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Design And Synthesis Of Isatin-Based Caspase Inhibitors For Ruthenium Caging Applications

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DESIGN AND SYNTHESIS OF ISATIN-BASED CASPASE INHIBITORS FOR RUTHENIUM CAGING APPLICATIONS

by

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_____

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DEDICATION

To my loving parents and grandmother

We all have dreams. But in order to make dreams come into reality, it takes a lot of determination, dedication, self-discipline, and effort

ACKNOWLEDGEMENTS

First, I would like to thank my parents for their unconditional support, love, care, inspiration and guidance which lead me to the person I am. I would like to thank my loving grandmother for inspiring me to achieve important things in my life. I would like to convey my sincere gratitude to my loving wife Ruwani Wasalathanthri for being with me and inspired me throughout my career.

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LIST OF ABBREVIATIONS

Caspases: Cysteinyl dependent Aspartate directed Specific Proteases

Boc-D-FMK: t-Butoxycarbonyl-Asp-fluoromethylketone

Z-VAD-FMK: Benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone

FACS: Fluorescence Activated Cell Sorter

OA: Osteoarthritis

Ru: Ruthenium

bpy: Bipyridine

terpy: Terpyridine

DMF: Dimethyl formamide

DMSO: Dimethyl sulfoxide

DIPEA: Diisopropylethylamine

THF: Tetrahydrofuran

$CHCl_3$: Chloroform

ESI-TOF: Electrospray ionization - time of flight

NMR: Nuclear Magnetic Resonance

FT-IR: Fourier Transform Infrared

CHAPTER 1

DESIGN AND SYNTHESIS OF ISATIN-BASED CASPASE INHIBITORS FOR RUTHENIUM CAGING APPLICATIONS

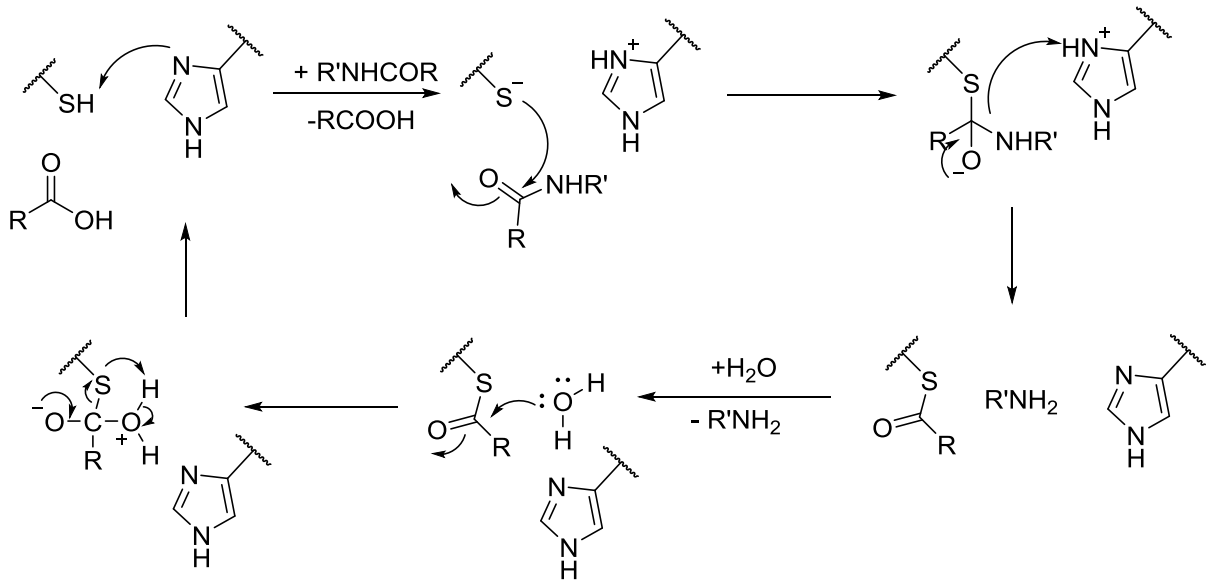
1.1 Introduction

1.1.1 Apoptosis and Caspases

Apoptosis is a process of cell degradation in eukaryotes. This process is mediated by specific signaling cascades. The ultimate result of apoptosis is programmed cell death. This significant cellular process is directly co-related with embryogenesis, the immune system, ageing and various diseases including cancers, ischemic vascular diseases and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease.[1]

An intracellular proteolytic cascade is involved in regulating apoptosis. This process depends on a protease enzyme class called caspases (**c**ysteinyl dependent **asp**artate directed specific prote**ases**). Caspases exist as inactive proenzymes (called zymogens) that are activated via proteolytic pathways to form two subunits. A characteristic feature of caspases is the presence of cysteine and histidine residues in the active site. These amino acids carry out cleavage of the peptide bond next to aspartic acid residues. The reaction mechanism of peptide hydrolysis is shown below (Figure 1-1). The cleaved peptide sequence is designated as an amino acid chain P_n-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_n' and its corresponding substrate binding site is designated as S_n-S_3-S_2-S_1-S_1'-S_2'-S_3'-S_n'.[2] Caspases have a unique preference for aspartic acid at P_1 that binds specifically to the S_1 subsite. Caspases specifically cleave the peptide from its C-terminal next to this aspartic acid. The catalytic efficiency is drastically reduced if a different amino acid is substituted at P_1 in the substrate. The cleavage preference for aspartic acid over glutamic acid in the S_1 subsite is more than 20,000 fold.[3] It is also reported that the S_3 subsite prefers glutamine

residue in all mammalian caspases. Therefore, the specific tetrapeptide sequence can be identified as X-Glu-X-Asp for mammalian caspases.[2]



Scheme 1-1: Mechanism of the peptide bond hydrolysis by cysteine thiol

Caspases are divided into different classes according to their roles in the signaling cascades of apoptosis (Table 1-1). Caspases 6, 8, 9 and 10 are initiators and found at the beginning of the signaling cascade. Those caspases activate the effector caspases. Caspases 2, 3 and 7 are executioner/effector caspases which are found downstream in the signaling cascade.[4] Caspases 1, 4, 5 and 13 are involved in inflammation and they are not active in the cell death process. Caspases 2, 3 and 7 are the most significant and studied caspase types because they are involved in almost all apoptotic models.[5]

Table 1-1. Caspase types and classes

Enzyme class	Caspase type
1- Initiators	6, 8, 9, 10
2- Executioners	2, 3, 7
3- Inflammation	1, 4, 5, 13

1.1.2 Caspase inhibition

Extensive research has been conducted on caspases and their pharmacological activity because they are important targets in drug discovery.[2-8] Development of novel caspase inhibitors is attractive medicinally and pharmaceutically to prevent diseases caused by dysregulated apoptosis. Caspase inhibitors can be categorized as natural and synthetic inhibitors. Natural inhibitors are sub-categorized as viral and cellular caspase inhibitors. Synthetic inhibitors are based on peptidomimetic and non-peptidyl compounds.[7]

1.1.2.1 Natural Caspase inhibitors

Previous studies showed the involvement of viral and cellular caspase inhibitors in apoptosis. For example, Cytokine Response modifier A (CrmA), a product of cowpox virus and a member of the serpin superfamily of protease inhibitors, has been identified as a Caspase-1 inhibitor. A baculoviral protein, p35, is an inhibitor for both insect and mammalian caspases. Mammalian caspase inhibitors such as Survivin and XIAP are reported as specific caspase-3 inhibitors.[7]

Various synthetic caspase inhibitors have also been reported.[6-14] Development of synthetic caspase inhibitors is important to gain specificity in caspase inhibition. These inhibitors are categorized as either peptide and non-peptide-based compounds.

1.1.2.2 Peptidomimetic caspase inhibitors

Peptidomimetic caspase inhibitors have been developed according to their structural properties and interactions with the substrate cleavage sites of caspases. Caspases identify specific binding regions of peptide substrates according to the peptide sequence of the substrate. Caspases preferentially cleave the peptide bonds next to aspartate residues due to the stability

caspases gain from interactions of cysteine and histidine residues in the active binding region. Thus, an aspartate is typically included at the P_1 position in most peptidic inhibitors.

Various caspase inhibitors have been reported which inhibit different caspases.[6] For example Ac-YVAD-CHO is a potent caspase-1 inhibitor which prevents release of interleukin-1β (IL-1β) from monocytes. Peptide-based caspase inhibitors can be categorized according to the number of aspartyl residues present in the peptide and the number of peptide units present in the compound.[7] For example Boc-Asp-fluoromethylketone (Boc-D-FMK) is a broad-range caspase inhibitor containing a single aspartyl residue. Z-VAD-FMK is a tri-peptide that competitively and irreversibly inhibits caspases 1-10.[7] Tetrapeptides have also been developed and their structures are significant for substrate specificity. The tetrapeptide inhibitors generally have higher potency against caspases because they contain a tetrapeptide region which is specifically recognized by the enzyme (Table 1-2).[6,7]

According to previously reported caspase inhibition data (Table 1-2), the significance of peptide aldehyde sequences has been established. For example, Ac-WEHD-CHO **1** specifically binds to group I caspases and Ac-DEVD-CHO **2** binds with group II caspases.

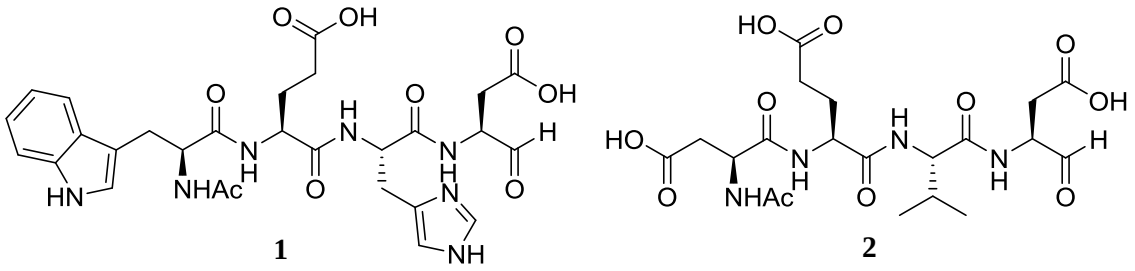
Peptide aldehydes such as Ac-WEHD-CHO **1**, Ac-DEVD-CHO **2**, Boc-IETD-CHO **3** and Boc-AEVD-CHO **4** are caspase-3 inhibitors (Figure 1-1).[6,7] The specificity of these inhibitors for different caspases has been desired for many years. Halomethyl ketone containing peptide derivatives are highly reactive irreversible inhibitors. Thus they lack specificity towards a specific caspase. Peptides containing aldehyde, ketone and nitrile groups are reversible inhibitors of caspases.[11] Some peptide-based caspase inhibitors have not shown good efficacy (IC_{50} < 1

μM) in cellular apoptotic models.[6-8] Lower cell permeability of these peptidyl inhibitors limits the efficacy. Thus, use of peptidyl compounds as caspase inhibitors *in vitro* is limited.

Table 1-2. Inhibition of caspases by peptide aldehydes[6]

Enzyme	**Aldehyde K_i (nM)**				
	WEHD	YVAD	DEVD	IETD	AEVD
Group I					
Caspase-1	0.056	0.76	18	<6	<12
Caspase-4	97	362	132	400	375
Caspase-5	43	163	205	223	438
Group II					
Caspase-3	1960	>10,000	0.23	195	42
Caspase-7	>10,000	>10,000	1.6	3280	425
Caspase-2	>10,000	>10,000	1710	9400	>10,000
Group III					
Caspase-6	3090	>10,000	31	5.6	52
Caspase-8	21.1	352	0.92	1.05	1.6
Caspase-9	508	970	60	108	48
Caspase-10	330	408	12	27	320

Amino acid sequences are shown as Ac-XXXX-CHO (Refer Figure 1-1 for structures)

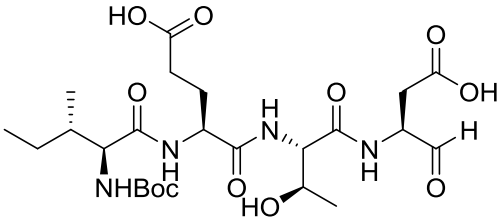


1

>50 µM

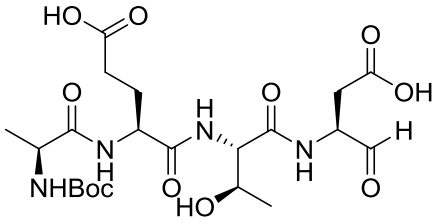
2

1 µM



3

>50 µM

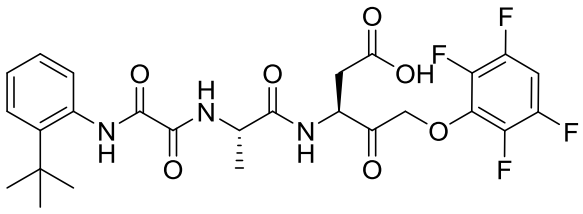


4

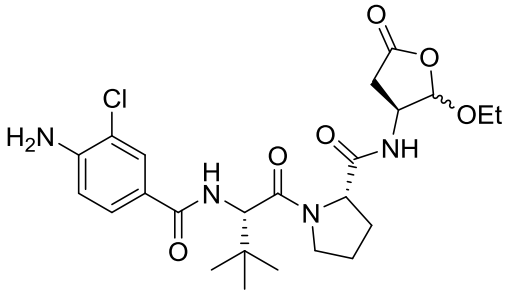
0.6 µM

Figure 1-1: Previously reported tetrapeptide-based caspase-3 inhibitors

Research has been conducted to develop peptidomimetic caspase inhibitors to increase their potencies and selectivities against specific caspases. Peptidomimetic caspase-1 inhibitors such as IDN-6556 and VX-765 are already being investigated in clinical trials (Figure 1-2).[10]



IDN-6556



VX-765

Figure 1-2: Caspase-1 inhibitors IDN-6556 and VX-765

Prodrug VX-765 has been used to design nitrile based peptidomimetic caspase inhibitors using SAR studies (Figure 1-3).[11] For example, compounds **6** and **8** have reported IC_{50} values of

2.58 nM and 0.023 nM against caspase-1, respectively. Compounds **6** and **8** show different potencies against other caspases (Table 1-3).

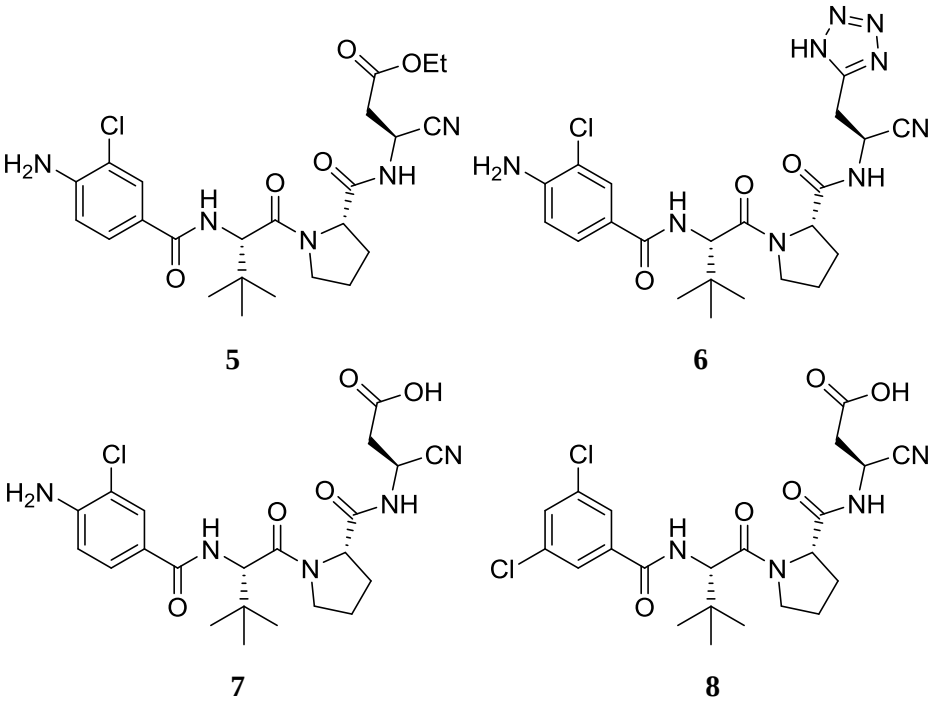


Figure 1-3: Nitrile-based Caspase inhibitors

Table 1-3. IC_{50} values for **6** and **8** against different caspases[11]

Compound	**Caspase type, nM**									
	1	3	4	5	6	7	8	9	10	14
6	2.58	>10000	1380	1300	>10000	>10000	>10000	91.5	>10000	>10000
8	0.023	>10000	13.8	3.60	>10000	>10000	25.2	2.17	89.7	801

Later studies revealed that a greater selectivity and efficacy could be achieved using non-peptidomimetic compounds as caspase inhibitors.[9-14] Molecular modeling with computational studies has been used to design potent small molecular caspase inhibitors.[14,15] Structure activity

relationship studies have also been conducted in order to develop highly potent and specific caspase inhibitors. Small molecular caspase inhibitors have shown greater specificity towards various caspases than peptidomimetic inhibitors. These inhibitors contain various functional groups such as isoquinoline, sulfonamide and nitriles.[9,13,14]

1.1.2.3 Caspase-3 inhibitors

Caspase-3 is one of the executioner caspases frequently involved in most pharmacological models of apoptosis and their signaling cascades. It is known to be activated in most of the apoptotic cellular processes.[5] Therefore caspase-3 is a significant molecular target for studying apoptosis and its functions. Selective caspase-3 inhibitors have potential as therapeutic agents. Isatin- and isoquinoline-based caspase-3 inhibitors have been reported.[9,13-17] Some of the reported caspase-3 inhibitors have been further investigated in preclinical studies.[10]

1.1.2.4 Modified isatin sulfonamides as non-peptidomimetic caspase-3 inhibitors

In 2000, SmithKline Beecham conducted a high-throughput screen using their compound library in order to identify non-peptidic small molecules that inhibit caspase-3.[14] Several 5-nitroisatins were identified (Figure 1-4). Later studies showed that the presence of electron-withdrawing groups (EWG) such as nitro and cyano groups were necessary for higher potency.[13] The higher potency is attributed to the interaction of the active site cysteine thiol with the C-3 ketone of isatin. The thiol-ketone interaction is enhanced upon increasing the electrophilicity of C-3 (Scheme 1-2). Thus, an EWG at C-5 of isatin is required for higher inhibition, which makes the C-3 more susceptible for nucleophilic attack. Compounds **9** and **14** contain EWGs such as nitro and cyano on C-5 and show higher potency against caspase-3. Compound **13** has a

carboxylate group at C-5, which limits the electrophilicity of C-3 and shows lower potency (Figure 1-5).

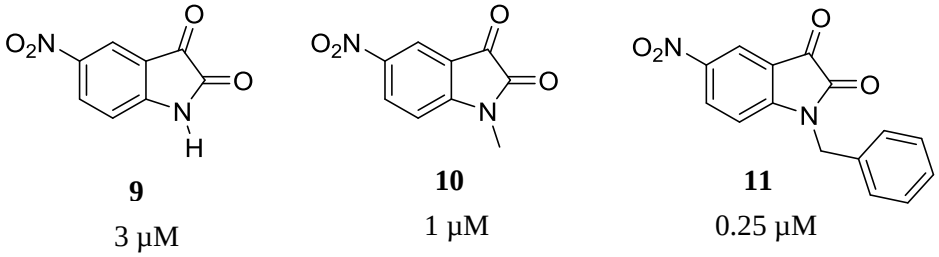
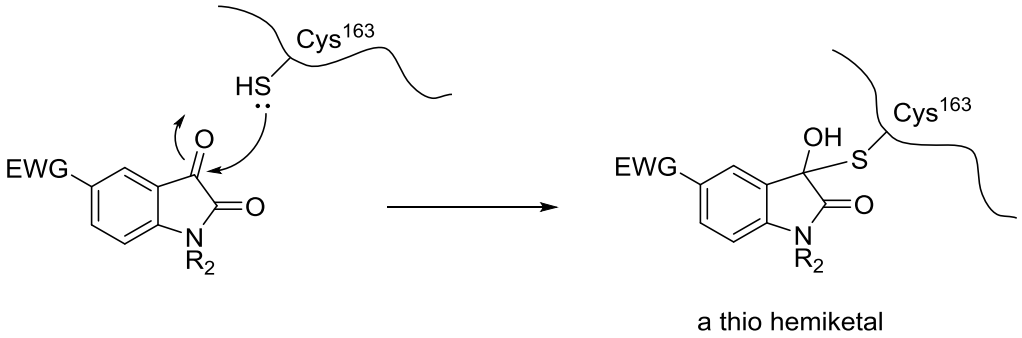


Figure 1-4. IC_{50} values of 5-nitroisatins as caspase-3 inhibitors[14]



Scheme 1-2. Thio hemiketal formation between C-3 ketone and cysteine-163 thiol

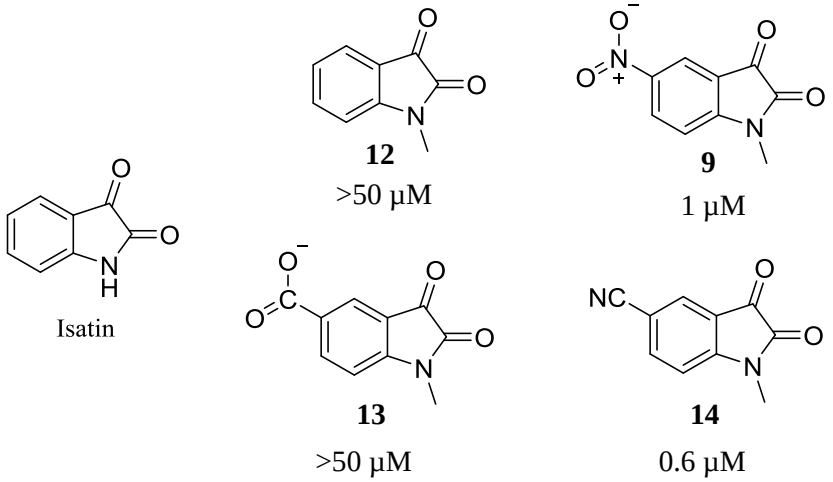


Figure 1-5. IC_{50} values of previously reported caspase-3 inhibitors[13]

Use of 5-*N*,*N*-dialkylisatin sulfonamides as potential caspase-3 inhibitors has been investigated.[13,14] The nitro group at C-5 has been substituted with a 5-sulfonamide group to

increase metabolic stability. Different amine substituents have also been used to investigate the caspase-3 potency and found that the incorporation of a pyrrolidine moiety is favorable for higher potency (Figure 1-6).[13] The stereochemistry of this pyrrolidine moiety (**15** and **17**), cyclic vs acyclic structures (**15** and **19**), and ring sizes (**16**, **18** and **20**) have been examined in inhibition studies. Incorporation of an *S*-2-methoxymethyl group in the pyrrolidine ring (**15**) yields a higher potency than its enantiomer, *R*-2-methoxymethyl (**17**). The ring size of pyrrolidine does not significantly change the potency (**16**, **18** and **20**). The acyclic analog of the pyrrolidine moiety (**19**) is less potent than its cyclic analog (**15**).[13]

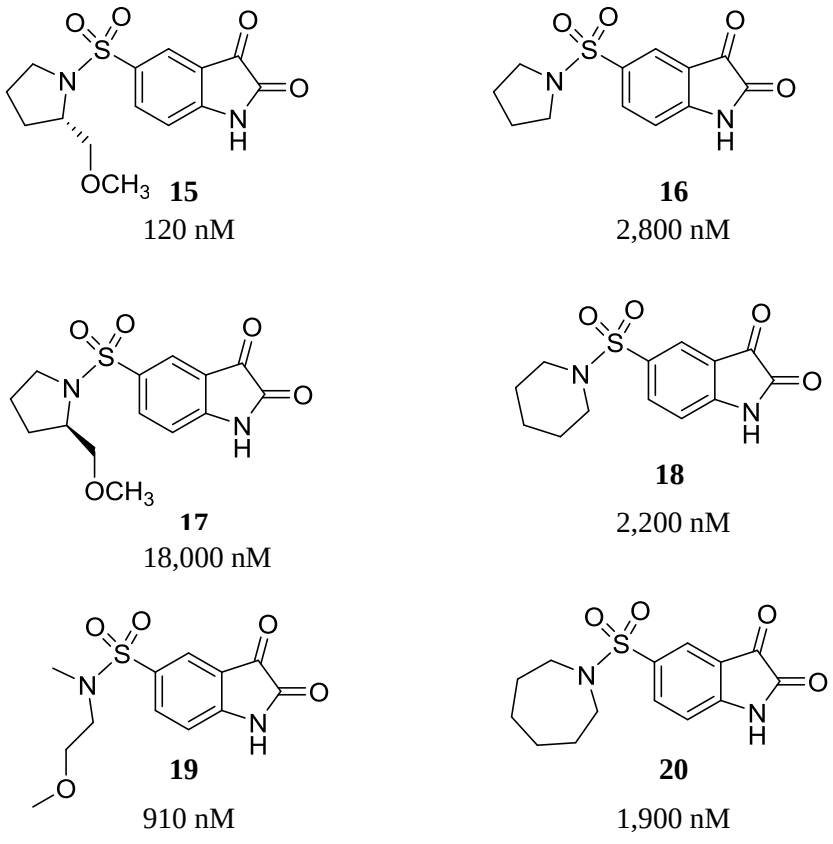


Figure 1-6. IC_{50} values of Pyrrolidinyl-5-sulfoisatin analogs against caspase-3 according to their structural properties[13]

Isatin sulfonamides inhibits executioner caspases (caspase-3 and -7).[13] In 2000, X-ray crystallographic data of caspase-3 bound with an isatin analog, 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1h-indole-2,3-dione **21** in the active site of the enzyme were reported (Figure 1-7).[14] Some key interactions between the inhibitor and caspase-3 were identified. Formation of a tetrahedral thio hemiketal intermediate was observed between the thiol of cysteine-163 and the C-3 ketone of **21**. Interaction with His-121 was also observed. Hydrophobic interactions occurred between the S_2 pocket and the pyrrolidine ring of **21**. A hydrophobic interaction of Tyr-204 with one face of the isatin core enhances the binding of inhibitor (Figure 1-7 and Figure 1-8).[14]

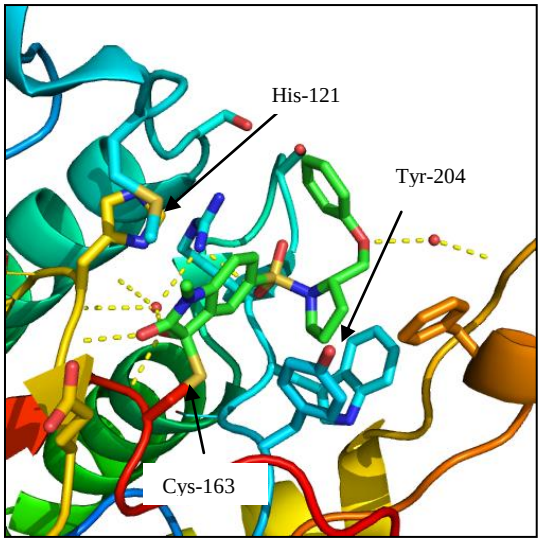


Figure 1-7. X-ray crystallographic data of **21** bound within active site of caspase-3[14]

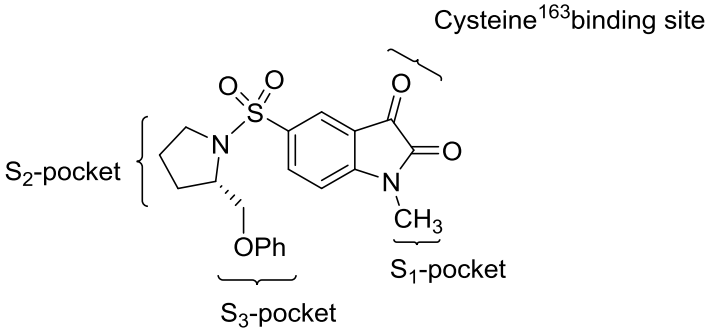
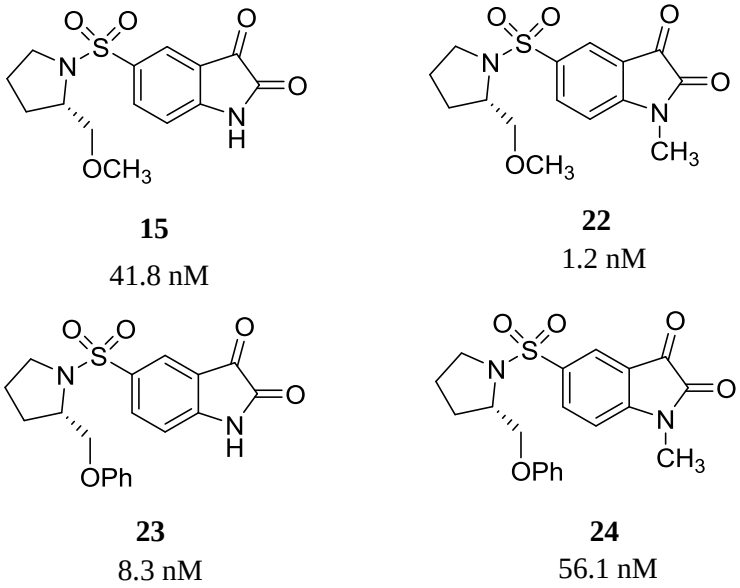


Figure 1-8. Enzyme binding pockets and binding site of isatin analog **21**

Modifying isatin sulfonamide analogues with pyrrolidine groups has significant effects on caspase inhibition.[13] For example, various pyrrolidinyl-5-sulfoisatins inhibit caspase-3 and -7 (Figure 1-9). Isatin sulfonamide analogs were modified according to structure activity relationships and their potencies in cellular apoptosis assays were determined. The inhibition of executioner caspases (caspase-3 and -7) is critical to prevent apoptosis in multiple cell types. Human Jurkat T cells and chondrocyte cells have been used to evaluate the cell death process in a previous study.[13] FACS (Fluorescence Activated Cell Sorter) analysis was used to investigate camptothecin-induced apoptosis in Jurkat and chondrocyte cells. Compound **15** inhibited apoptosis of Jurkat cell by 54% at 50 μM which was in good correlation with *in vitro* studies.[13]

Chondrocyte apoptosis was investigated because of its relevance to osteoarthritis (OA). Osteoarthritis is a degenerative joint disease in which erosion of articular cartilage occurs. Chondrocytes, the only cell type specific to cartilage tissue, are a good candidate to monitor increased levels of apoptosis. A gene, bcl-2 is involved in apoptosis has been highly expressed in chondrocytes adjacent to OA lesions. The fibrillar collagen network is degraded upon progression of OA. Thus, chondrocyte cell death is increased.[13] Similar results were obtained for compound **15** for inhibition of chrondocyte apoptosis which showed 98% inhibition at 50 μM. Recent cell apoptosis studies show that modified isatin sulfonamide analogs inhibit apoptosis in human umbilical vein endothelial cells (HUVEC).[17] Western blot analysis indicates the cleavage of a general caspase substrate PARP (poly ADP ribose polymerase), which correlates with caspase activity in cellular apoptosis.[13,17] PARP is a well known DNA repair enzyme and a substrate for caspase-3 cleavage in cellular apoptosis.[12] PARP is cleaved into 29 kDa and 85 kDa subunits by caspase-3 during apoptosis and cleavage can be monitored using Western blot analysis. This analysis has been extensively used in research to monitor cellular apoptosis.

The 2-phenoxymethyl pyrrolidinyls did not show cellular apoptosis inhibition activity up to 300 µM and are less potent than 2-methoxymethyl pyrrolidinyl compounds.[17] This observation implies that phenoxymethyl pyrollidinyls (**24**) have lower cell penetration properties[17] than that of methoxymethyl pyrollidinyls. The lipophilicity of these compounds is a major factor for their cell penetration properties. These properties were taken into account in the designing process of pyrollidinyl sulfo isatins as caspase-3 inhibitors.[13-17]



15
41.8 nM

22
1.2 nM

23
8.3 nM

24
56.1 nM

Figure 1-9. Previously reported K_i values of pyrrolidinyl-5-sulfoisatin analogs with caspase-3[17]

1.1.3 Ruthenium complexes for caging applications

Metal complexes are important therapeutics and tools for biological applications.[18-31] The caging of biologically active molecules with metal complexes has been explored recently.[31-35] Photocaging is an established method in biological and medicinal research. It enhances our ability to investigate living systems by providing spatial and temporal control over the release of the biologically active molecules.[31-35]

Ruthenium compounds have been used to develop caged complexes for several reasons. Ru-based compounds have unique photochemical properties which allow photoreactions to proceed with visible light, which is less harmful towards biological systems than UV light. Various biologically active compounds have been caged with Ru-complexes.[31-35] Those bio-active compounds contain functional groups that cannot be caged with organic photolabile groups. Recently reported bioactive molecules contain nitriles, *N*-containing heteroaromatics and thioethers that strongly bind to the metal center. Recently, neuroactive biomolecules (4-aminopyridine **25**) [31-33], bio-active thioethers[34] (D-biotin **26** and *N*-acetyl-L-methionine **27**) as well as small molecular enzyme inhibitors (**28**)[35] have been reported to be caged with these Ru-based complexes (Figure 1-10). A long-term goal of compounds like these is to achieve spatial and temporal release over drug release that could be used to treat various diseases.

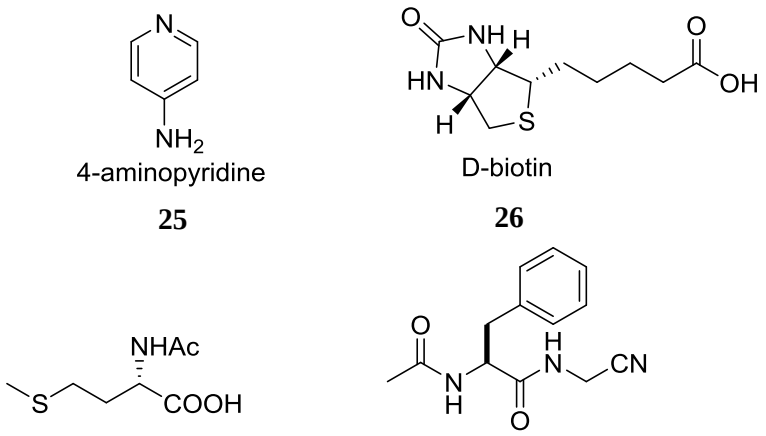


Figure 1-10. Previously reported photocagable biologically active compounds

Ruthenium polypyridyl compounds are excellent candidates for caging of small molecules. Various ruthenium polypyridyl groups have also been developed as efficient caging groups. The complexes $Ru(bpy)_2$ (bpy = 2,2'-bipyridne) (**29, 30**) and Ru(terpy)(bpy) (**31**) are

leading examples.[31-35] They show attractive photochemical properties, including stability in the dark and efficient release upon irradiation with visible light. Importantly, their Ru-caged byproducts show low toxicity. These Ru-polypyridyl complexes consist of planar heteroaromatic ligands with highly conjugated, extended π-electron systems that enhance their photolabile properties (Figure 1-11).

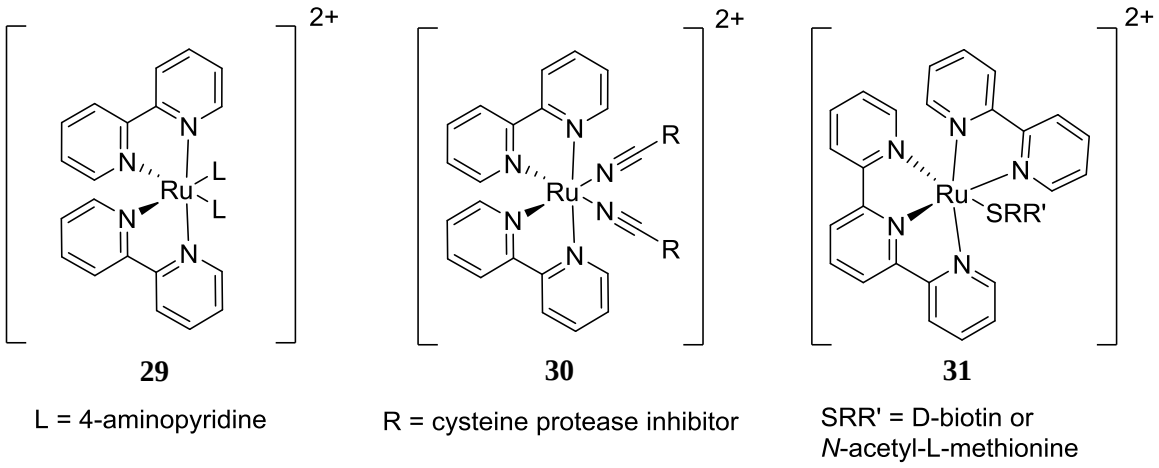


Figure 1-11: Chemical structures of caged ruthenium complexes

1.2 Research objectives

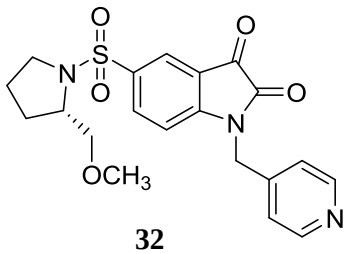
The aim of this study is to develop novel caspase-3 inhibitors which can be caged with ruthenium complexes. These light activated Ru complexes can then be used as tools for chemical biology and as potential therapeutics. Development of light activated compounds has advantages when compared to general small molecules. A unique advantage of light activation is the ability to control time, location and concentration of the biologically active compounds *in vitro* and *in vivo*. Therefore, light activated caspase-3 inhibitors could be used as a chemical tool to block apoptotic induction at specific sites *in vivo*. In the long term, the spatial and temporal control could be used to treat various diseases caused by dysregulation of apoptosis.

CHAPTER 2

2.1 Results

2.1.1 Designing of Caspase-3 inhibitors

The novel caspase-3 inhibitors developed in this study were designed based on several factors. Recent studies show that various 5-pyrrolidinylsulfonyl isatins act as caspase-3 inhibitors. Thus, we decided to design compounds based on a 5-pyrrolidinylsulfonyl isatin core. This small molecule inhibitor could be modified accordingly to develop light activated ruthenium complexes. Higher caspase-3 inhibition was desired in the designing process. Several findings in previous studies were considered when designing high potent caspase-3 inhibitors.[13,15-17] Use of specific stereochemistry in the pyrrolidine moiety is important since *S*-alkoxypyrrolidine is more potent than its *R*-stereoisomer against caspase-3. Methoxymethyl pyrrolidinyl analogs show higher cell permeability and lower lipophilicity than phenoxymethyl pyrrolidines, thus methoxymethyl pyrrolidine analogs were chosen for further studies. When designing the ruthenium-caged inhibitors, the chosen analogs should contain a group that has caging and photolabile properties. Therefore, pyridyl and cyano groups were introduced into these isatin sulfonamide analogs. Therefore 4-methylpyridine and cyanoethyl groups were selected to be attached to the N-1 position, resulting in compounds **32** and **33** (Figure 2-1).



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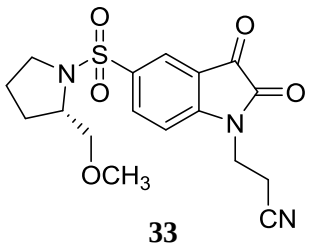
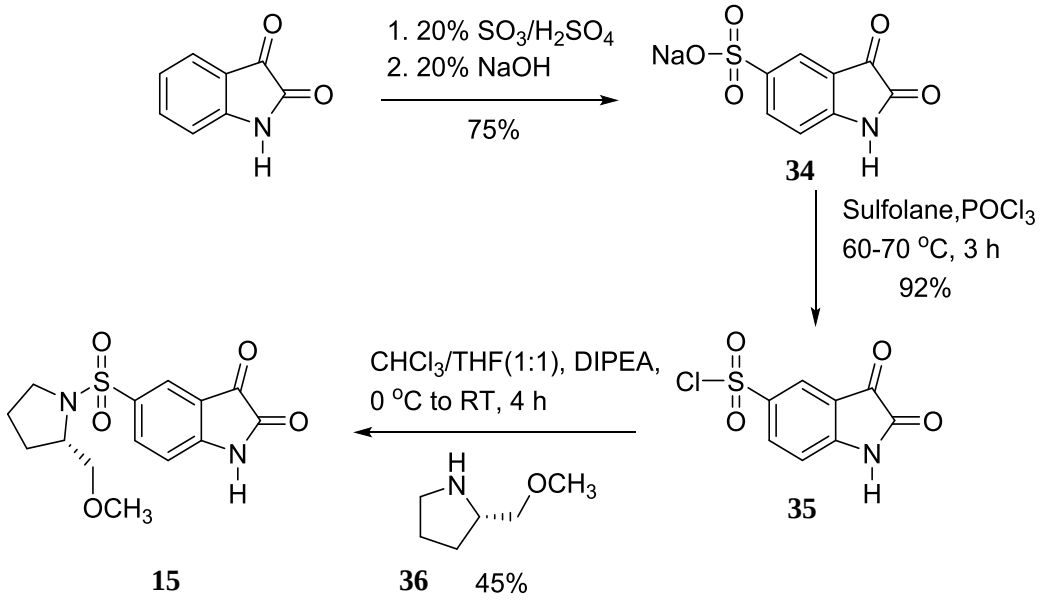


33

Figure 2-1. Proposed pyrrolidinyl-5-sulfoisatin analogs for caging studies

2.2 Synthesis of designed isatin sulfonamide analogs

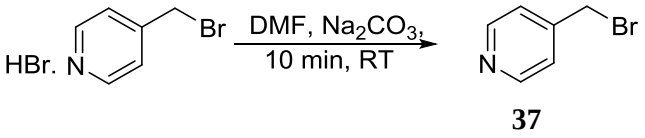
The designed analogs were synthesized using literature and modified procedures[13-17, 36-39] (Scheme 2-1). Compound **15** was synthesized as the precursor for the final analogs **32** and **33**. First, isatin was sulfonated at its C-5 position using fuming sulfuric acid containing 20% SO_3 to obtain **34**. Then according to the procedure reported previously,[17] 5-chlorosulfonyl isatin **35** was synthesized. The procedure was optimized to increase the yield. The condensation of **35** with **36** was performed in a 1:1 $CHCl_3$/THF solvent system in the presence of diisopropylethylamine (DIPEA). The reaction from **35** to **15** was challenging because **15** has lower solubility in most of the solvents thus giving low yields for **15**. Compound **15** was purified and isolated using flash column chromatography in 45% yield.



Scheme 2-1. Synthesis route for compound **15**

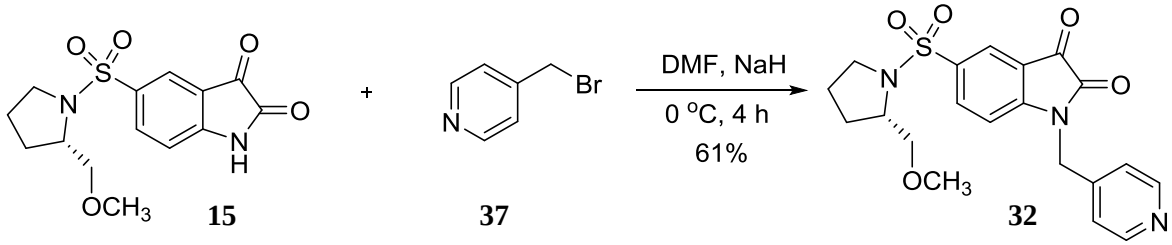
Compounds **32** and **33** were synthesized using modified and optimized procedures (Scheme 2-3 and Scheme 2-4). Compound **32** was synthesized using N-alkylation of **15** with 4-bromomethylpyridine (**37**). In this reaction compound **37** needed to be used as a free base

(Scheme 2-2) to avoid formation of an undesired side products which could lower yield of **32**. The synthesis of **33** proceeds through a base-catalyzed addition reaction of **15** with acrylonitrile. The reason for the low yield obtained for **33** might be the formation of polymers with acrylonitrile, consistent with TLC analysis which showed a complex mixture of products. The proposed reaction mechanisms for the formation of **32** and **33** are outlined below (Scheme 2-5 and Scheme 2-6).



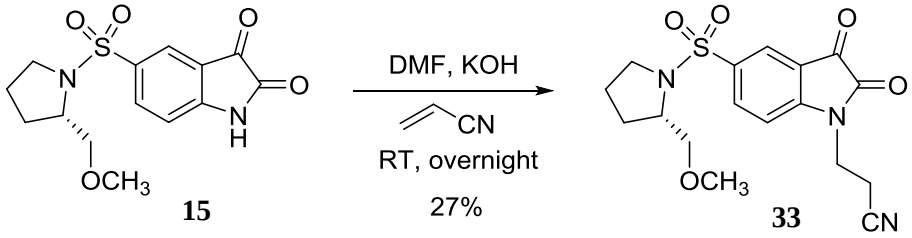
37

Scheme 2-2. Synthesis of **37** as a free base



15 **37** **32**

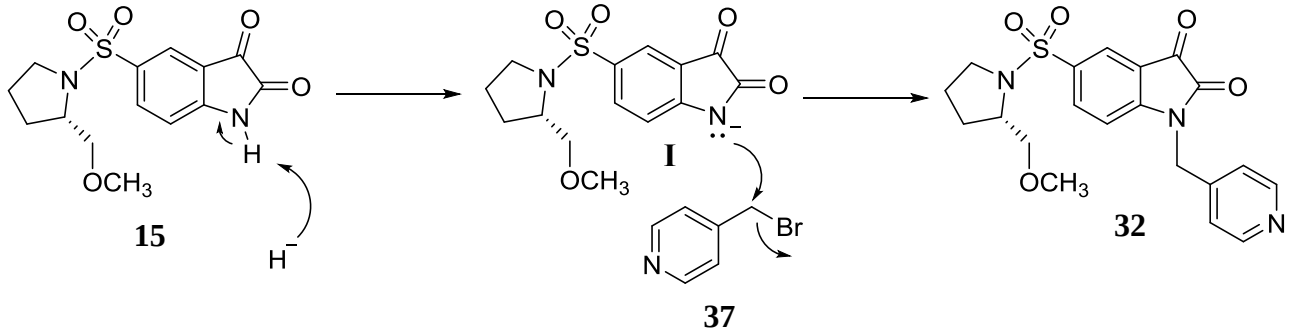
Scheme 2-3. Synthesis of analog **32**



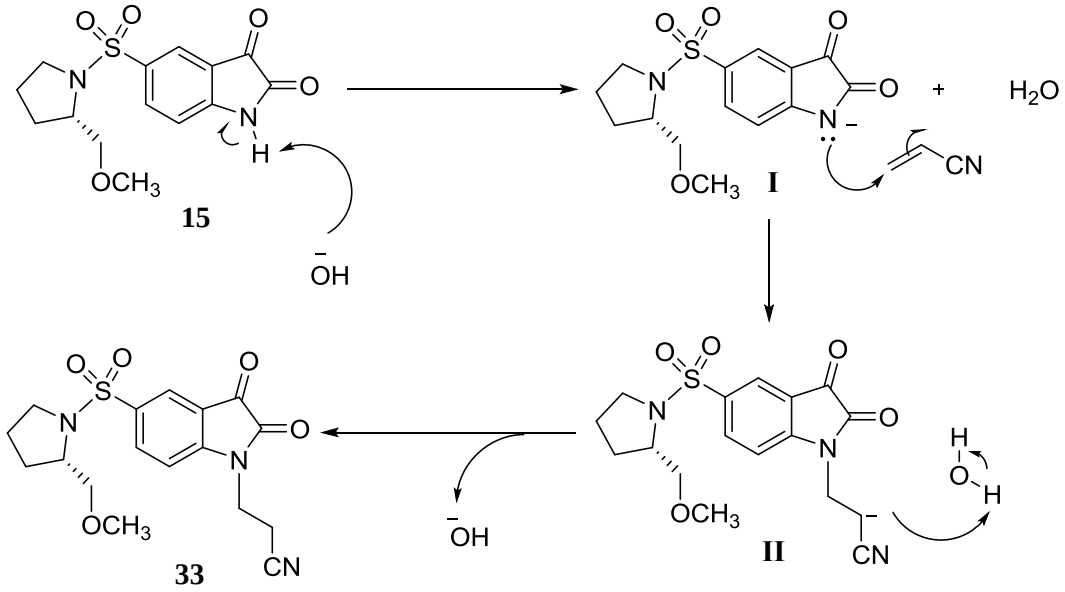
15 **33**

Scheme 2-4. Synthesis of analog **33**

Inhibition studies were done on **32** and **33** with human recombinant caspase-3. These enzyme inhibition assays were performed by the undergraduate researcher Veronica Lewalski. IC_{50} values were calculated according to the enzyme inhibition data. Compound **32** showed an IC_{50} of 89 nM and **33** showed IC_{50} of 560 nM.[40]



Scheme 2-5. Mechanism for formation of **32**



Scheme 2-6. Mechanism for formation of **33**

CHAPTER 3

3.1 Conclusion

The novel compounds **32** and **33** were synthesized successfully and their inhibitory effects were studied. The compounds **32** and **33** were both potent inhibitors of caspase-3. Compound **32** was more potent than compound **33**. Thus compound **32** was selected for further ruthenium caging studies.

3.2 Future directions

The designed compound **32** is expected to be caged with ruthenium polypyridyl complexes. The caged ruthenium complexes will be subjected to light activation experiments where the IC_{50} values of caged complexes under light and dark conditions will be determined and compared. Higher stability and lower potency will be desired for caged Ru-complexes in dark conditions. A higher dark/light inhibition ratio is desired and expected for **32**. Then photoinduced cell toxicity studies could be performed with these ruthenium caged complexes to monitor caspases-3 inhibition in different cell models. Cellular models of apoptosis such as Jurkat cells, chondrocytes, neutrophils from mouse bone marrow and HUVEC cells can be used to evaluate cellular toxicities of the designed compounds.

The spatial and temporal controlling experiments could be done for the developed Ru-complexes in various biological systems and animal models and apoptosis in these systems could be monitored. The reduction of the cellular apoptosis is desired upon light activation of these Ru-caged complexes. The cellular death could be monitored using various imaging methods. These combined experiments and results could lead to the development of novel tools to treat dysregulated apoptosis in biological systems that can be used to prevent various diseases. For

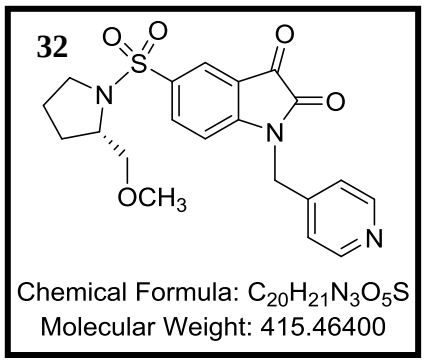
example, *in vivo* experiments could be carried out to study cardiomyocyte apoptosis (in myocardial ischemia model) and evaluate the heart muscle and tissue damage in mouse models. These designed compounds can also be used to evaluate apoptosis in bacterial meningitis models to study neuronal cell degaradation and also in hepatitic models to study liver damage in mouse models. These experiments could be carried out to develop efficient therapeutic methods such as photodynamic therapy to treat diseases caused by apoptosis.

3.3 Experimental

3.3.1 General considerations

All reagents were purchased from commercial suppliers and used as received. NMR spectra were recorded on a Varian FT-NMR Mercury-400 Spectrometer. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer (KBr pellet). High resolution (HRMS) electrospray (ESI-TOF) mass spectra were recorded on a Micromass LCT spectrometer. Melting points were recorded on Thermo scientific 9100 melting point apparatus. Enzymatic assays were performed on a Tecan Infinite F200Pro microplate reader. All reactions were performed under ambient atmosphere unless otherwise noted. Anaerobic reactions were performed by purging the reaction solutions with Ar or N_2.

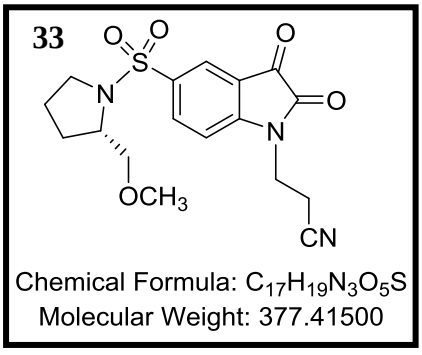
3.3.2 (S)-5-((2-(methoxymethyl) pyrrolidin-1-yl)sulfonyl)-1-(pyridin-4-ylmethyl)indoline-2,3-dione (32)



To a stirred solution of **15** (0.936 g, 2.89 mmol) in DMF (40 mL), 60% NaH in mineral oil (0.174 g, 7.23 mmol) was added at 0 °C. The reaction was stirred for 30 min under Ar atmosphere. Then a solution of 4-bromomethyl pyridine **37** was prepared as follows. 4-bromomethyl pyridine hydrobromide salt (0.873 g, 3.46 mmol) was dissolved in DMF (15 mL) and Na_2CO_3 (0.367 g, 3.46 mmol) was added and stirred for 10 min. The filtrate of **37** was added dropwise to the reaction mixture over 10 min and stirred for additional 4 h at 0 °C under Ar

atmosphere. The reaction was diluted with EtOAc (60 mL) and washed with saturated aqueous NaCl (3 × 40 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel chromatography using 1% MeOH:CH_2Cl_2 solvent system. The isolated fractions containing product were combined and concentrated in vacuo and further crystallized using EtOAc:hexanes. The product was isolated as a bright yellow solid. Yield: 0.730 g (1.76 mmol, 61%). mp = 172-174 °C. ^{1}H NMR (400 MHz, DMSO): δ 8.51 (d, 2H, *J* = 4.4 Hz), 8.01 (d, 1H, *J* = 8.4 Hz), 7.84 (s, 1H), 7.46 (d, 2H, *J* = 4.4 Hz), 7.08 (d, 1H, *J* = 8.0 Hz), 4.99 (s, 2H), 3.67 (m, 1H), 3.41 (dd, 1H, *J* = 9.6 Hz, 3.6 Hz), 3.32 (s, 3H), 3.26 (t, 2H), 3.06 (m, 1H), 1.73 (m, 2H), 1.48 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 181.7, 159.2, 153.3, 150.2, 144.8, 136.9, 132.3, 123.1, 122.6, 118.9, 111.8, 74.9, 59.1, 58.9, 49.4, 42.7, 28.7, 24.0. IR (ν_{max}) (KBr): 3443, 2929, 2361, 2342, 1747, 1616, 1478, 1450, 1417, 1365, 1344, 1330, 1199, 1181, 1154, 1130, 1115, 1070, 1041, 994. HRMS (ESI-TOF): calc'd for $C_{21}H_{25}N_3O_6NaS$ $(M+MeOH+Na)^+$ 470.1362, found: 470.1339.

3.3.3 (S)-3-(5-((2-(methoxymethyl) pyrrolidin-1-yl)sulfonyl)-2,3-dioxoindolin-1 yl)propanenitrile (33)



Chemical Formula: $C_{17}H_{19}N_3O_5S$
Molecular Weight: 377.41500

To a stirred solution of **15** (0.200 g, 0.620 mmol) in DMF (10 mL), KOH (0.004 g, 0.062 mmol) was added and stirred for 10 min at RT. Then acrylonitrile (45 µL, 0.68 mmol) was added dropwise and stirred for 2 days at RT under Ar atmosphere. The reaction mixture was added to H_2O (30 mL), and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed

with 10% aqueous NaCl (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel chromatography with 1% MeOH:CH_2Cl_2 to afford a yellowish-orange solid. Yield: 0.064 g (0.169 mmol, 27%). mp = 134-138 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, 1H, *J* = 2 Hz, 8.4 Hz), 8.11 (d, 1H, *J* = 2 Hz), 7.19 (d, 1H, *J* = 8.0 Hz), 4.10 (t, 2H), 3.77 (m, 1H), 3.57 (dd, 1H, *J* = 4.0 Hz, 9.2 Hz), 3.43 (m, 1H), 3.38 (d, 1H, *J* = 2.4 Hz, 10 Hz), 3.36 (s,3H), 3.14 (m, 1H), 2.86 (t, 2H), 1.92 (m, 2H), 1.69 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.8, 157.8, 152.3, 137.6, 134.7, 124.9, 117.5, 116.8, 110.4, 74.8, 59.3, 59.1, 49.4, 36.8, 28.8, 24.1, 16.7. IR (ν_{max}) (KBr): 3422, 2921, 2852, 2361, 2251, 1742, 1717, 1647, 1612, 1558, 1542, 1508, 1475, 1456, 1418, 1373, 1364, 1340, 1314, 1268, 1234, 1195, 1175, 1153, 1133, 1063, 1046, 991, 970, 905, 877. HRMS (ESI-TOF): calc'd for $C_{17}H_{19}N_3O_5NaS$ $(M+Na)^+$ 400.0943, found: 400.0958.

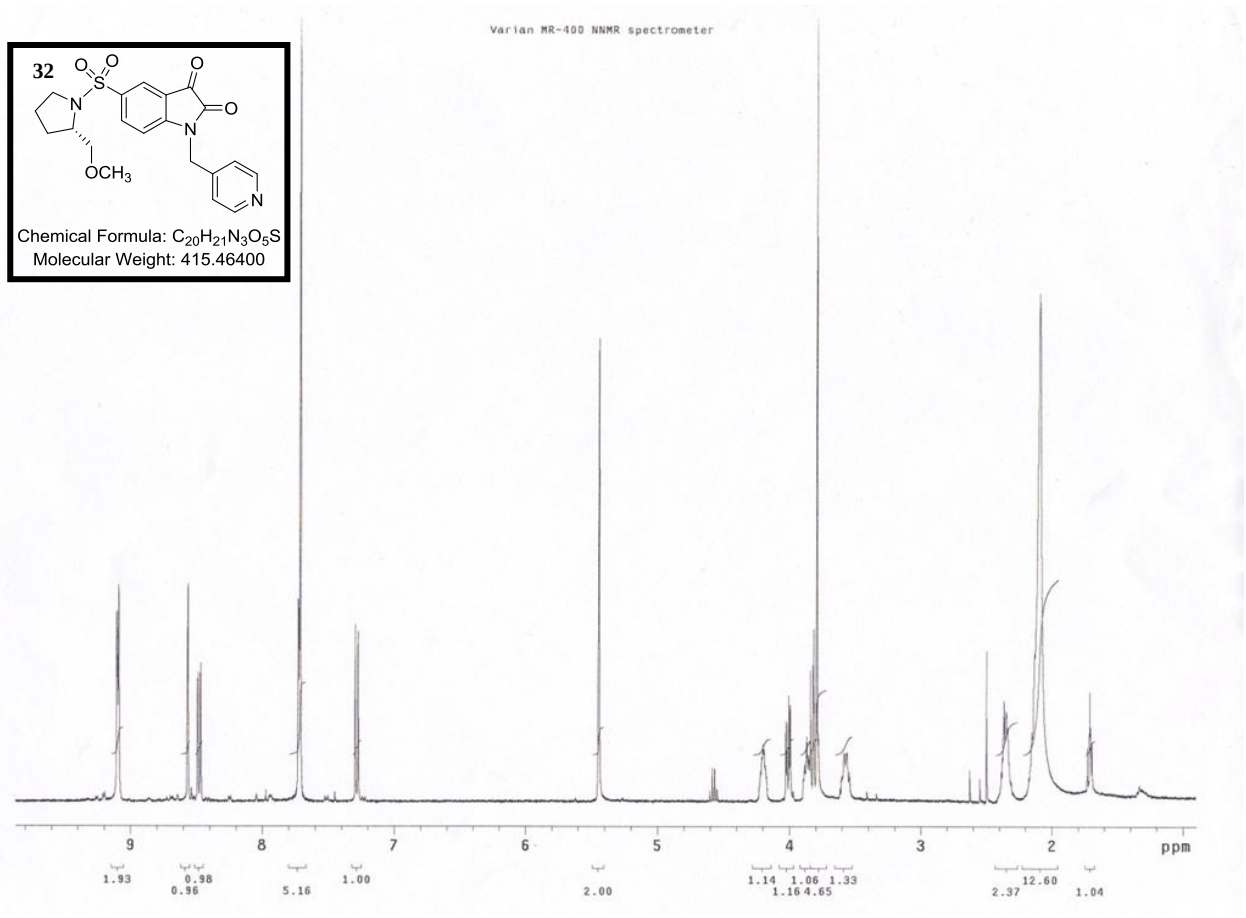


Figure 3-1: ^{1}H-NMR spectrum of **32**

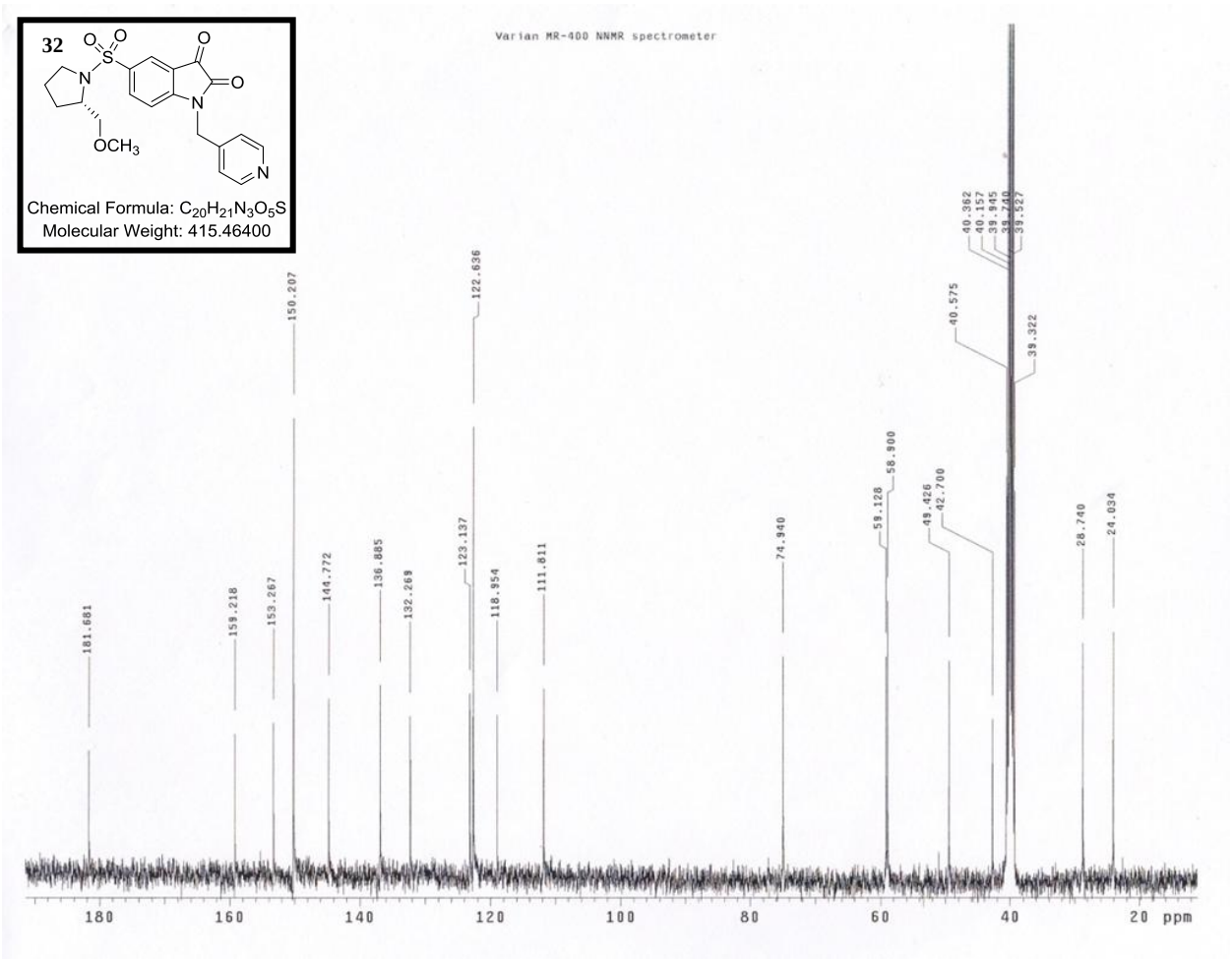


Figure 3-2: ^{13}C-NMR spectrum of **32**

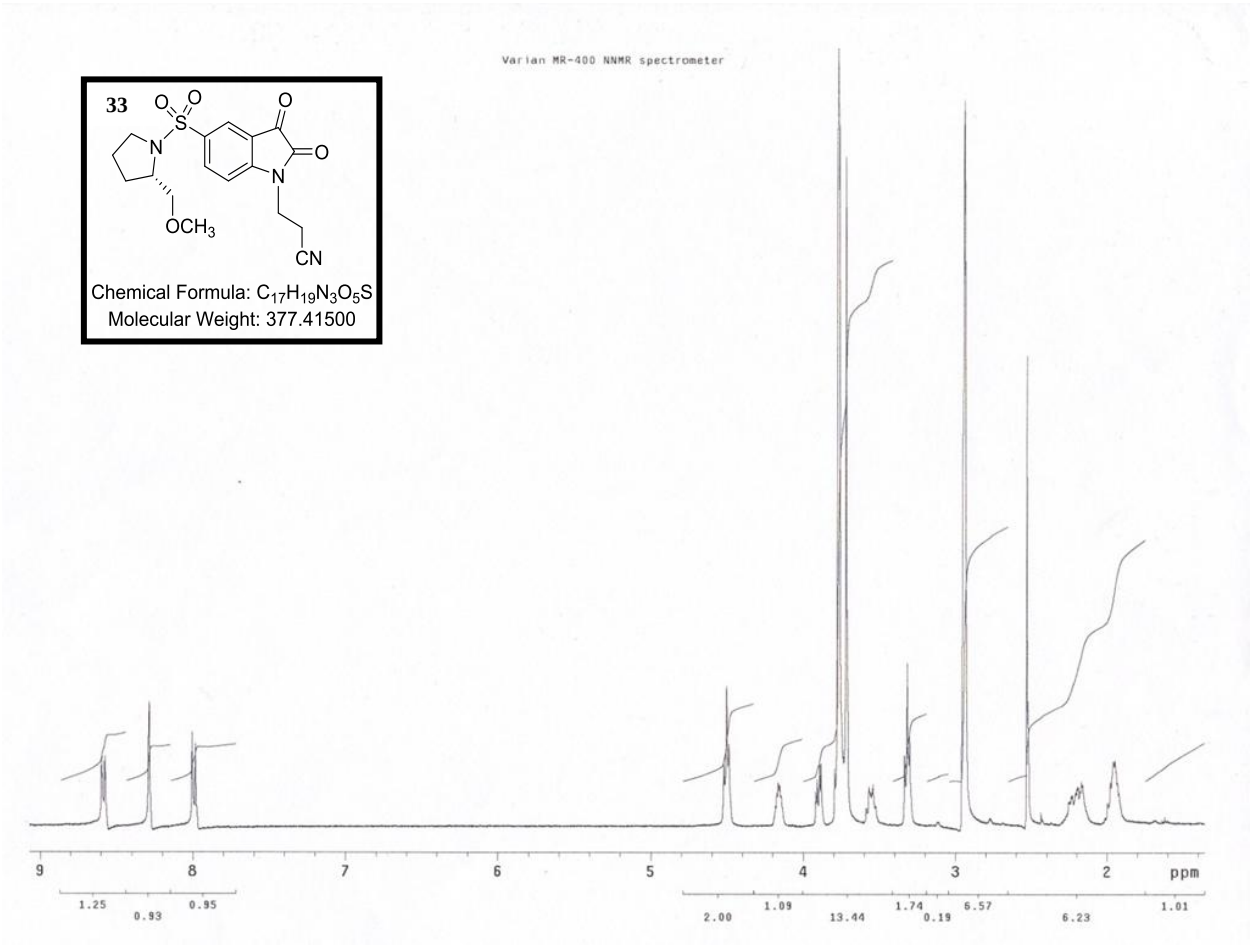


Figure 3-3: ^{1}H-NMR spectrum of **33**

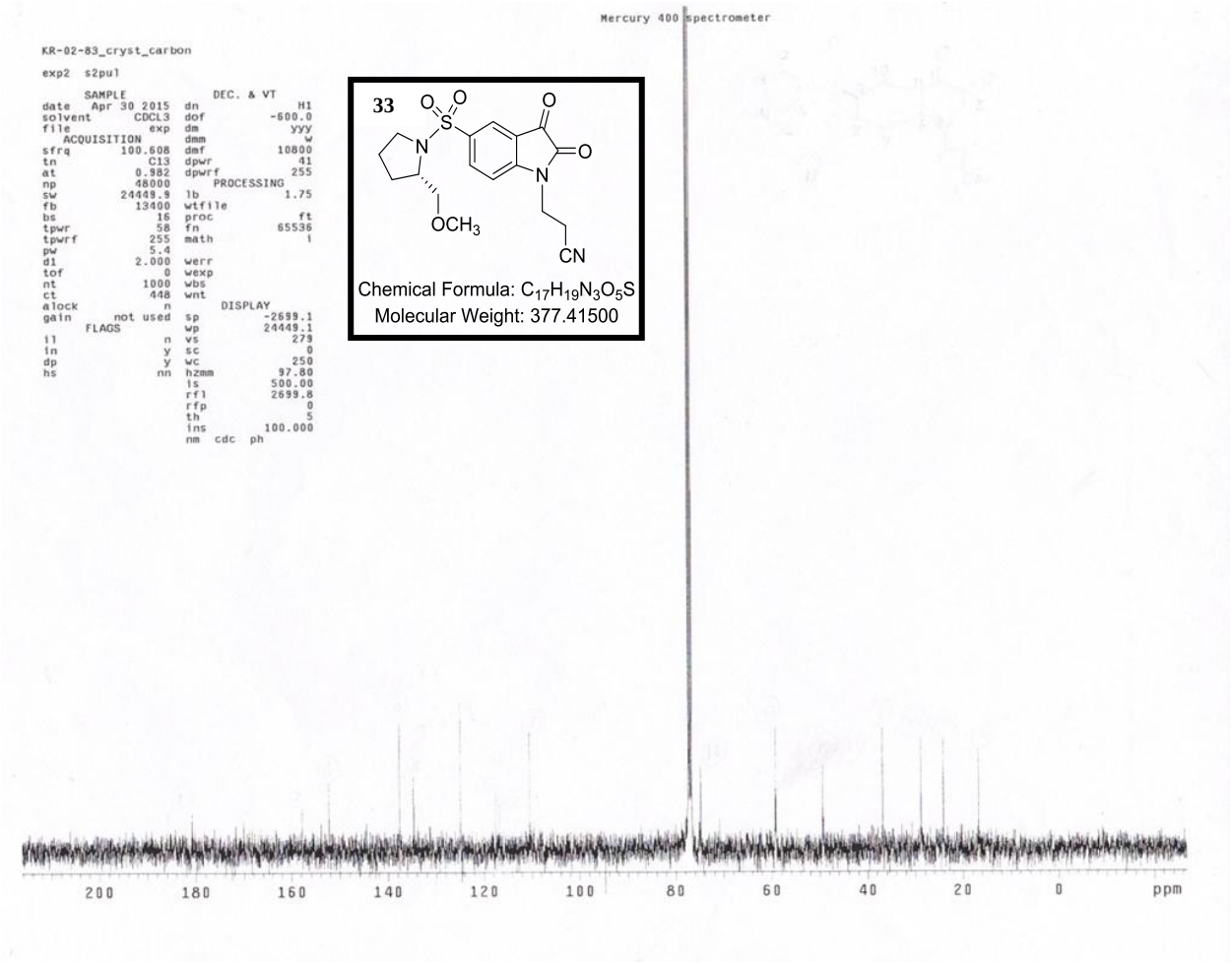


Figure 3-4: ^{13}C-NMR spectrum of **33**

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40. Enzymatic inhibition assays were conducted by Veronica Lewalski, Department of Chemistry, Wayne State University.

ABSTRACT

DESIGN AND SYNTHESIS OF ISATIN BASED CASPASE INHIBITORS FOR RUTHENIUM CAGING APPLICATIONS

by

KASUN CHINTHAKA RATNAYAKE

August 2015

Advisor: Jeremy J. Kodanko, Ph.D.

Major: Chemistry (Organic)

Degree: Master of Science

Apoptosis is the energy dependent programmed cell death. Improper function of apoptosis could lead to diseases such as cancers, strokes, Alziemer's disease. Caspases are the enzymes involved in the later stage of this process. Peptidyl and non-peptidyl caspase inhibitors have been synthesized recently. These non-peptidyl compound classes which consist of pyrrolidinyl-5-sulfo isatins have showed a greater potency against executioner caspases, caspase-3 and -7. According to literature and for further caging studies, two compounds were designed, synthesized and evaluated their inhibition against caspase-3 in this study. The analog in which its N-1 position alkylated with a 4-methyl pyridine moiety (**32**) showed higher inhibition than the analog in which N-1 was alkylated with cyanoethyl group (**33**). Thus, compound **32** was selected for further caging studies with ruthenium.

AUTOBIOGRAPHICAL STATEMENT

Kasun Chinthaka Ratnayake

Mr. Kasun Ratnayake was born in Kandy, Sri Lanka. He received his BSc. degree in chemistry from University of Peradeniya (UoP), Sri Lanka with first class honors. He worked as a teaching assistant at UoP between January-July, 2013. He enrolled in the department of chemistry at Wayne State University in August, 2013 to obtain his Master of Science degree in chemistry. He worked as a graduate research and teaching assistant from 2013-August to 2015-August.

Education

2013-2015	Master of Science (Chemistry), Wayne State University, Detroit, MI, USA Advisor: Prof. Jeremy Kodanko
2008-2013	Bachelor of Science (Chemistry), First class honors (UoP)

Honors and Awards

2013 - University Award for Academic Excellence (UoP)

2013 - Bhikaji Framji Khan Gold Medal for Chemistry (UoP)

2013 - Sultanbawa Prize for the Best Performance in Organic Chemistry (UoP)

2013 - Inter University Chemistry competition - Bronze medal (Royal Society of Chemistry-Sri Lanka section)

2013 - Newton A Dias Weerasinha Memorial Scholarship (Royal Society of Chemistry-Sri Lanka section)

Publications

PURSE 2013 (Peradeniya University Annual Research Sessions): Abstract on “Extraction of alkaloids of *Catharanthus roseus* into interlayer nanospaces of cation-exchanged montmorillonite clays”