A yellow precipitate was collected by vacuum filtration and then (1.3309 g, 69%): mp 233-238°C, (lit.⁷⁷ mp 213-218°C); $R_f = 0.37$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 10.72 (s, N-H), 7.65-7.55 (m, 3H), 7.35 (s, H_{vinyl}), 7.29 (t, $J = 8.46\text{Hz}$, 1H), 6.88 (d, $J = 8.13\text{Hz}$, 1H); $^{13}\text{C NMR}$ δ : 167.5, 161.3, 158.0, 143.2, 132.3, 132.0, 130.8, 123.1, 121.5, 120.7, 119.7, 112.1, 112.0, 110.1 ppm; MS (m/z): 257 (M^+ , 100%).

1-Benzylindoline-2,3-dione (80)

To a solution of isatin (7.08 mmol, 1.042 g) in acetonitrile (70 mL), was added 15 equiv. of KF/alumina (42.3 mmol, 6.47 g) and the resulting mixture was allowed to stir for 5 min until the initial orange solution turned to a brownish color. Benzyl chloride (1.5 equiv., 10.62 mmol, 1.22 mL) was then added dropwise to the stirred solution after which time the mixture was then refluxed under acetonitrile for 2 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then evaporated under reduced pressure to afford a solid which was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as orange crystals (1.47 g, 81%): mp 130-132°C; (lit.⁶⁸ mp 126-127°C); $R_f = 0.4$ (EtOAc/Hexanes, 1:1); $^1\text{H NMR}$ (300 MHz CDCl_3) δ 7.62 ppm (dd, $J = 7.2\text{Hz}$, 0.75Hz, 1H), 7.5 (td, $J = 7.83\text{Hz}$, 1.32Hz, 1H) 7.41-7.3 (m, $5 \times \text{Ar-H}$), 7.10 (td, $J = 7.53\text{Hz}$, 0.75Hz, 1H), 6.8 (d, $J = 7.92\text{Hz}$, 1H), 4.95 (s, 1H); $^{13}\text{C NMR}$ δ : 183.2, 158.3, 150.7, 138.3, 134.5, 129.1, 128.2, 127.4, 125.4, 123.8, 117.7, 111.0, 44.1 ppm; MS (m/z): 237 (M^+), 146 (100%).

Ethyl 2-(2,3-dioxindolin-1-yl)acetate (81)

To a solution of isatin (3.4 mmol, 0.503 g) in acetonitrile (35 mL), was added KF/alumina (20.39 mmol, 3.201 g) and the resulting mixture was allowed to stir for 5 min until the initial orange solution turned to a brownish color. Ethyl bromoacetate (1.5 equiv., 5.09 mmol, 5.28 g, 0.567 mL) was pipetted into the round bottom flask after which the mixture was then refluxed under acetonitrile for 21 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then allowed to dry under the fume hood overnight to afford the pure (TLC, GC/MS) yellow solid product (0.77 g, 97%): mp 124-129°C; (lit.⁶⁸ mp 132-133°C); $R_f = 0.51$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz CDCl_3) δ 7.64 ppm (dd, $J = 7.56\text{Hz}$, 0.75Hz , 1H), 7.6 (td, $J = 7.83\text{Hz}$, 1.35Hz , 1H), 7.16 (td, $J = 7.54\text{Hz}$, 0.72Hz , 1H), 6.81 (d, $J = 7.98\text{Hz}$, 1H), 4.49 (s, 2H), 4.25 (q, $J = 7.15\text{Hz}$, 2H), 1.29 (t, $J = 7.08\text{Hz}$, 3H); $^{13}\text{C NMR}$ (300 MHz $\text{DMSO-}d_6$) δ : 182.6, 167.3, 158.2, 150.3, 138.5, 124.5, 123.7, 117.2, 111.2, 61.3, 41.2, 14.0 ppm; MS (m/z): 233 (M^+), 132 (100%).

1-(2,6-Dichlorobenzyl)indoline-2,3-dione (82)

To a solution of isatin (3.39 mmol, 0.5 g) in acetonitrile (115 mL), was added KF/alumina (20.4 mmol, 3.12 g) and the resulting mixture was allowed to stir for 5 min until the initial orange solution turned to a brownish color. Then 2,6-dichlorobenzyl bromide (1.5 equiv., 5.1 mmol, 1.22 g) was added to the round bottom flask after which time the mixture was then refluxed under acetonitrile for 8 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then evaporated under reduced pressure to afford a solid which was recrystallized from chloroform/hexanes to afford the pure (TLC, GC/MS) product as orange crystals (3.04 g, 66%): mp 175-180°C; $R_f = 0.73$

(EtOAc/hexanes, 1:1); ^1H NMR (300 MHz CDCl_3) δ 7.61 ppm (dd, $J = 7.47\text{Hz}$, 0.75Hz , 1H), 7.46 (td, $J = 7.83\text{Hz}$, 1.35Hz , 1H), 7.39 (d, $J = 8.31\text{Hz}$, 1H), 7.28 (d, $J = 7.23\text{Hz}$, 1H), 7.26 (t, $J = 6.51\text{Hz}$, 1H), 7.08 (t, $J = 7.56\text{Hz}$, 1H), 6.75 (d, $J = 7.89\text{Hz}$, 1H), 5.26 (s, 2H); ^{13}C NMR δ : 182.9, 157.7, 150.4, 138.4, 136.4, 130.3, 129.2, 129.1, 125.3, 123.6, 117.9, 111.1, 40.3 ppm; MS (m/z): 305 (M^+), 270 (100%); Anal. Calcd for $\text{C}_{15}\text{H}_9\text{Cl}_3\text{NO}_2$: C, 58.85; H, 2.96; N, 4.58 Found: C, 58.77; H, 3.04; N, 4.57.

1-(2,6-Difluorobenzyl)indoline-2,3-dione³⁵ (83)

To a solution of isatin (6.84 mmol, 1.01 g) in acetonitrile (70 mL), was added 15 equiv. of KF/alumina (40.7 mmol, 6.40 g) and the resulting mixture was allowed to stir for 5 min until the initial orange solution turned to a brownish color. Then 2,6-difluorobenzyl bromide (1.5 equiv., 10.2 mmol, 2.11 g) was added to the round bottom flask after which time the mixture was then refluxed under acetonitrile for 20.5 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then evaporated under reduced pressure to afford a solid which was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as brown crystals (1.64 g, 88%): mp 154-156°C; $R_f = 0.46$ (EtOAc/hexanes, 1:1); ^1H NMR (300 MHz CDCl_3) δ 7.60 ppm (dd, $J = 7.47\text{Hz}$, 0.75Hz , 1H), 7.53 (t, $J = 7.83\text{Hz}$, 1H), 7.32 (m, 1H), 7.10 (t, $J = 7.52\text{Hz}$, 1H), 6.94 (t, $J = 8.22\text{Hz}$, 1H), 5.03 (s, 1H); ^{13}C NMR δ : 182.9, 163.2, 159.9, 157.5, 150.4, 138.4, 130.5, 125.3, 123.8, 117.7, 111.8, 110.3, 110.3, 32.2 ppm; MS (m/z): 273 (M^+ , 100%); (This is a known compound as referenced in literature above, however there were no physical properties reported).

3-(2,3-Dioxoindolin-1-yl)propanoic acid (85)

To a solution of isatin (2.500 mmol, 0.3678 g) in acetonitrile (30 mL), was added KF/alumina (19.0 mmol, 2.91 g) and the resulting mixture was allowed to stir for 10 minutes until the initial orange solution turned to a dark brownish color, inside a 250 mL round bottom flask. *Tert*-butyl acrylate (3.0 equiv., 7.50 mmol, 0.961g, 1.089 mL) was then added to the stirred solution after which time the mixture was then refluxed under acetonitrile for 1.5h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution, to give a impure red oil. A silica gel column (hexanes/EtOAc, 70:30) was used to purify separate the product from impurities to give a red solid. The solid was then recrystallized from 2-propanol to afford pure (TLC) product as red crystals (0.1256 g, 18%): mp 144-149°C; $R_f = 0.53$ (EtOAc/hexanes, 1:1) $^1\text{H NMR}$ (300 MHz DMSO- d_6) δ 12.41 ppm (s, 1H), 7.66 (d, $J = 7.76\text{Hz}$, 1H), 7.54 (d, $J = 7.32\text{Hz}$, 1H), 7.24 (d, $J = 7.89\text{Hz}$, 1H), 7.12 (t, $J = 7.47\text{Hz}$, 1H), 3.89 (t, $J = 7.28\text{Hz}$, 2H), 2.62 (t, $J = 7.19\text{Hz}$, 2H); $^{13}\text{C NMR}$ δ : 183.4, 172.2, 158.0, 150.4, 138.1, 124.4, 123.1, 117.5, 110.9, 35.6, 31.4 ppm; Anal. Calcd for $\text{C}_{11}\text{H}_9\text{NO}_4$: C, 60.27; H, 4.14; N, 6.39 Found: C, 60.34; H, 4.36; N, 6.30.

1-Benzyl-5-chloroindoline-2,3-dione⁴⁰ (88)

To a solution of 5-chloroisatin (5.51 mmol, 1.00 g) in acetonitrile (70 mL), was added KF/alumina (33.04 mmol, 5.19 g) and the resulting mixture was allowed to stir for 10 min until the initial orange solution turned to a dark brownish color. Benzyl chloride (1.5 equiv., 8.26 mmol, 0.952 mL) was then added dropwise to the stirred solution after which time the mixture was then refluxed under acetonitrile for 47 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then evaporated

under reduced pressure to afford a solid which was recrystallized from ethanol to afford the pure (TLC, GC/MS) product as orange crystals (1.20 g, 65%): mp 134°C; $R_f = 0.65$ (EtOAc/hexanes, 1:1) ^1H NMR (300 MHz CDCl_3) δ 7.57 ppm (d, $J = 2.22\text{Hz}$, 1H), 7.45 (dd, $J = 8.43\text{Hz}$, 2.2Hz, 1H), 7.37-7.31 (m, $5 \times \text{Ar-H}$), 6.75 (d, $J = 8.34\text{Hz}$, 1H), 4.94 (s, 2H); ^{13}C NMR δ : 182.2, 157.7, 149.0, 137.7, 134.2, 129.7, 129.2, 128.4, 127.4, 125.3, 118.6, 112.3, 44.1 ppm; MS (m/z): 271 (M^+), 180 (100%). (This is a known compound as referenced in literature above, however there were no physical properties reported).

Ethyl 2-(5-chloro-2,3-dioxindolin-1-yl)acetate³⁷ (89)

To a solution of 5-chloroisatin (2.76 mmol, 0.501 g) in acetonitrile (35 mL), was added KF/alumina (16.5 mmol, 2.61 g) and the resulting mixture was allowed to stir for 10 min until the initial orange solution turned to a dark brownish color. Ethyl bromoacetate (1.5 equiv., 4.13 mmol, 0.734 mL) was pipette into the reaction mixture, and then refluxed under acetonitrile for 9 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then allowed to dry under the fume hood overnight to afford the pure (TLC, GC/MS) yellow solid (0.64 g, 87%): mp 130-135°C; $R_f = 0.68$ (EtOAc/hexanes, 1:1); ^1H NMR (300 MHz $\text{DMSO-}d_6$) δ 7.75 ppm (dd, $J = 8.55\text{Hz}$, 2.22Hz, 1H), 7.67 (d, $J = 2.01\text{Hz}$, 1H), 7.25 (d, $J = 8.46\text{Hz}$, 1H), 4.63 (s, 2H), 4.17 (q, 7.08Hz, 2H), 1.22 (t, $J = 7.13\text{Hz}$, 3H); ^{13}C NMR δ : 181.5, 167.3, 157.8, 148.9, 137.4, 127.9, 124.1, 118.6, 112.9, 61.4, 41.3, 14.0; MS (m/z): 267 (M^+), 166 (100%). (This is a known compound as referenced in literature above, however there were no physical properties reported).

1-(2,6-Dichlorobenzyl)-5-chloroindoline-2,3-dione (90)

To a solution of 5-chloroisatin (2.78 mmol, 0.504 g) in acetonitrile (35 mL), was added KF/alumina (16.4 mmol, 2.50 g) and the resulting mixture was allowed to stir for 10 min until the initial orange solution turned to a dark brownish color. Then 2,6-dichlorobenzyl bromide (1.5 equiv., 4.16 mmol, 0.99 g) was added to the round bottom flask and then refluxed under acetonitrile for 24 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then allowed to dry under the fume hood for several days to afford the pure (TLC, GC/MS) orange solid (20.55g, 58%): mp 233-235°C; R_f = 0.67 (EtOAc/hexanes, 1:1); ^1H NMR (300 MHz DMSO- d_6) δ 7.71 ppm (dd, J = 8.49Hz, 2.30Hz, 1H), 7.66 (d, J = 2.31Hz, 1H), 7.54 (d, J = 8.01Hz, 1H), 7.42 (t, 7.92Hz, 1H), 7.00 (d, J = 8.37Hz, 1H), 5.13 (s, 2H); ^{13}C NMR δ : 181.7, 157.5, 149.2, 137.3, 135.4, 130.8, 129.5, 129.1, 127.7, 124.2, 119.0, 112.6, 40.4; MS (m/z): 339 (M^+), 180 (100%); Anal. Calcd for $\text{C}_{15}\text{H}_8\text{Cl}_3\text{NO}_2$: C, 52.90; H, 2.37; N, 4.11 Found: C, 52.76; H, 2.46; N, 4.07.

1-(2,6-Difluorobenzyl)-5-chloroindoline-2,3-dione³⁵ (91)

To a solution of 5-chloroisatin (2.76 mmol, 0.502 g) in acetonitrile (35 mL), was added KF/alumina (17 mmol, 2.6 g) and the resulting mixture was allowed to stir for 10 min until the initial orange solution turned to a dark brownish color, inside a 250 mL round bottom. Then 2,6-difluorobenzyl bromide (1.5 equiv., 4.15 mmol, 0.85 g) was added to the round bottom flask and then refluxed under acetonitrile for 24 h. The mixture was then allowed to cool to rt and the suspended KF/alumina was vacuum filtered from the solution. The filtrate was then evaporated under reduced pressure to afford a solid which was recrystallized from DCM/hexanes to afford the pure (TLC, GC/MS) product as reddish-violet crystals (0.76 g, 89%): mp 172-177°C; R_f =

0.46 (EtOAc/hexanes, 1:1); ^1H NMR (300 MHz CDCl_3) δ 7.57 ppm (d, $J = 2.07\text{Hz}$, 1H), 7.50 (dd, $J = 8.25\text{Hz}$, 2.27Hz, 1H), 7.39-7.28 (m, 1H), 6.96 (t, $J = 8.15\text{Hz}$, 1H), 6.75 (d, 8.55Hz, 1H), 4.94 (s, 2H); ^{13}C NMR δ : 181.8, 163.2, 159.7, 156.9, 148.5, 137.8, 130.8, 129.7, 125.2, 118.6, 111.9, 111.6, 109.9, 32.4; MS (m/z): 307 (M^+ , 100%). (This is a known compound as referenced in literature above, however there were no physical properties reported).

5-Nitroindolin-2-one (95)

To a 25-mL Erlenmeyer flask in an ice bath containing sulfuric acid (3.84 mL) and oxindole (7.54 mmol, 1.0042 g) was added nitric acid (0.323 mL) dropwise with stirring. The mixture was stirred for 30 min after which the mixture was poured over ice. A brown colored solid (0.6030 g) was collected via vacuum filtration. Recrystallization using 50% acetic acid/water gave the pure product (brown color) (0.3125 g, 23%); mp 240-243°C, (lit.⁸² mp 240-241°C); $R_f = 0.28$ (EtOAc/hexanes, 1:1); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.03 (s, N-H), 8.13 (d, $J = 8.4$ Hz, 1H), 8.07 (s, 1H), 6.97 (d, $J = 8.4$ Hz, 1H), 3.62 (s, 2H); ^{13}C NMR δ : 176.7, 150.3, 141.7, 127.1, 125.0, 119.9, 108.9, 35.5 ppm; MS (m/z): 178 (M^+ , 100%).

1-(2,6-Difluorobenzyl)indolin-2-one (98)

To a 250 mL round bottom flask was added 1-(2,6-difluorobenzyl)indoline-2,3-dione (7.32 mmol, 2.0 g) with 80%/wt hydrazine-hydrate (45 mL). The round bottom flask was covered with aluminum foil and then allowed to stir under reflux for 4h. The reaction mixture was allowed to cool to room temperature and then distilled water (80 mL) was added with 6N HCl (60 mL) to the flask. The reaction mixture was then extracted into EtOAc (75 mL) and the organic layer was washed with brine 3-times and dried with NaSO_4 . The organic layer was then evaporated under the reduced pressure to afford the pure (GC/MS, TLC) tan solid (1.801 g, 95%); mp 157-162°C;

$R_f = 0.41$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.20–7.07 (m, 3H), 6.91 (t, $J = 7.52\text{Hz}$, 1H), 6.82 (d, $J = 8.07\text{Hz}$, 1H), 6.76 (t, $J = 7.32\text{Hz}$, 1H), 4.93 (s, 2H), 3.48 (s, 2H); $^{13}\text{C NMR}$ δ : 174.3, 163.3, 160.0, 143.7, 129.9, 127.9, 124.4, 124.4, 122.3, 111.6, 111.5, 108.4, 35.6, 32.0 ppm; MS (m/z): 259 (M^+ , 100%); Anal. Calcd for $\text{C}_{15}\text{H}_{11}\text{F}_2\text{NO}$: C, 69.49; H, 4.28; N, 5.40 Found: C, 68.58; H, 4.45; N, 5.66.

1-Benzyl-5-chloroindolin-2-one (99)

To a 250 mL round bottom flask was added 1-benzyl-5-chloroindoline-2,3-dione (3.68 mmol, 1.00 g) with 80%/wt hydrazine-hydrate (25 mL). The round bottom flask was covered with aluminum foil and then allowed to stir under reflux for 5h. The reaction mixture was allowed to cool to room temperature and then distilled water (20 mL) was added with 6N HCl (40 mL) to the flask. The reaction mixture was then extracted into EtOAc (50 mL) and the organic layer was washed with brine 3-times and dried with NaSO_4 . The organic layer was then evaporated under reduced pressure to afford the pure (GC/MS, TLC) tan solid, which was recrystallized from DCM/hexanes to give a tan solid (0.7224 g, 76%): mp 103-105°C; (lit.⁸³ mp 103-105°C); $R_f = 0.38$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.20–7.07 (m, 3H), 6.91 (t, $J = 7.52\text{Hz}$, 1H), 6.82 (d, $J = 8.07\text{Hz}$, 1H), 6.76 (t, $J = 7.32\text{Hz}$, 1H), 4.93 (s, 2H), 3.48 (s, 2H); $^{13}\text{C NMR}$ δ : 174.3, 163.3, 160.0, 143.7, 129.9, 127.9, 124.4, 124.4, 122.3, 111.6, 111.5, 108.4, 35.6, 32.0 ppm; MS (m/z): 257 (M^+), 91 (100%).

(Z)-3-(4-Nitrobenzylidene)indolin-2-one (103)

To a vial was added oxindole (1.877 mmol, 0.2500 g) with 4-nitrobenzaldehyde (1.2 equiv., 2.253 mmol, 0.3405 g), piperidine (0.1 equivalents, 0.1877 mmol, 0.0159 g, 0.01856 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 3 h while stirring. The heat was

then turned off and the mixture was allowed to continue stirring for 24 h. An orange precipitate formed (0.4109 g, 81%) and was collected by vacuum filtration: mp 251-252°C, (lit.⁷³ mp 245-250°C); ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.68 (s, N-H), 8.33 (d, *J* = 8.37 Hz, 1H), 7.93 (d, *J* = 8.37Hz, 1H), 7.65 (s, H_{vinyl}), 7.39 (d, *J* = 7.59Hz, 1H), 7.25 (t, *J* = 7.49Hz, 1H), 6.87 (m, 2H); ¹³C NMR δ: 168.1, 147.4, 143.4, 141.4, 132.8, 131.0, 130.4, 130.0, 123.9, 122.8, 121.3, 120.2, 110.3 ppm; MS (m/z): 266 (M⁺, 100%).

(*E/Z*)-3-(3-Nitrobenzylidene)indolin-2-one (104)

To a vial was added oxindole (1.877 mmol, 0.2500 g) with 3-nitrobenzaldehyde (1.2 equivalents, 2.253 mmol, 0.3405 g) piperidine (0.1 equivalents, 0.1877 mmol, 0.0159 g, 0.01856 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 3 h while stirring. A yellow precipitate formed (0.4486 g, 90%) and was collected by vacuum filtration: mp 244-246°C, (lit.⁷⁴ mp 258°C); ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.71 (s, N-H), 10.67(s, N-H), 9.37 (s, 1H), 8.62 (d, *J* = 7.68Hz, 1H), 8.50 (s, 1H), 8.27 (t, *J* = 9.33Hz, 1H), 8.11 (d, *J* = 7.44Hz, H_{2,6}), 7.92 (s, 1H), 7.79 (t, *J* = 8.01Hz, 1H) 7.72 (d, *J* = 8.01Hz, H_{2,6}), 7.67 (s, H_{vinyl}), 7.40 (d, *J* = 7.62Hz, 1H), 7.25 (t, *J* = 7.61Hz, 1H) 7.00 (t, *J* = 7.61Hz, 1H), 6.90-6.81 (m, 1H); ¹³C NMR δ: 168.2, 166.9, 147.9, 147.6, 143.4, 141.3, 137.8, 136.2, 135.5, 135.3, 133.7, 132.8, 130.8, 130.3, 129.7, 129.5, 129.1, 125.8, 124.3, 123.9, 123.5, 122.5, 121.3, 120.3, 110.3, 109.6 ppm; MS (m/z): 266 (M⁺, 100%).

(*Z*)-3-((Pyridine-2-yl)methylene)indolin-2-one (105)

To a vial was added oxindole (3.76 mmol, 0.50 g) with 2-pyridinecarboxaldehyde (1.2 equivalents, 4.51 mmol, 0.483 g, 0.428 mL) piperidine (0.1 equivalents, 0.451 mmol, 0.0384 g, 0.0446 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 6 h while stirring.

A yellow precipitate (0.6287 g, 75%) was collected by vacuum filtration: mp 206-209°C, (lit.⁷⁵ mp 202-203°C); $R_f = 0.075$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 10.64 (s, N-H), 9.02 (d, $J = 7.77\text{Hz}$, 1H), 7.96 (t, $J = 7.59\text{Hz}$, 1H), 7.88 (d, $J = 7.5\text{Hz}$, 1H), 7.60 (s, H_{vinyl}), 7.48 (t, $J = 5.96\text{Hz}$, 1H), 7.30 (t, $J = 7.67\text{Hz}$, 1H), 7.00 (t, $J = 7.68\text{Hz}$, 1H), 6.89 (d, $J = 7.74\text{Hz}$, 1H); $^{13}\text{C NMR}$ δ : 169.2, 153.2, 149.6, 143.6, 137.2, 133.6, 130.8, 129.2, 128.4, 127.9, 124.1, 121.2, 109.6 ppm; MS (m/z): 221 (M^+ , 100%).

(E/Z)-3-((Pyridine-3-yl)methylene)indolin-2-one⁸⁴ (106)

To a vial was added oxindole (3.76 mmol, 0.50 g) with 3-pyridinecarboxaldehyde (1.2 equivalents, 4.51 mmol, 0.483 g, 0.423 mL) piperidine (0.1 equivalents, 0.451 mmol, 0.0384 g, 0.0446 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 5 h while stirring. A yellow precipitate (0.3032 g, 36%) was collected by vacuum filtration: mp 161-165°C; $R_f = 0.043$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 10.67 (s, N-H), 9.20 (s, N-H), 8.88 (t, $J = 9.59\text{Hz}$, 1H), 8.66 (d, $J = 4.74\text{Hz}$, 1H), 8.59 (d, $J = 4.71\text{Hz}$, 1H), 8.12 (d, $J = 7.83\text{Hz}$, 1H), 7.83 (s, 1H), 7.73 (d, $J = 7.44\text{Hz}$, 1H), 7.64 (s, 1H), 7.59-7.47 (m, 1H), 7.39 (d, $J = 7.83\text{Hz}$, 1H), 7.26 (t, $J = 7.65\text{Hz}$, 1H), 7.02 (t, $J = 7.65\text{Hz}$, 1H), 6.91-6.84 (m, 1H); $^{13}\text{C NMR}$ δ : 168.1, 167.0, 152.3, 150.2, 150.1, 149.7, 143.2, 141.1, 137.8, 136.3, 132.7, 132.0, 130.6, 129.9, 129.5, 129.4, 128.8, 124.4, 123.6, 123.1, 122.2, 121.3, 121.2, 120.6, 120.2, 110.3, 109.5 ppm; MS (m/z): 222 (M^+ , 100%). (This is a known compound as referenced in literature above, however there were no physical properties reported).

(Z)-3-(2,6-Dichlorobenzylidene)indolin-2-one (107)

To a vial was added oxindole (1.877 mmol, 0.2500 g) with 2,6-dichlorobenzaldehyde (1.2 equivalents, 2.253 mmol, 0.3943 g) piperidine (0.1 equivalents, 0.2253 mmol, 0.0192 g, 0.0223

mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 3 h while stirring. A brown precipitate was collected by vacuum filtration and was recrystallized from DCM/hexanes to give a brown solid (0.3114 g, 57%) was collected by vacuum filtration: mp 182-184°C, (lit.⁷⁶ mp 179-181°C); $R_f = 0.45$ (EtOAc/hexanes, 1:1); ¹H NMR (300 MHz, CDCl₃) δ 9.30 (s, N-H), 7.66 (s, H_{vinyl}), 7.46 (d, $J = 7.89$ Hz, 1H), 7.41-7.31 (m, 2H), 7.24 (t, $J = 7.43$ Hz, 1H), 6.96 (d, $J = 7.62$ Hz, 1H), 6.85 (t, $J = 7.59$ Hz, 1H), 6.72 (d, $J = 7.59$ Hz, 1H); ¹³C NMR δ : 169.4, 141.9, 134.5, 133.0, 131.8, 130.5, 130.2, 129.7, 128.3, 123.8, 122.3, 121.6, 110.3 ppm; MS (m/z): 289 (M⁺), 254 (100%).

(Z)-3-(3,5-Difluorobenzylidene)indolin-2-one (108)

To a vial was added oxindole (3.75 mmol, 0.4990 g) with 3,5-difluorobenzaldehyde (1.2 equivalents, 4.56 mmol, 0.6480 g) piperidine (0.1 equivalents, 0.456 mmol, 0.0388 g, 0.0451 mL) and EtOH (4 mL). The vial was placed on a hot plate at 90°C for 3 h while stirring. A yellow precipitate was collected by vacuum filtration and then recrystallized from ethanol to give a yellow/orange solid (0.5716 g, 58%): mp 206-210°C, (lit.⁷⁷ mp 202-205°C); $R_f = 0.43$ (EtOAc/hexanes, 1:1); ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.66 (s, N-H), 7.56 (s, H_{vinyl}), 7.41 (d, $J = 7.05$ Hz, 1H), 7.42-7.32 (m, 2H), 7.26 (t, $J = 7.70$ Hz, 1H), 6.88 (t, $J = 7.7$ Hz, 1H); ¹³C NMR δ : 167.5, 161.3, 158.0, 143.2, 132.7, 132.0, 130.8, 123.1, 121.5, 120.7, 119.7, 112.1, 110.1 ppm; MS (m/z): 257 (M⁺, 100%).

(E)-3-(2-Hydroxybenzylidene)indolin-2-one (109)

To a vial was added oxindole (1.877 mmol, 0.2500 g) with salicylaldehyde (1.2 equivalents, 2.253 mmol, 0.2751 g, 0.2401 mL), piperidine (0.1 equivalents, 0.2253 mmol, 0.0192 g, 0.0223 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 3 h while stirring. A

yellow precipitate (0.1965 g, 44%) was collected by vacuum filtration: mp 193-195°C; (lit.⁷⁴ mp 195-196°C); $R_f = 0.25$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ 10.56 (s, N-H), 10.17 (s, O-H), 7.72 (s, H_{vinyl}), 7.64 (d, $J = 7.56\text{Hz}$, 1H), 7.51 (d, $J = 7.56\text{Hz}$, 1H), 7.32 (t, $J = 7.77\text{Hz}$, 1H), 7.22 (t, $J = 7.68\text{Hz}$, 1H), 7.00 (d, $J = 8.02\text{Hz}$, 1H), 6.93 (t, $J = 7.37$, 1H), 6.89-6.83 (m, 2H); $^{13}\text{C NMR}$ δ : 168.8, 156.4, 142.6, 132.4, 131.5, 129.6, 129.5, 126.5, 122.3, 121.3, 121.3, 120.9, 118.7, 116.0, 109.9 ppm; MS (m/z): 237 (M^+ , 100%).

(Z)-3-Benzylidene-2-one (110)

To a vial was added oxindole (3.77 mmol, 0.5022 g) with benzaldehyde (1.2 equivalents, 4.52 mmol, 0.4803 g, 0.460 mL), piperidine (0.1 equivalents, 0.4526 mmol, 0.0385 g, 0.0448 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 3.5h while stirring. A yellow precipitate (0.5518g, 66%) was collected by vacuum filtration: mp 172-175°C; (lit.⁷⁹ mp 180-181°C); $R_f = 0.46$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, CDCl_3) 9.30 (s, N-H), 7.88 (s, 1H), 7.69 (d, $J = 7.2\text{Hz}$, 1H), 7.64 (s, H_{vinyl}), 7.53-7.45 (m, 5H), 7.24 (t, $J = 7.67\text{Hz}$, 1H), 6.97 (d, $J = 7.71\text{Hz}$, 1H), 6.89 (t, $J = 7.64\text{Hz}$, 1H); $^{13}\text{C NMR}$ (300 MHz, DMSO- d_6) 168.6, 143.0, 135.7, 134.5, 130.1, 129.6, 129.2, 128.7, 127.7, 122.3, 121.1, 120.9, 110.1 ppm; MS (m/z): 221 (M^+ , 100%).

(E)- 3-(2,6-Difluorobenzylidene)-1-benzylindolin-2-one (111)

To a solution of Z-3-(2,6-difluorobenzylidene)indolin-2-one (1.579 mmol, 0.4062 g) in acetonitrile (30 mL), was added KF/alumina (39.2 mmol, 6.00 g) and the resulting mixture was allowed to stir for 10 minutes and formed an orange color. Benzyl chloride (1.5 equivalents, 2.368 mmol, 0.273mL) was added to the round bottom flask dropwise and then refluxed under acetonitrile for 12 h. The mixture was then allowed to cool to rt and the suspended KF/alumina

was vacuum filtered from the solution. The filtrate was then evaporated under the reduced pressure to afford a orange oil, upon which after the addition of a small amount of methanol to afforded the pure (TLC, GC/MS) product as orange crystals (0.2130 g, 39%): mp 94-99°C; $R_f = 0.58$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.70 (s, H_{vinyl}), 7.49-7.28 (m, 1H), 7.19 (td, $J = 7.74\text{Hz}$, 1.08Hz , 1H), 7.11 (d, $J = 7.47\text{Hz}$, 1H), 7.06 (t, $J = 7.95\text{Hz}$, 1H), 6.90 (td, $J = 7.65\text{Hz}$, 0.96Hz , 1H), 6.74 (d, $J = 7.86\text{Hz}$, 1H), 5.02 (s, 2H); $^{13}\text{C NMR}$ δ : 167.6, 162.2, 158.9, 143.7, 135.9, 131.4, 131.1, 130.3, 128.8, 127.6, 127.4, 123.7, 122.2, 121.7, 121.2, 113.0, 111.8, 109.1, 43.9 ppm; MS (m/z): 347 (M^+ , 100%); Anal. Calcd for $\text{C}_{22}\text{H}_{15}\text{F}_2\text{NO}$: C, 76.07; H, 4.35; N, 4.03 Found: C, 76.05; H, 4.49; N, 4.05.

(E)-1-(2,6-Difluorobenzyl)-3-(4-methoxybenzylidene)indolin-2-one (112)

To a vial was added 1-(2,6-difluorobenzyl)indolin-2-one (1.16 mmol, 0.300 g) with *p*-anisaldehyde (1.2 equivalents, 1.39 mmol, 0.1891 g, 0.1689 mL), piperidine (0.2 equivalents, 0.278 mmol, 0.0236 g, 0.0275 mL) and EtOH (8 mL). The vial was placed on a hot plate at 90°C for 50 h while stirring. A yellow precipitate (0.1225 g, 28%) was collected by vacuum filtration: mp 142-143°C; $R_f = 0.66$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.89 (s, H_{vinyl}), 7.76 (d, $J = 7.8\text{Hz}$, 1H), 7.67 (d, $J = 8.58\text{Hz}$, 1H), 7.31-7.17 (m, Ar-H), 7.00 (d, $J = 8.79\text{Hz}$, 1H), 6.94-6.87 (m, Ar-H), 5.12 (s, 2H); $^{13}\text{C NMR}$ δ : 168.3, 163.4, 160.9, 160.1, 142.7, 137.8, 134.5, 131.4, 129.8, 129.3, 127.3, 125.0, 122.4, 121.7, 114.1, 111.6, 108.5, 55.4, 32.1 ppm; MS (m/z): 377 (M^+ , 100%); Anal. Calcd for $\text{C}_{23}\text{H}_{17}\text{F}_2\text{NO}_2$: C, 73.20; H, 4.54; N, 3.71 Found: C, 73.23; H, 4.65; N, 3.74.

(E)-1-(2,6-Difluorobenzyl)-3-((pyridin-4-yl)methylene)indolin-2-one (113)

To a vial was added 1-(2,6-difluorobenzyl)indolin-2-one (1.16 mmol, 0.300 g) with 4-pyridincarboxaldehyde (1.2 equivalents, 1.39 mmol, 0.1487 g, 0.1308 mL), piperidine (0.1 equivalents, 0.1390 mmol, 0.0118 g, 0.0137 mL) and EtOH (6 mL). The vial was placed on a hot plate at 90°C for 20 h while stirring. A light-green precipitate (0.2121 g, 53%) was collected by vacuum filtration: mp 180-181°C; $R_f = 0.08$ (EtOAc/hexanes, 1:1); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.74 (d, $J = 6.03\text{Hz}$, 1H), 7.76 (s, H_{vinyl}), 7.49 (d, $J = 5.34\text{Hz}$, 1H), 7.42 (d, $J = 7.29\text{Hz}$, 1H), 7.31-7.20 (m, Ar-H), 6.95-6.86 (m, Ar-H), 5.09 (s, 2H); $^{13}\text{C NMR}$ δ : 167.2, 163.3, 160.0, 150.3, 143.5, 143.2, 133.1, 130.8, 130.0, 129.7, 123.2, 123.0, 122.1, 120.5, 111.7, 111.3, 108.9, 32.2 ppm; MS (m/z): 348 (M^+), 127 (100%); Anal. Calcd for $\text{C}_{21}\text{H}_{14}\text{F}_2\text{N}_2\text{O}$: C, 72.41; H, 4.05; N, 8.04 Found: C, 72.46; H, 4.10; N, 7.99.

References

- 1) Silva, J. F.; Garden, S. J.; Pinto, A. C. "The chemistry of isatins: a review from 1975 to 1999" *J. Braz. Chem. Soc.* **2001**, *12*, 273-324.
- 2) Pandeya, S. N.; Smitha, S.; Stables, J. P. "Anticonvulsant and sedative-hypnotic activities of N-substituted isatin semicarbazones" *Arch. Pharm. Med. Chem.* **2002**, *4*, 129-134.
- 3) Pandeya, S. N.; Sriram, D.; Yogeewari, P.; Ananthan, S. "Antituberculous activity of norfloxacin mannich bases with isatin derivatives" *Chemotherapy* **2001**, *47*, 266-269.
- 4) Brewerton, T. D.; Zealberg, J. J.; Lydiard, R. B.; Glover, V.; Sandler, M.; Ballenger, J. C. "CSF isatin is elevated in bulimia nervosa" *Biol. Psychiatry* **1995**, *37*, 481-483.
- 5) Sandmeyer, T. "Isonitrosoacetanilides and their condensation to form isatin derivatives" *Helv. Chim. Acta.* **1919**, *2*, 234-232.
- 6) Smolders, R.R.; Waefelaer, A.; Francart, D. "7-Fluoroisatin" *Ing. Chim. (Brussels)* **1982**, *64*, 5-6.
- 7) Jnaneshwara, G.K.; Bedekar, A.V.; Deshpande, V.H. "Microwave assisted preparation of isatins and synthesis of (±)-convolutamydine" *Synth. Commun.* **1999**, *29*, 3627-3633.
- 8) Stolle, R. "N-Substituted oxindoles and isatins" *J. Prak. Chem.* **1922**, *105*, 137-148.
- 9) Loloiu, G.; Maior, O. "Isatin chemistry. Synthesis of N-methyl-2,3-dioxo-2,3-dihydropyrrolo[2,3-b]phenoxatiin" *Rev. Roum. Chim.* **1997**, *42*, 67-69.
- 10) Fukuda, Y.; Itoh, Y.; Nakatani, K.; Terashima, S. "Synthetic studies on duocarmycin. 1. Total synthesis of dL-duocarmycin A and its 2-epimer" *Tetrahedron* **1994**, *50*, 2793-2808.
- 11) Gassman, P.G.; van Bergen, T.J. "A new, general method of synthesis" *J. Am. Chem. Soc.*, **1974**, *96*, 5508-5512.

- 12) Gassman, P.G.; Cue Jr., B.W.; Luh, T.Y.; "A general method for the synthesis of isatins" *J. Org. Chem.* **1977**, *42*, 1344-1348.
- 13) Hewawasam, P.; Meanwell, N. "A general method for the synthesis of isatins: preparation of regiospecifically functionalized isatins from anilines" *Tetrahedron Lett.* **1994**, *35*, 7303-7306.
- 14) Smith, K.; El-Hiti, G. A.; Hawes, A. C. "A novel procedure for the formation of isatins via carbonylation of lithiated *N'*-Aryl-*N,N*-dimethylureas" *Synlett* **1999**, *S1*, 945-947.
- 15) Mironov, M.A.; Mokrushin, V.S. "Reaction of aromatic isocyanides with triethylamine: a new method for the synthesis of indole betaines" *Mendeleev Commun.* **1998**, *8*, 242-243.
- 16) Mironov, M.A.; Kleban, M.I.; Mokrushin, V.S. "Novel method for synthesis of isatins" *Chem. Heterocycl. Comp.* **2001**, *36*, 368.
- 17) Otto-Meth Cohn; S. Goon, "Synthetic applications of unpoled vilsmeier reagents- a new simple one pot route to isatins from formanilides" *Tetrahedron Letters*, **1996**, *37*, 9381-9384.
- 18) Calvery, H.O.; Noller, C.R.; Adams, R. "Arsonophenyl-cinchoninic acid (arsonocinchophen) and derivatives II" *J. Am. Chem. Soc.* **1925**, *47*, 3058-3060.
- 19) Kara L. Vine, Julie M. Locke, Marie Ranson, Stephen G. Pyne and John B. Bremner "In vitro cytotoxicity evaluation of some substituted isatin derivatives" *Bioorg. Med. Chem.* **2007**, *15*, 931-938.
- 20) de Souza, S.P.L.; da Silva, J.F.M.; de Mattos, M.C.S.; "SiO₂ mediated reaction of isatin with N-halosaccharins: A regiospecific preparation of 5-haloisatins" *Heterocycl. Commun.* **2003**, *9*, 31-34.

- 21) Mendonça, G.F.; Magalhães, R.R.; de Mattos, M.C.S.; Esteves, P.M. "Trichloroisocyanuric acid in H₂SO₄: An efficient superelectrophilic reagent for chlorination of isatin and benzene derivatives" *J. Chem. Braz. Soc.* **2005**, *16*, 695-698.
- 22) Lindwell, H.G.; Bandes, J.; Weinberg, I. "Preparation of certain brominated cincophens" *J. Am. Chem. Soc.* **1931**, *53*, 317-319.
- 23) 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.
- 24) Kopka, K.; Faust, A.; Keul, P.; Wagner, S.; Breyholz, H.; Hoßltke, C.; Schober, O.; Schäfers, 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.
- 25) Chu, W.; Rothfuss, J.; d'Avignon, 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.
- 26) Chu, W.; Zhang, J.; Zeng, C.; Rothfuss, J.; Tu, Z.; Chu, Y.; Reichert, D.E.; 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.
- 27) Podichetty, A.K.; Faust, A.; Kopka, K.; Wagner, S.; Schober, O.; Schäfers, 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.
- 28) Rekhter, M.A. "Direct N-alkylation of isatin by halomethyl ketones" *Chem. Heterocycl. Compd.* **2005**, *41*, 1119-1120.

- 29) 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.
- 30) Bariwal, J.B.; Trivedi, J.C.; Van der Eycken, E.V. "Microwave irradiation and multicomponent reactions" *Top. Heterocycl. Chem.* **2010**, *25*, 169-230.
- 31) 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.
- 32) 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.
- 33) Torres, J. C.; Pinto, A. C.; Garden, S. J. "Application of a catalytic palladium biaryl synthesis reaction via C-H functionalization, to the total synthesis of amaryllidaceae alkaloids" *Tetrahedron* **2004**, *60*, 9889-9900.
- 34) 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.
- 35) Bridges, T.M.; Marlo, J.E.; Niswender, C.M.; Jones, C.K.; Jadhav, S.B.; Gentry, P.R.; Plumley, H.C.; Weaver, C.D.; Conn, P.J.; Lindsley, C.W. "Discovery of the first highly M5-preferring muscarinic acetylcholine receptor ligand, an M5 positive allosteric modulator derived from a series of 5-trifluoromethoxy N-benzyl isatins" *J. Med. Chem.* **2009**, *52*, 3445-3448.
- 36) Torisawa, Y.; Nishi, T.; Minamikawa, J. "An efficient conversion of 5-nitroisatin into 5-nitroindole derivative" *Bioorg. Med. Chem. Lett.* **2001**, *11*, 829-832.

- 37) Bouhfid, R.; Joly, N.; Ohmani, F.; Essassia, E.M.; Lequart, V.; Banoub, J.; Kheddid, K.; Charof, R.; Massoui, M.; Martin, P. "Synthesis and antibacterial activity of new (2,3-dioxo-indolin-1-yl) acetamides" *Lett. Org. Chem.* **2008**, *5*, 3-7.
- 38) Majumdar, K.C.; Kundu, A.K.; Chatterjee, P. "1-alkylisatin via aldol-retro-aldol condensation" *J. Chem. Research* **1996**, 460-461.
- 39) Di Carlo, F.J.; Lindwall, H.G. "Synthesis and properties of 1-Cyanoethylisatin" *J. Am. Chem. Soc.* **1945**, *67*, 199-201.
- 40) 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.
- 41) 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.
- 42) Fraile, J. M.; Garcia, J.I.; Mayoral, J.A.; Figueras, F. "Comparison of several heterogeneous catalysts in the epoxidation of α -isophorone with hydroperoxides" *Tetrahedron Lett.* **1996**, *37*, 5995-5996.
- 43) Alloum, B.A.; Villemin, D. "Potassium fluoride on alumina: an easy preparation of diazo carbonyl compounds" *Synth. Commun.* **1989**, *19*, 2567-2571.
- 44) Kabalka, G. W.; Wang, L.; Namboodiri, V.; Pagni, R. M. "Rapid microwave-enhanced, solventless Sonogashira coupling reaction on alumina" *Tetrahedron Lett.* **2000**, *41*, 5151-5154.
- 45) Kabalka, G.W.; Pagni, R.M.; Hair, C.M. "Solventless Suzuki coupling reactions on palladium-doped KF/Al₂O₃" *Org. Lett.* **1999**, *1*, 1423-1425.

- 46) Nakano, Y.; Shi, W.; Nishii, Y.; Igarashi, M. "Knoevenagel reaction of malononitrile with methyl ketones followed by double cyclization catalyzed by potassium fluoride-coated alumina" *J. of Heterocycl. Chem.* **1999**, *36*, 33-40.
- 47) Texier-Boullet, F.; Villemin, D.; Ricard, M.; Moison, H.; Foucaud, A. "Wittig, Wittig-Horner and Knoevenagel reactions by anionic activation with alumina or alumina-supported potassium fluoride in the absence of solvent" *Tetrahedron* 1985, *41*, 1259-1266.
- 48) Ando, T.; Clark, J.H.; Cork, D.G.; Hanafusa, T.; Ichihara, J.; Kimura, T. "Fluoride-alumina reagents: the active basic species" *Tetrahedron Lett.* **1987**, *28*, 1421-1424.
- 49) Shi, Y. "Activation of initiator caspases: history, hypothesis, and perspectives" *J. Cancer Mol.* **2005**, *1*, 9-18.
- 50) Thornberry, N.A. "Caspases: key mediators of apoptosis" *Chem. Biol.* **1998**, *5*, R97-R103.
- 51) Adrain, C.; Creagh, E. M.; Martin, S.J. "Caspase cascades in apoptosis. Caspases-their role in cell death and cell survival" Ed. Marek Los and Henning Walczak. Molecular Biology Intelligence Unit 24, New York, New York, **2002**, pp 41-51.
- 52) Maly, D.J.; Huang, L.; Ellman, J.A. "Combinatorial strategies for targeting protein families: application to the proteases" *ChemBioChem*, **2002**, *3*, 16-37.
- 53) Van Noorden, C.J. "The history of Z-VAD-FMK, a tool for understanding the significance of caspase inhibition" *Acta Histochem* **2001**, *103*, 241-251.
- 54) Yoshikawa, H.; Tasaka, K. "Caspase-dependent and -independent apoptosis of mast cells induced by withdrawal of IL-3 is prevented by Toll-like receptor 4-mediated lipopolysaccharide stimulation" *Eur. J. Immun.* **2003**, *33*, 2149-2159.

- 55) T. M. Caserta, A. N. Smith, A. D. Gultice, M. A. Reedy and T. L. Brown *Apoptosis* **2003**, *8*, 345-352.
- 56) Southerland, B.; Kulkarni-Datar, K.; Keoni, C.; Bricker, R.; Grunwald, Jr., W.C.; Ketcha, D.M.; Hern, E.; Cool, D.R.; Brown, T.L. "Q-VE-OPh, a negative control for o-phenoxy-conjugated caspase inhibitors" *J. Cell Death* **2010**, *3*, 33-40.
- 57) 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, W.E. Jr. "Potent and selective nonpeptide inhibitors of caspases 3 and 7" *J. Med. Chem.* **2001**, *44*, 2015-2026.
- 58) 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.
- 59) Somasekhara, S.; Dighe, V.S.; Suthar, G.K.; Mukherjee, S.L. "Chlorosulphonation of isatin" *Curr. Sci.* **1965**, 508.
- 60) Martinez, F.; Naarmann, H. "New isatin derivatives: Synthesis and reactions" *Synth. Met.* **1990**, *39*, 195-203.
- 61) Chu, W.; Rothfuss, J.; d'Avignon, 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.

- 62) 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.
- 63) Nguyen, Q.; Smith, G.; Glaser, M.; Perumal, M.; Arstad E.; Aboagye, E. "Positron emission tomography imaging of drug-induced tumor apoptosis with a caspase-3/7 specific [¹⁸F]-labeled isatin sulfonamide" *Proc. Natl. Acad. Sci.* **2009**, *106*, 16375-16380.
- 64) Nefzi, A.; Ostresh, J.M.; Houghten, R.A. "Solid phase synthesis of mixture-based acyclic and heterocyclic small molecule combinatorial libraries from resin-bound polyamides" *Biopolymers (Peptide Science)* **2001**, *60*, 212-216.
- 65) 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.
- 66) Loupy, A. "Microwaves in organic synthesis" Wiley-VCH, Weinheim, **2002**.
- 67) Yadav, V.K.; Kapoor, K.K. "KF adsorbed on alumina effectively promotes the epoxidation of electron deficient alkenes by anhydrous t-BuOOH" *Tetrahedron* **1996**, *52*, 3659-3668.
- 68) Loetter, A.N.C.; Pathak, R.; Sello, T.S.; Fernandes, M.A.; van Otterlo, W.A.L.; de Koning, C.B. "Synthesis of the dibenzopyrrocoline alkaloid skeleton: indolo[2,1-a]isoquinolines and related analogues" *Tetrahedron* **2007**, *63*, 2263-2274.
- 69) Kniess, T.; Kuchar, M.; Wuest, F. "Facile synthesis of various nitro-substituted derivatives of semaxinib (SU5416)" *Synth. Commun.* **2008**, *38*, 3017-3022.
- 70) Sumpter, W.C.; Miller, M.; Magan, M.E. "The structure of baeyer's nitro-oxindole" *J. Am. Chem. Soc.* **1945**, *67*, 499-500.

- 71) 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)methylidene]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases" *J. Med. Chem.* **1999**, *42*, 5120-5130.
- 72) Hung, C.; Hsu, M.; Huang, L.; Hwang, C.; Lee, O.; Wu, C.; Chena, C.; Kuob, S. "Synthesis of 1-substituted 3-pyridinylmethylideneindolin-2-ones and 1-substituted 3-quinolinylmethylideneindolin-2-ones as the enhancers of ATRA-induced differentiation in HL-60 cells" *Bioorg. Med. Chem.* **2008**, *16*, 4222-4232.
- 73) Kniess, T.; Bergmann, R.; Kuchar, M.; Steinbach, J.; Wuest, F. "Synthesis and radiopharmacological investigation of 3-[4'-[¹⁸F]fluorobenzylidene]indolin-2-one as possible tyrosine kinase inhibitor" *Bioorg. Med. Chem.* **2009**, *17*, 7732-7742.
- 74) Olgen, S.; Akaho, E.; Nebioglu, D. "Synthesis and anti-tyrosine kinase activity of 3-(substituted-benzylidene)-1, 3-dihydro-indolin derivatives: investigation of their role against p60c-Src receptor tyrosine kinase with the application of receptor docking studies" *Farmaco* **2005**, *60*, 497-506.
- 75) Zhang, W.; Go, M. "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.
- 76) 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 Org. Chem. J.* **2009**, *3*, 1-10.

- 77) Abdallah, H.M. *Small Molecule Caspase Inhibitors Using Isatin and Oxindole Scaffolds and a Combinatorial Approach*. MS Thesis. Wright State University, Dayton, 2010.
- 78) Fasimoni, G.; Ita, G.; Righetti, P.; Tacconi, G. "Catalysis with inorganic cations. V. Intramolecular hetero Diels-Alder versus ene reactions: effect of magnesium perchlorate on chemoselectivity" *Tetrahedron* **1996**, *52*, 12009-12018.
- 79) Lathourakis, G.E.; Litinas, K.E. "Synthesis and study of 3-(triphenylphosphoranylidene)-2,3-dihydro-1H-indole-2-one" *J. Chem. Soc., Perkin Trans. I*: **1996**, *5*, 491-493.
- 80) 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.
- 81) 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.
- 82) Itoh, K. "99mTc-MAG3: Review of pharmacokinetics, clinical application to renal diseases and quantification of renal function" *Ann. Nucl. Med.* **2001**, *15*, 179-190.
- 83) Hennessy, E.J.; Buchwald, S.L. "Synthesis of substituted oxindoles from α -chloroacetanilides via palladium-catalyzed C-H functionalization" *J. Amer. Chem. Soc.* **2003**, *125*, 12084-12085

84) Lubkoll, J.; Millemaggi, A.; Perry, A.; Taylor, R.J.K. "Tandem Horner-Wadsworth-Emmons/Heck procedures for the preparation of 3-alkenyl-oxindoles: the synthesis of semaxanib and GW441756" *Tetrahedron* **2010**, *66*, 6606-6612.