Synthesis and Characterization of Spirooxindole Derivatives as Potential Antimalarial Agents

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Declaration

I declare that the work presented in this dissertation was carried out exclusively by myself under the supervision of Dr. Amanda Rousseau and Dr. Moira Bode. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Kamogelo Butsi

Abstract

Spirooxindoles are an important class of spirocycles in organic and medicinal chemistry. They are characterised by a spiro-ring fused with the oxindole scaffold and have a wide range of biological activity. We are particularly interested in spirooxindoles because of their antimalarial activity. Malaria is a major health problem in many parts of the world and the burden caused by the disease is still of great concern. In 2015 alone, an estimated 438 000 deaths due to malaria were reported across the world, with 90% of the deaths occurring in Africa. The increase in drug resistance to currently used antimalarial agents has rendered most of them ineffective, thereby contributing to the high mortality rates. As a result, there is a need for the development of new effective antimalarial agents. In the search for a new class of antimalarial chemotypes, cipargamin, introduced as NITD609 by norvatis in 2010 was synthesised. This compound is a novel synthetic antimalarial candidate, with an IC_{50} of ~1 nM against *P. falciparum* strains, including multi drug-resistant strains.

Previously in our laboratory, several spirooxindole derivatives were synthesised using an imino Diels-Alder reaction, also known as the Povarov reaction. Of all the compounds synthesised, only those derived from a *para*-substituted aniline displayed activity in the low micromolar range (~5µM) against *P. falciparum in vitro*. In this project, we aimed to further explore the antimalarial activity of these compounds by designing and synthesising ring-opened analogues. The analogues were successfully synthesised by a Grignard addition reaction using *N*-Boc protected arylimines as electrophiles. Despite several attempts, we were unable to remove the Boc-protecting group in the final step.

The second series of compounds we aimed to synthesise were ring closed analogues lacking one aromatic ring. The compounds were synthesised starting from an imine condensation reaction between benzyl protected isatin with *para*-substituted 2-allyanilines. The 2-allylanilines were prepared by subjecting *N*-allylanilines to an aza-Cope rearrangement. The arylimines prepared were then subjected to a nucleophilic Grignard addition reaction with commercially available vinylmagnesium bromide to yield the intermediate necessary for the ring closure step. Unfortunately, the nucleophilic

addition reaction was unsuccessful. The ring-closure step is very crucial during the synthetic route as it gives rise to the desired ring closed analogues via ring-closing metathesis. Although we were unable to reach the final step in the synthesis of ring-closed analogues, some progress was made in developing methodology for the synthesis of these analogues.

The synthesised ring-opened analogues were screened for antimalarial activity against *P. falciparum in vitro*. Six hit compounds were identified from the series of compounds tested with *tert*-butyl 3-(2,4-dichlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **60** being the most active compound in the series with an IC_{50} value of 0.60 nM against the FCR 3 Strain. In general, compounds derived from *p*-toluidine displayed the most potent activity.

Acknowledgements

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A special Thank you to the most important people in my life, Atlegang and Sello. Thank you for being my source of strength during good and hard times. I will forever treasure your presence in my life.

I thank God for the endless blessings, it is for his mercy that everything was possible.

List of abbreviations

Ac_2O	acetic anhydride
Boc	tert-butyloxycarbonyl
Cl_3CCN	trichloroacetonitrile
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DDT	dichlorodiphenyltrichloroethane
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EtOAc	ethyl acetate
Hex	hexane
HRMS	high resolution mass spectrometry
m.p	melting point
NMR	nuclear magnetic resonance
R_f	retardation factor
rt	room temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Contents

1 INTRODUCTION

The recent world malaria report (2015) has indicated that the mortality rates caused by malaria have decreased by 48% worldwide since the year 2000, and by 66% in Africa.[1] However, despite efforts made to eradicate the disease, it is still a major health problem in many parts of the world and the burden caused by the disease is still of great concern. In 2015 approximately 214 million malaria cases were reported and an estimated 438 000 people died from the disease across the world, with 90% of the deaths occurring in Africa. The majority of deaths were children under the age of 5 (~ 292 000).[1]

Since its discovery by Charles Louis Alphonse Laveran in 1800,[2] there has been a significant effort in search of strategic interventions to control, treat and eradicate the disease. South Africa has been successful in controlling malaria transmission in all three malaria-endemic provinces: Limpopo, Mpumalanga and Kwa-Zulu Natal. This was achieved by implementing Indoor Residual Spraying, a strategy aimed at preventing transmission of the malaria parasite from the vector, *Anopheles* mosquito, to humans. Insecticides such as dichlorodiphenyltrichloroethane (DDT), deltamethrin and carbamates are utilised for vector control.[3] DDT is sprayed on inner walls of households at coverage of 2 g/m^2 yearly[4] and has displayed successful toxic activity against the mosquito vector. Despite its advantages, it has been banned in many other industrialized countries since the early 1970s. The bans are consequent to environmental and health concerns posed by the organochlorine compound. DDT is a persistent organic pollutant that accumulates in the environment and can cause significant health problems when in contact with skin or ingested. There have been reports of the presence of DDT in breast milk of lactating women in Kwa-Zulu Natal,[4] which is passed on to breastfed infants. The DDT concentration also increased in the infants' blood with age.[4] Despite the alarming reports that DDT poses serious problems to human health and the environment, it is still continuously used to control malaria in South Africa.

1.1 The Parasite

1.1.1 *Plasmodium falciparum*

Malaria is caused by parasitic protists belonging to the genus *Plasmodium*, with five species affecting humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi* and *P. malariae*. Of these, *P. falciparum* causes the most severe cases and is prevalent in Africa. Characteristic symptoms associated with severe malaria include coma, severe anaemia, multi-organ failure, hypoglycemia, convulsions, cerebral malaria and metabolic acidosis to name a few.[5,6] Manifestation of the symptoms occurs during the blood stage of infection, indicated in the life cycle of the parasite (**Figure 1**), as a result of the parasite replicating and growing in the human host erythrocytes. Mature erythrocytes infected by *P. falciparum* sequester in the microvasculature of vital organs throughout the host body. Sequestration occurs as adhesions derived by the parasite, expressed on the surface of mature infected erythrocytes, bind to receptors expressed on the surface of human cells (vascular endothelium).[5] Sequestrations of infected erythrocytes leads to microvascular obstruction and metabolic acidosis.[6] Blocking the microvascular system results in a decrease in perfusion of oxygen and nutrients required by the internal organs or tissues as a consequence, anaerobic glycolysis occurs and lactate concentrations are increased leading to acidosis. Sequestration is the pathological trademark to severe *P. falciparum* malaria.

1.1.2 *Plasmodium vivax*

P. vivax has a wider geographical distribution compared to *P*. *falciparum*, and predominates in Asia and South America.[7] It can survive over long periods because of its dormant liver stage, called the hypnozoite. The dormant stage can be activated months after the first infection, causing a relapse in symptoms. Although malaria infections caused by *P. vivax* can occur throughout Africa, the risk of infection is reduced because of the Duffy negativity trait among Africans,[1] which results in the lack of a protein necessary for *P. vivax* to invade red blood cells.

1.1.3 *Plasmodium knowlesi*

Malaria infections due to *P. knowlesi* can lead to death because of the explosive asexual erythrocytic cycle,[8] but this *Plasmodium* species has a more limited distribution as it is vector-restricted to the *Anopheles leucosphyrus* group, a rare mosquito only found in South-Western India, Southern China, Taiwan, South-East Asia, Indonesia and Philippines.[9,10] Until recently, this *Plasmodium* species was known to cause malaria among monkeys only, but recent reports have indicated that *P. knowlesi* can cause malaria among humans. It is passed on to humans *via* zoonotic transmission, a type of transmission that occurs when an *Anopheles* mosquito infected by a monkey bites and infects humans.[1]

1.1.4 *Plasmodium ovale and Plasmodium malariae*

Similar to *P. vivax*, *P. ovale* causes a relapse of symptoms and enlarges the host hepatocyte nucleus during the liver stage.[11] *P. malariae* is known to cause quartan malaria, the name is brought about because the fever resulting from the malaria infection reoccurs at 3-days intervals.[11]

1.2 The general life cycle of malaria parasite

The general life cycle of the malaria causing species is illustrated in **Figure 1**.[2,5,8] It has a complex life cycle involving both the human host and the mosquito vector. The female mosquito of the genus *Anopheles* plays a vital role in the widespread transmission of malaria.[1] Knowledge of the life cycle can help identify new biological targets at a molecular level, which in turn leads to the identification of new antimalarial compounds. The infection stage (**1**) begins by inoculation of sporozoites from an infected mosquito while feeding on the human host blood stream. They are then carried through the body until they reach liver cells, where asexual multiplication takes place to produce merozoites. This is a stage specifically known as exoerythrocytic schizogony (**2**). The merozoites are released into the blood stream and invade erythrocytes where they undergo the second asexual multiplication, i.e, erythrocytic schizogony (**3**) to produce merozoites. The resulting merozoites could repeat the cycle indefinitely ultimately leading to parasitaemia. This stage is responsible for the manifestation of malaria.

During progression of the disease, other merozoites develop into female and male gametocytes (**4**) taken up by the mosquito while feeding (**5**). The gametocytes will develop into mature gametes inside the gut of the insect. Fertilization occurs progressing towards the development of ookinetes which develop into mature sporozoites found in the saliva of the insect vector.

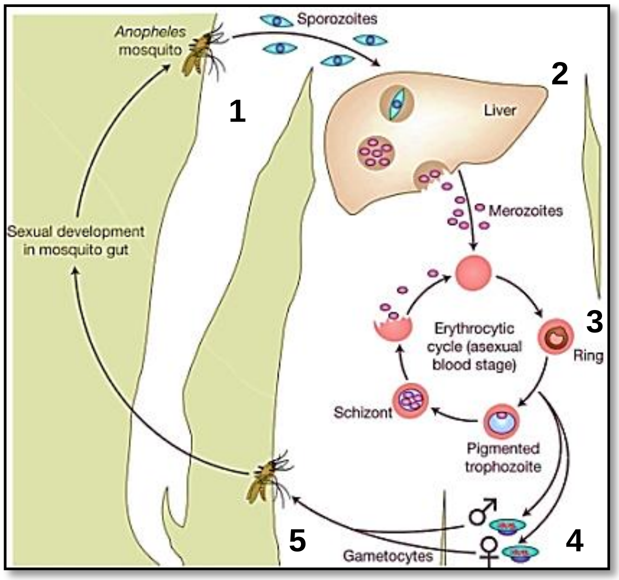


Figure 1. General life cycle of *Plasmodium*. Extracted from: J.A Rowe *et al*., *Expert Rev. Mol. Med.,* **2009**, 11, 1-29

Processes leading towards gametocytogenesis are good targets for malaria chemotherapy. Inhibition of the sexual reproduction stage of merozoites to gametocytes which are taken up by the mosquito vector can prevent transmission of the parasite from person to mosquito vector, and therefore reduce the spread of the disease. A successful antimalarial drug should ideally have gametocytocidal activity or be capable of terminating the erythrocytic cycle phase (**3** in **Figure 1**) with minimal side effects.[8]

1.3 Treatments

1.3.1 Quinolones

Chemotherapy is an essential tool utilised to treat and prevent transmission of malaria. A variety of quinolones have played a crucial role as antimalarial agents including quinine (**1**), chloroquine (**2**), mefloquine (**3**), amodiaquine (**4**) and primaquine (**5**). Structural differences between these compounds introduce variation in their respective mode of action.

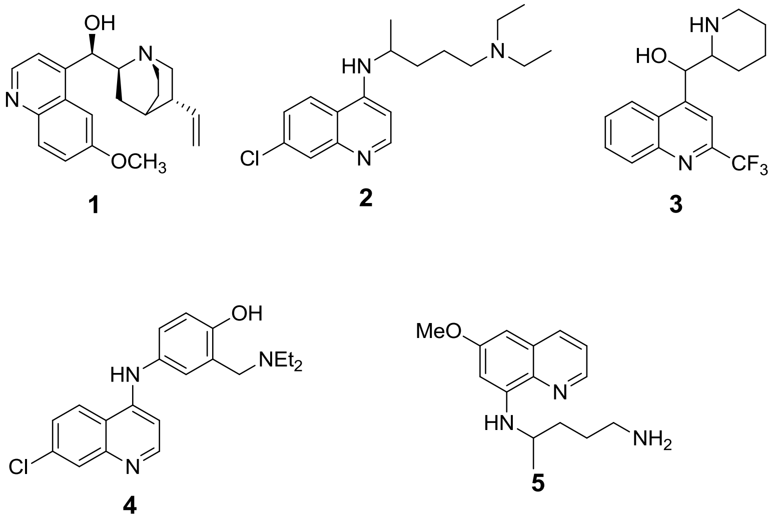


Figure 2. Chemical structures of quinine **1**, chloroquine **2**, mefloquine **3**, amodiaquine **4** and primaquine **5**

1.3.1.1 Quinine

The Spanish Countess of Chinchon visited Peru during the 1600s and contracted a fever. She was given a tree bark to treat the fever by the natives of Peru. On her return to Europe in 1638, she introduced Europeans to the tree bark, now commonly known as the bark of the *Cinchona* tree.[12] In 1820, the active alkaloid was extracted from the tree bark and named quinine by Pierre Joseph Pelletier and Joseph Carventou.[7,12] Since its isolation, quinine has been used as a drug candidate for the treatment of malaria, replacing the tree bark. The limited availability of large quantities of purified quinine during the World Wars prompted scientists to develop and synthesise alternative antimalarial agents,[13] leading to the identification of chloroquine and pyrimethamine in the 1940's. Significantly, quinine is still used to date as a second line of treatment in severe cases of malaria, largely owing to its ability to cross the blood brain barrier

1.3.1.2 Chloroquine

Chloroquine is one of the earliest synthetic quinolones used to treat malaria. It was introduced during the late 1940s and became the predominant anti-malarial drug through that particular period. Unlike many malaria drugs for which the mode of action is poorly understood, the mode of action of chloroquine has been rigorously studied. During the erythrocytic cycle in the host red blood cells, the parasite degrades haemoglobin in an acidic digestive vacuole to generate haem monomers. However, free haem is toxic to cells.[12] The liberation of haem from haemoglobin leads to haem-iron undergoing oxidation from Fe^{2+} to Fe^{3+}.[12] This results in the production of reactive oxygen species such as peroxide giving rise to oxidative stress that can lead to DNA damage and degradation of proteins to peptides.[14] The parasite protects itself from the toxic haem by polymerizing haem monomers to inert biocrystals named hemozoin.[15]

Chloroquine is a weak base with pKa values of 8.1 and 10.2. In its non-protonated form it can readily diffuse through the membrane of the digestive vacuole.[7] Once in the acidic vacuole, it is diprotonated and accumulates (**Figure 3**).[16] Chloroquine then binds to hematin (haem dimer) forming a chloroquine-hematin complex. The complex prevents detoxification of haem by inhibiting polymerization of haem monomers, which leads to accumulation of toxic haem monomers. The inhibition of polymerization ultimately causes the death of the parasite with its own metabolic debris.[12]

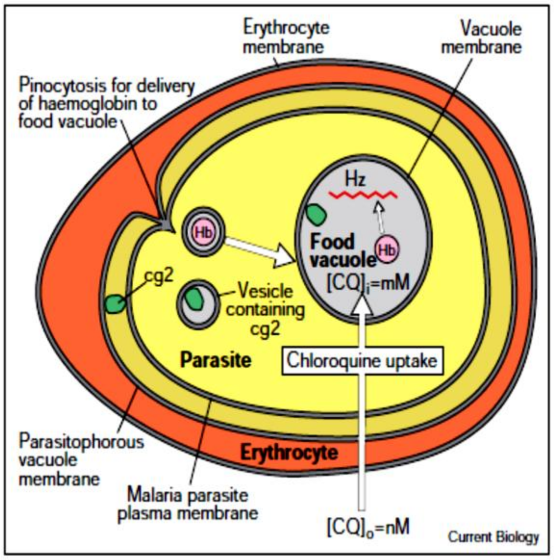


Figure 3. Schematic representation of mature malaria parasite inside invaded erythrocyte and the accumulation of chloroquine in the digestive vacuole. Extracted from: R.G. Ridley, *Curr. Biol*., **1998**, 8, 346-349

The unfortunate emergence of resistance towards chloroquine ten years after its introduction was believed to be caused by prolonged exposure of the parasite to sub-lethal doses of the drug. *P. falciparum* chloroquine resistance transporter (PfCRT) is a transmembrane protein located within the digestive vacuole membrane.[7] It has been predicted to be a member of the drug transporter family and reports show that the parasites with mutant PfCRT accumulate less chloroquine in the digestive vacuole than parasites expressing wild type PfCRT. [7] This is likely the reason why resistant strains of the *Plasmodium* parasite accumulate chloroquine less effectively in the acidic digestive vacuole compared to susceptible strains.[12] The widespread resistance against chloroquine has resulted in its replacement with more effective drugs such as sulfadoxine-pyrimethamine in some parts of the world. Despite this, it is still frequently used in sub-Saharan Africa countries.[8]

1.3.1.3 Mefloquine, Amodiaquine and Primaquine

Mefloquine and amodiaquine are quinoline derivatives with a similar mode of action to chloroquine. Reports suggest that they display antimalarial actvity due to their ability to bind to haem and inhibit haem detoxification.[7] Primaquine is an 8-aminoquinoline and is currently used for the treatment of dormant liver stage of *P. vivax* and *P. ovale*.

1.3.2 Antifolates

Antifolates are a class of antimalarial agents which target folate metabolism. The parasites utilises folate derivatives such as tetrahydrofolate (THF) as essential cellular cofactors for DNA synthesis, biosynthesis and/or catabolism of several amino acids and the initiation of protein synthesis.[17,18,19] Although most microorganisms can synthesise the folates they require for replication and growth from the precursors guanosine triphosphate (GTP), *p*-aminobenzoic acid (pABA) and L-glutamate using the *de novo* pathway, *P. falciparum* can utilise both the *de novo* pathway and the human salvage pathway.[19]

The *de novo* pathway is initiated by the catalytic conversion of GTP to dihydroneopterin triphosphate by GTP cyclohydrolase (GTPC) (**Figure 4**, a).[18] This undergoes further catalytic conversions to 6-hydroxy-7-8-dihydropterin pyrophosphate (pterin). The enzyme dihydropteroate synthase (DHPS) then facilitates the reaction between the activated pterin and pABA to give 7,8-dihydropteroate. This in turn is converted to dihydrofolate (DHF) by addition of a mono L-glutamate residue by dihydrofolate synthase (DHFS) (**Figure 4**, b).[18] DHF produced by the parasite then enters the human salvage pathway, where it is converted to THF by dihydrofolate reductase (DHFR).[18] THF is a folate cofactor essential in DNA synthesis. It is converted to 5,10-

methylenetetrahydrofolate (methylene THF) by serine hydroxymethyl transferase. Methylene THF provides the methyl group required for the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). dTMP is an essential nucleotide for DNA synthesis. DHF is produced during the synthesis of dTMP, where the reduction of the methylene group to methyl is accompanied by simultaneous oxidation of THF to DHF.[19]

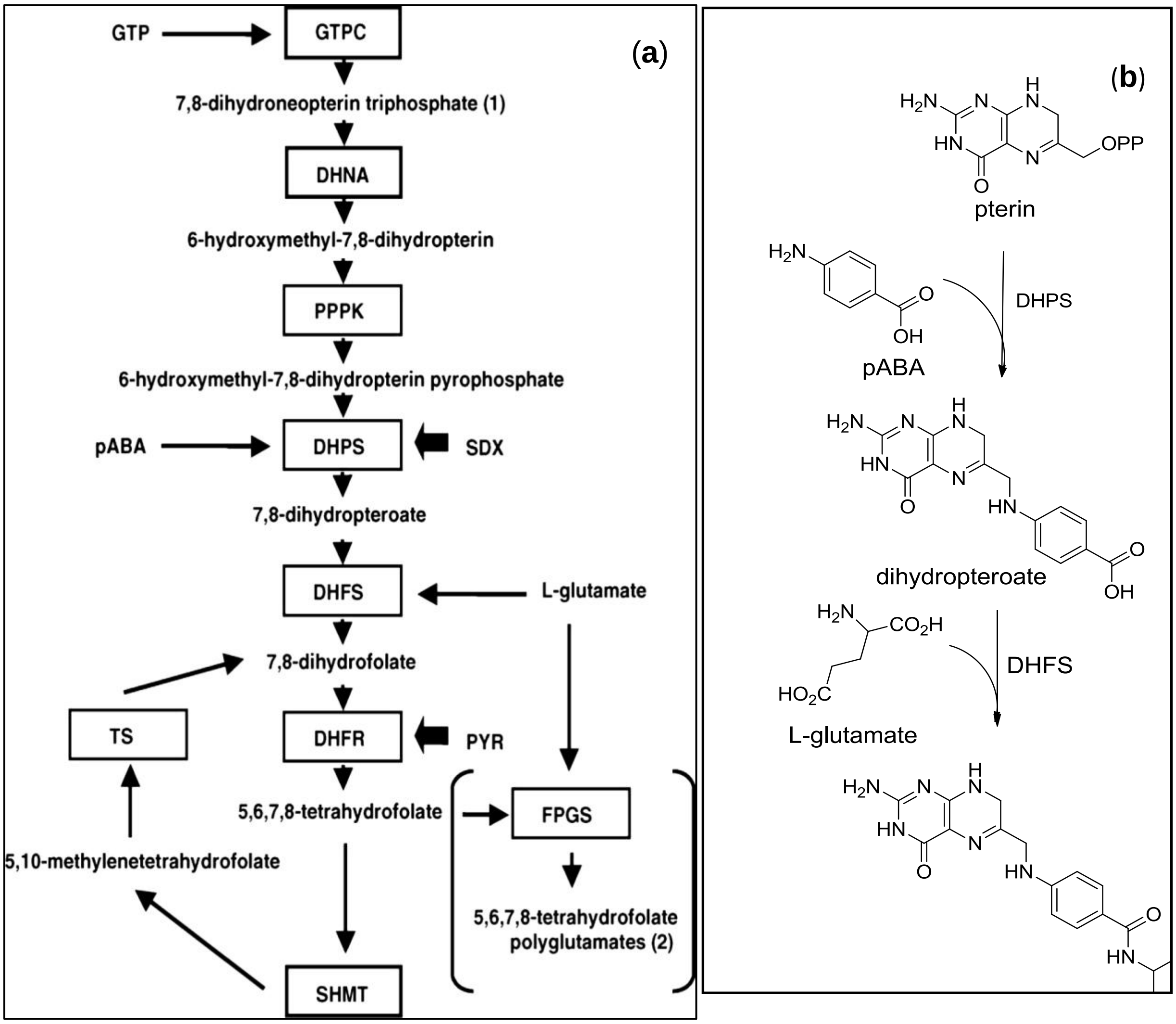
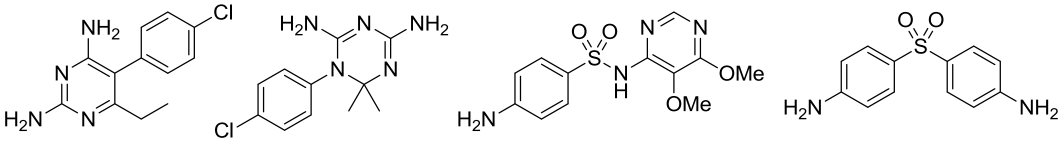


Figure 4. a) Principal enzymes and folate cofactors in the human salvage pathway and the *de novo* pathway, extracted from: J.E. Hyde, *Acta Trop.*, **2005**, 94, 191-206, b) Synthesis of DHF from pterin, pABA and L-glutamate

Enzymes of the folate metabolic pathway make good targets for antimalarial agents. Inhibiting the function of these enzymes will halt metabolic processes such as DNA synthesis and therefore growth of the parasite. DHFR, which is responsible for reduction of DHF in the human salvage pathway is the drug target of the well known antimalarials pyrimethamine **6** and cycloguanil **7** (**Figure 5**).[18] Sulfur-based antimalarial drugs such as sulfadoxine **8** and dapsone **9** inhibit the function of DHPS present only in the *de novo* pathway. Pyrimethamine and sulfadoxine are used in combination and they act synergistically, therefore inhibiting two essential enzymes in the biosynthesis and utilization of both folate pathways.[19] Resistance to antifolates is thought to be brought about by point mutations occurring in the active sites of DHFR and DHPS.[18] These arise in response to excessive drug pressure over extended periods of time.[20] Resistance to these drugs is common world-wide, but they are still used in some sub-Saharan African countries as the first-line of treatment because they still remain affordable and effective alternatives.

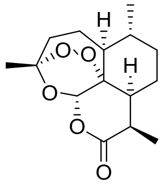


6 **7** **8** **9**

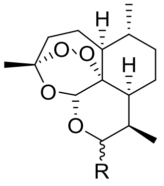
Figure 5. Antifolates used in the treatment of malaria; pyrimethamine **6**, cycloguanil **7**, sulfadoxine **8** and dapsone **9**

1.3.3 Artemisinin

Artemisinin **10** was introduced during the 1970s, as a result of the emergence of resistance against most of the available antimalarial drugs. The antimalarial agent was extracted from a plant called *Artemisia annua*. The plant was used by Chinese herbalists for medicinal purposes for centuries. The World Health Organization has recommended artemisinin-based combination therapy as the first-line of treatment for uncomplicated *P. falciparum* malaria in all malaria endemic areas.[1] Artemisinin derivatives used for malaria treatment are artesunate **11**, artemether **12**, arteether **13** and dihydroartemisinin **14**.



10



11. R= $OC(O)CH_2CH_2COOH$
12. R= OCH_3
13. R= OCH_2CH_3
14. R= OH

Figure 6. Artemisinin and its derivatives used in the treatment of malaria

Artemisinins are successful antimalarial agents because of their peculiar structure. They contain a peroxide bridge, which is the basis for the mode of action (**Figure 7**).[21] Peroxides are known to be a source of reactive oxygen species (ROS). Artemisinin acts by oxidizing reduced flavin cofactors in the *Plasmodium* cytosol, giving rise to rapid accumulation of reactive oxygen species.[21] The reduced state of these flavin cofactors is important for the function of flavoenzymes responsible for maintaining the levels of reduced glutathione (GSH), an antioxidant in the malaria parasite preventing damage of cellular components caused by reactive oxygen species. Maintenance of GSH requires NADPH,[22] provided by the hexose monophosphate shunt. The supply of NADPH is rate-limiting and therefore catalyzed by glucose-6-phosphate dehydrogenase (G6PD). Administration of artemisinin results in consumption of NADPH and reduced production of GSH, consequently resulting in an increase in buildup of ROS.

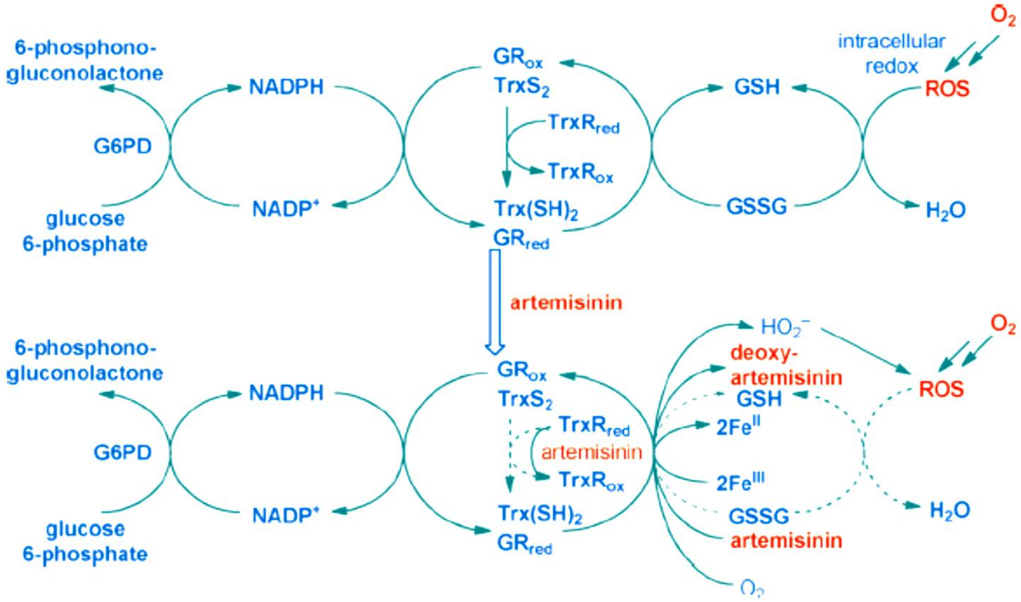
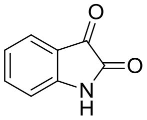


Figure 7. Biological mechanism of artemesinin against antimalarial parasite. Extracted from: R.K. Haynes *et al.*, *ChemMedChem*, **2012**, 7, 2204-2226

1.4 Spirooxindoles

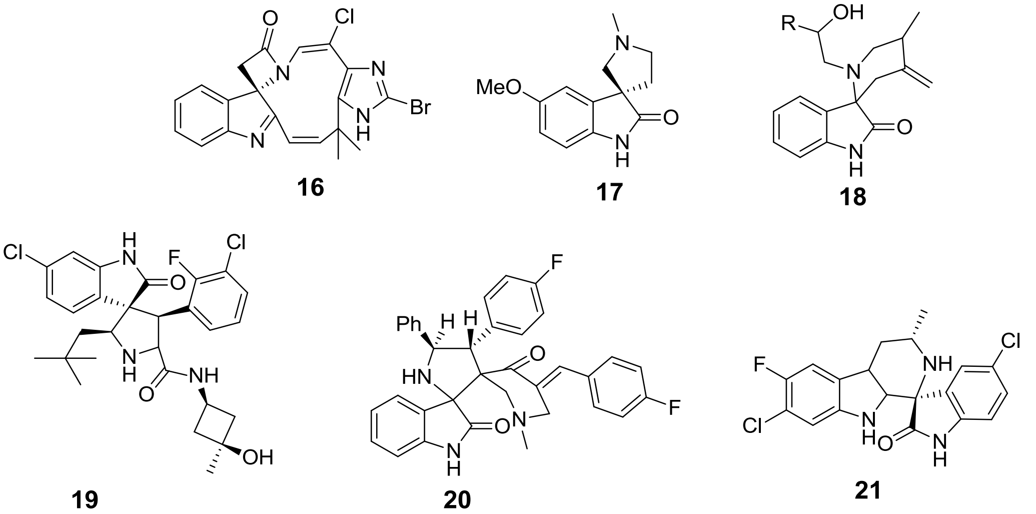
Spirooxindoles are an important class of spirocycles in organic and medicinal chemistry. These alkaloids contain a quaternary spiro carbon at the C-3 position of an oxindole core which has been indicated to be an important feature for their biological activity (**Figure 8**).[23]



15

Figure 8. Structure of the oxindole **15** scaffold

The spiro-ring infused with the oxindole heterocycle makes up the basic chemical structure for diverse natural and synthetic spirooxindoles. Naturally occurring alkaloids (**Figure 9**) such as chartellines **16** extracted from the marine bryozoan *Chartella papyracea* and horsfiline **17** isolated from *Horsfieldia superba* are biologically active and used in medicine.[24] Much attention has been paid to this class of compounds because of their vast range of biological activity. Spirooxindole **18** has been shown to act as an HIV protease inhibitor.[25] MI-888 **19** is currently in preclinical evaluation for treatment of human cancer.[26] It is a potent inhibitor of p53-MDM2 interaction and therefore able to achieve tumour regression. The tumour suppressor, p53, is an essential mediator of growth arrest, senescence, and apoptosis in response to cellular damage.[27] MDM2 functions as an E3 ubiquitin ligase that recognises the N-terminal trans-activation domain of p53 and is an inhibitor of p53 transcriptional activation. Inhibition of p53-MDM2 interaction enables MI-888 to achieve tumour regression. Other synthetic spirooxindoles include spiro-piperidin-4-ones **20,** synthesised by a 1,3-dipolar cycloaddition reaction of azomethine ylides, generated *in situ* by decarboxylative condensation of isatin and various amino acids. They were tested against *Mycobacterium tuberculosis*, the bacterium causing tuberculosis in humans. Spirooxindole **20** was most potent *in vitro* and able to drastically reduce the bacterial load in lung and spleen tissues *in vivo*.[28]



16 17 18

19 20 21

Figure 9. Synthetic and naturally occuring spirooxindoles which display biological activity

We are particularly interested in spiro-fused oxindoles because of their antimalarial activity. They are a new class of antimalarial agents with a novel mode of action. This is an important factor in the discovery of new antimalarial drugs as there is widespread resistance to most of the currently used antimalarial drugs.

1.4.1 NITD609

In search of a new class of antimalarial chemotypes, 12 000 natural products and synthetic compounds with structural similarities to the natural products were screened using *Plasmodium* whole-cell proliferation assays. The leads obtained yielded the synthetically optimized candidate NITD609 **21**, now known as cipargamin.[29] This compound is a novel synthetic antimalarial candidate, with an IC_{50} of 1 nM against *P. falciparum* strains, including multi drug-resistant strains.[29] It is potent against the asexual erythrocytic stage of *P. falciparum* and *P. vivax*, the stage in the parasite life cycle which leads to parasitaemia, as mentioned earlier. The development of antimalarial drug candidates has been focused on targeting the asexual erthrocytic stage, mainly because it is responsible for morbidity and mortality. However, the *Anopheles* mosquito takes up gametocytes emerging from the sexual blood stage, thereby spreading the parasite. Targeting the nonpathogenic gametocytogenesis stage will therefore prevent transmission of the parasite from human host to the mosquito vector. *In vitro* assays conducted to test the effects of NITD609 on the development of gametocytes has shown that the spirooxindole is an effective dose-dependent inhibitor of gametocytogenesis[30] and the most potent compound against early and late gametocyte development in comparison to lumefantrine, artemether and primaquine, giving a significant reduction in gametocytemia at the lowest tested concentration of 5 nM. Treatment with NITD609 also displayed damaging morphological effects, with early gametocytes showing swollen rounded forms.[30]

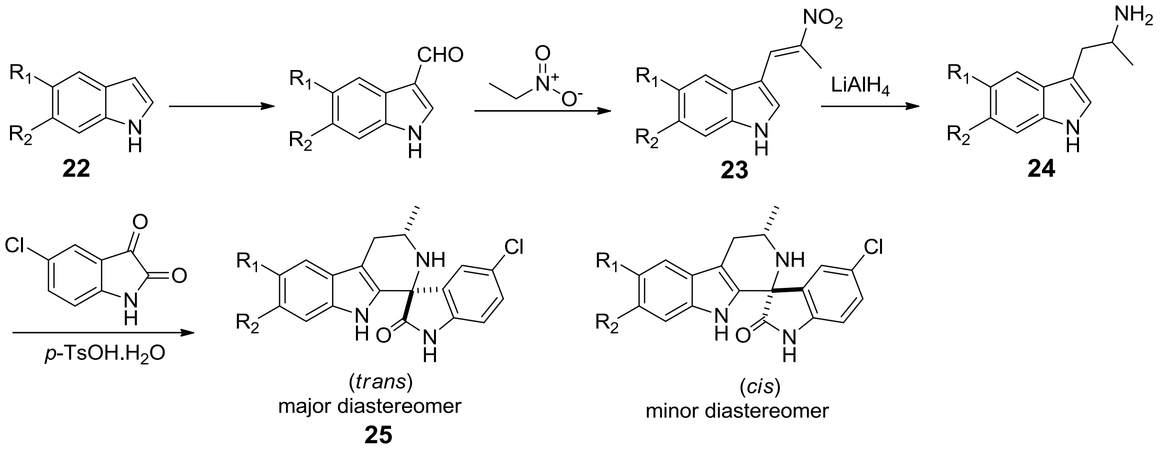
To further understand NITD609 and its mode of action, an assay incorporated with radiolabelled methionine and cysteine indicates that the spirooxindole rapidly inhibits protein synthesis in *P. falciparum* within an hour of exposure.[31] The proposed molecular target is a P-type cation-transporter, ATPase4 (PfATP4), a parasite plasma membrane protein.[30] This is assumed because *P. falciparum* strains bearing mutations in the gene encoding for PfATP4 confer low-level resistance towards NITD609.[30] This is consistent with the results obtained by Spillman *et al*., in their study of the regulation of Na^+ in *P. falciparum*.[32] After invading erythrocytes, the parasites establish permeable pathways which allow for nutrient uptake. This simultaneously allows for influx of Na^+ and efflux of K^+ down their respective concentration gradients, causing an increase in Na^+ concentration in the cytosol of the infected erythrocyte. Surprisingly, the parasite maintains a low Na^+ concentration in its cytosol by an unknown mechanism. Lower eukaryotes and some protozoa use P-type Na^+ ATPase to mediate Na^+ extrusion. Based on the sequence similarities between Na^+ ATPase and PfATP4, it was hypothesized that *P. falciparum* relies on PfATP4 for the regulation of Na^+. The results obtained from the study support the hypothesis that PfATP4 is a plasma membrane Na^+ efflux pump in *P. falciparum* and therefore maintains the low Na^+ concentration in the parasite. Mutations in PfATP4 result in altered regulation of Na^+ concentration, where Na^+ efflux is reduced and Na^+ load increases within the parasite.[32] This results in suppressed growth of the parasite. Treatment with NITD609 also disrupts regulation of Na^+ homeostasis by the parasite.[32]

The compound has displayed potent schizonticidal activity at nanomolar levels in *P. falciparum* and *P. vivax* and has been selected for Phase 2 clinical trials. The open-label study was conducted in Thailand on adults with uncomplicated *P. falciparum* and *P.*

vivax.[33] NITD609 cleared parasitemia in patients with *P. falciparum* and *P. vivax* at a dose of 30 mg daily for 3 days. This shows that the spiroindolone is fast-acting in clearing the parasite *in vivo* and therefore can be administered as a once-daily dosing regimen.

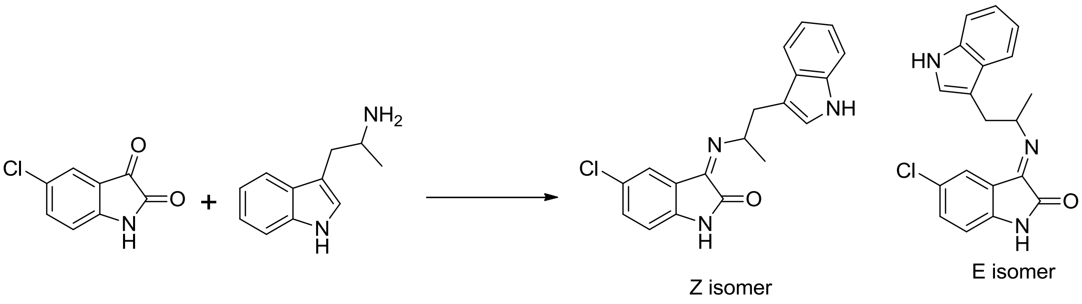
Considering that the majority of people susceptible to malaria are children and pregnant women, NITD609 has to be safe if it is to be considered as a drug-candidate for malaria chemotherapy. NITD609 has a good safety profile with no cardiotoxicity or genotoxicity identified to date.[30] This is also supported by results obtained from the Phase 2 study. No adverse event led to the discontinuation of treating the patients with NITD609. Fever and nausea were the major adverse events reported. The fever occurred after clearance of the parasite and the severity of the nausea was assessed as grade 2 in only 1 patient. It is important to take into account that vomiting is one of the symptoms patients experience during acute malaria and therefore the drug may not be responsible for the symptom.

The spirotetrahydro-β-carboline NITD609 and its analogues were synthesised in an 8 step synthetic route including chiral separation of the active enantiomer.[23,29] Resolution of the stereochemistry is of importance because the two enantiomers display different potencies against the parasite and also exhibit different pharmacodynamics *in vivo*. They were prepared by formylation of substituted indoles (**22**, **Scheme 1**) followed by condensation with nitroethane to give nitroalkenes **23**. The nitroalkenes were reduced with lithium aluminum hydride to give amines **24** which in turn were subjected to a Pictet-Spengler cyclisation reaction with isatin to yield the spirotetrahydro-β-carbolines **25** in a high degree of diastereoselectivity.[23] It was reported that only one of the diastereomers exhibited the desired potency for *P. falciparum* malaria *in vitro*. Fortunately, the diastereoselectivity favours the formation of the *trans* diastereomer which displayed potent antimalarial activity.



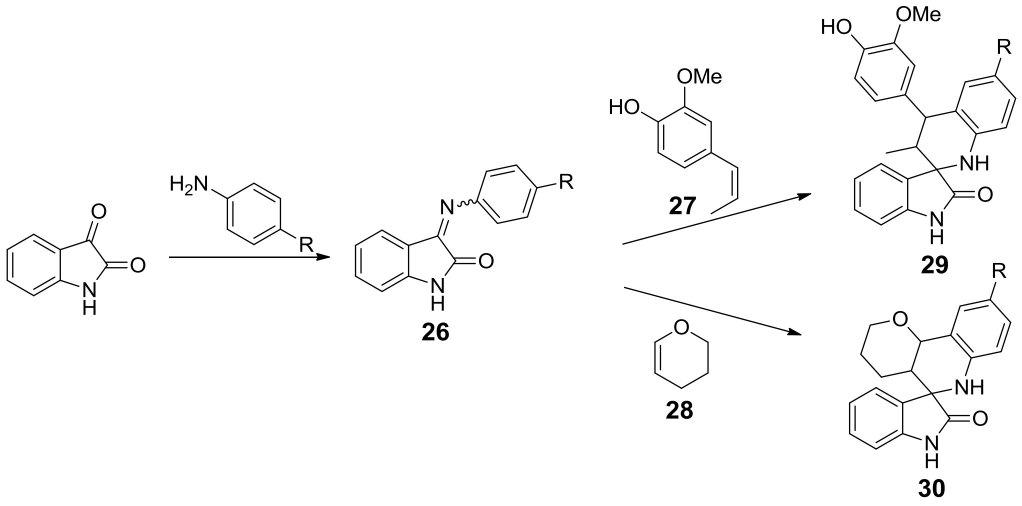
Scheme 1. Synthesis of NITD609 and its diastereomer

The observed diastereoselectivity in the Pictet-Spengler reaction was investigated further[34] and the isolation of the *E* and *Z* imine intermediates was essential in determining the mechanism of the cyclisation reaction. A thermodynamic mixture of *E/Z* imines prepared in DMSO at room temperature over 24 hours was isolated in a 1:3 ratio. The *Z* geometry was favoured mainly due to steric constraints experienced in the *E* isomer. Cyclisation of the *Z* isomer favoured formation of the *trans* diastereomer. In contrast, cyclisation of the *E* isomer selectively favoured the formation of the *cis* isomer at lower temperatures and the *trans* product at higher reaction temperatures.



Scheme 2. Synthesis of the imine intermediates in the formation of NIDT609.

Previously in our laboratory, several spirooxindole derivatives were synthesised using an imino Diels-Alder reaction, also known as the Povarov reaction. The reaction is a powerful tool for the synthesis of nitrogen containing six-membered heterocyclic compounds.[35] The spirooxindoles were synthesised by reacting isatin and a substituted aniline to produce the intermediate aryl-imines **26**. The imines were then used in the subsequent step along with either isoeugenol **27** or 3,4-dihydro-2*H*-pyran **28** to produce the spirooxindoles **29** and **30** respectively (**Scheme 3**). The imine acts as a heterodiene and undergoes an imino Diels-Alder reaction with electron rich dienophiles (**27** and **28**) catalyzed by Lewis acids.[36] Of all the compounds synthesised, only those derived from a *para*-substituted aniline displayed activity in the low micromolar range (~5µM) against *P. falciarum in vitro*.



Scheme 3. Synthesis of spirooxindoles using isoeugenol and dihydropyran

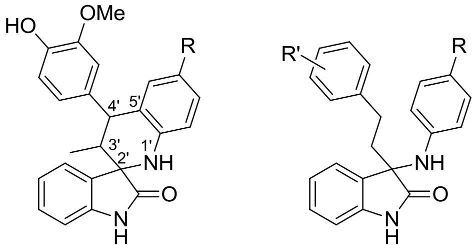
1.5 Aims

- We aim to further explore the antimalarial activity of this series of compounds by synthesising and characterizing analogues similar in structure to the ones that showed biological activity, specifically the analogues derived from *para*-substituted anilines.
- We plan to vary the ring systems surrounding the oxindole scaffold and synthesise ring-opened and closed-ring analogues. This will allow us to gain a better understanding of the structure-activity relationships of these compounds.
- All compounds prepared will be assessed for antimalarial activity in a primary whole cell *P. falciparum* assay in collaboration with colleagues at Wits Medical School.

1.6 Proposed Synthetic Route

1.6.1 Synthesis of ring-opened analogues

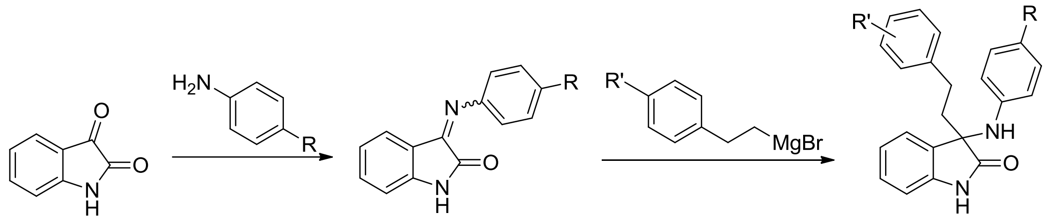
Previously, the Diels-Alder reaction was used as the key synthetic approach for the synthesis of spiroindole derivatives **29** containing a spiro-fused 6-membered ring. For this series of compounds, we intend to synthesise compounds lacking the covalent C-4' and C-5' bond present in the Diels-Alder products (**Figure 10**). This will afford ring-opened analogues with increased flexibility, and we hope to assess the effect of this change on the biological activity of the compounds. An increase in flexibility enables the compounds to adopt a number of conformations, and may result in improved binding to biological targets. This strategy has been employed across a number of disease classes to improve efficacy and overcome drug resistance, and in particular when the biological target is known.[37,38] However, in our case the target is not known, and this strategy will also be utilised to explore additional chemical space.



29 **31**

Figure 10. Chemical structure for the Spirooxindoles derived from the Povarov reaction **29** and the ring-opened analogues **31**

The flexible ring-opened analogues **31** will be synthesised starting from commercially available isatin and substituted anilines to yield the aryl imines (**Scheme 4**). The imines will be subjected to a nucleophilic addition reaction with a suitable Grignard reagent derived from substituted phenethyl bromides. Unlike carbonyl groups which readily react with nucleophiles because of their strong electrophilic nature, imines are weaker electrophiles and less reactive towards nucleophilic attack.[39] Therefore, the Grignard addition reaction will require a Lewis acid to activate the imine.[39]

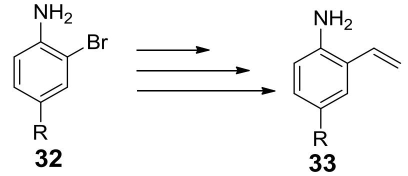


26 **31**

Scheme 4: Proposed synthetic route towards synthesis of ring-opened analogues

1.6.2 Synthesis of closed-ring analogues

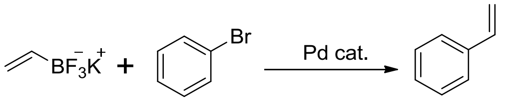
The closed-ring analogues are to be synthesised starting from commercially available *para*-substituted 2-bromoanilines **32** which are converted into 2-vinylanilines **33** by a series of chemical transformations (**Scheme 5**).



32 **33**

Scheme 5. Proposed chemical conversion of parasubstituted 2-bromoanilines to 2-vinylanilines

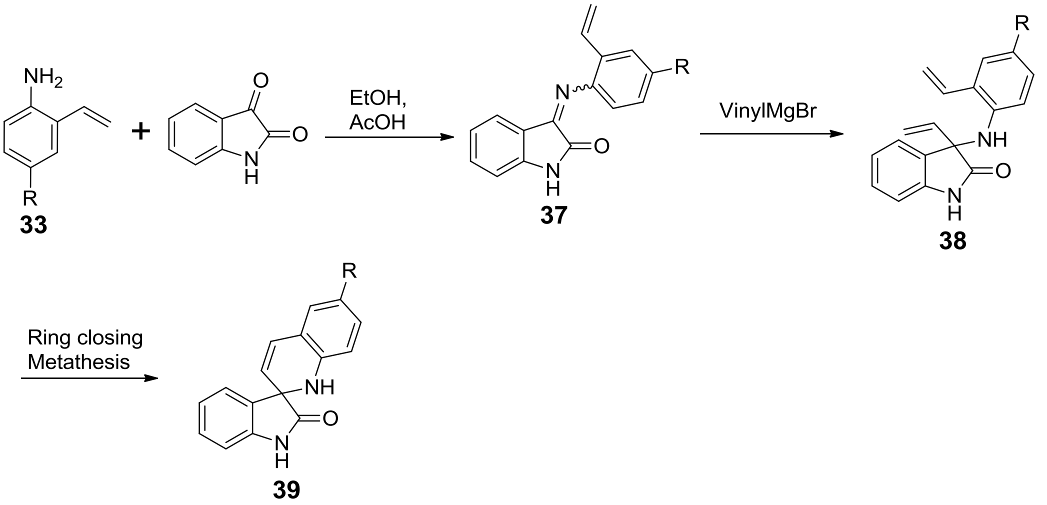
Synthesis of styrenes from aryl-halides using palladium or nickel catalyzed cross-coupling reactions has received much attention in literature (**Scheme 6**).[40,41] These reactions are carried out in the presence of various types of organometallic reagents due to their nucleophilic nature, such as boron derivatives for Suzuki coupling. The cross-coupling reaction of potassium vinyltrifluoroborate **34** with aryl-bromides **35** provides the desired styrenes in high yields. Although not much has been reported on the introduction of vinyl groups on to bromo-anilines, we will attempt to apply this methodology towards synthesis of subsituted 2-vinylanilines **33**, by Suzuki cross-coupling reaction in the presence of the optimized potassium vinyltrifluoroborate salt.[42]



34 **35** **36**

Scheme 6. Synthesis of styrenes using palladium catalyzed cross-coupling reactions

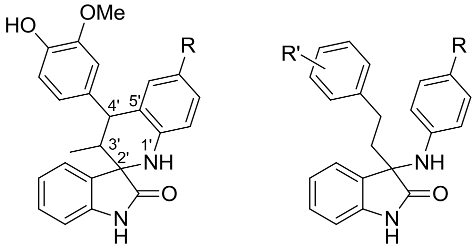
The *para*-substituted 2-vinylanilines **33** will undergo a condensation reaction with isatin to produce the desired imine intermediates **37 (Scheme 7)**. The intermediates will then be subjected to a Grignard reaction using vinyl magnesium bromide. The resulting substrates would then be ready for ring-closing metathesis (RCM). The C-C bond forming reaction catalyzed by molybdenum and ruthenium complexes is of utmost importance in organic synthesis.[42,43] We plan to apply RCM using a Grubbs II carbene catalyst to generate a six membered ring. The Grubbs II carbene is the catalyst of choice because it has a broad functional group tolerance, is air stable and has excellent metathesis activity.[44]



Scheme 7. Proposed synthetic route towards synthesis of ring-closed analogues.

2 RESULTS AND DISCUSSION

The aim of this project was to explore the structure-activity relationships of spirooxindole derivatives by synthesizing a range of substrates with variation in the core structure. This follows a research project conducted in our laboratory where a series of spirooxindole derivatives were synthesized and tested against *P. falciparum in vitro*, with the lowest recorded IC_{50} of ~5 µM for **29** (**Figure 10**). The spirooxindoles were synthesized using the Povarov reaction between aryl-imines and isoeugenol to construct N-containing six-membered heterocyclic compounds. The compounds of interest to this project are structurally similar to those previously synthesized, however without the six-membered heterocyclic ring in the compounds, i.e., compounds without the C-C covalent bond between C-4’ and C-5’, such as **31**. This will result in a set of more flexible compounds, a feature which has been shown to suppress the emergence of drug resistance.



29

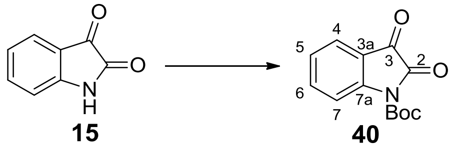
31

Figure 10. Spirooxindoles derived from the Povarov reaction **29** and the ring-opened analogues **31**

The proposed synthetic route for the synthesis of ring-opened analogues such as **31** (**Scheme 4**) involves formation of an aryl-imine from isatin and *para*-substituted anilines. The *para*-substituted anilines were chosen because only the spirooxindole derivatives prepared previously in our group derived from *para*-substituted anilines showed moderate activity against *P. falciparum in vitro*. The imines serve as intermediates for the nucleophilic Grignard addition reaction. Conventionally, Grignard addition reactions to carbonyl carbon atoms (C=O) occur readily as they are relatively strong electrophiles. In contrast, imine carbon atoms (C=N) are relatively weaker electrophiles, and the aryl-imines may tend to be less reactive towards nucleophilic addition.[39] To overcome this problem, a Lewis acid is to be used to activate the C=N bond.

2.1 Synthesis of ring-opened spirooxindoles

2.1.1 Synthesis of *tert*-butyl 2,3-dioxoindoline-1-carboxylate



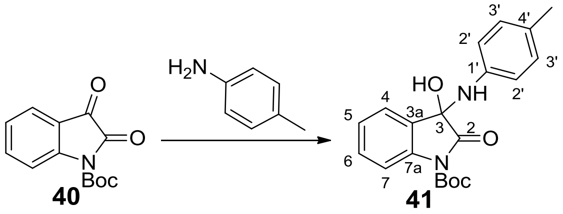
Scheme 8: Reagents and conditions: Boc_2O, THF, DMAP, rt, 50%

In order to prevent interference by the oxindole N-H in the nucleophilic addition reaction, we began the synthesis of the ring-opened analogues by protecting the nitrogen atom of commercially available isatin **15** with a *tert*-butoxycarbonyl (Boc) group (**Scheme 8**). The protecting group was specifically selected owing to its ability to be readily removed. To this end, isatin was treated with di-*tert*-butyl dicarbonate in the presence of a basic catalyst, (4-dimethylaminopyridine) DMAP, for 6 hours. After this time, the reaction mixture was quenched with water and the resulting precipitate was purified by recrystallization from dichloromethane/hexane. The product **40** was isolated as a yellow solid in 50% yield. The product is relatively unstable and decomposes back to the isatin starting material upon storage over an extended period of time, and therefore the compound was used in subsequent steps without delay. Storage at low temperatures retards the rate of decomposition, but does not prevent it.

Formation of *tert*-butyl 2,3-dioxoindoline-1-carboxylate **40** was confirmed by ^{1}H NMR spectroscopy owing to the presence of one distinct signal in the aliphatic region. The singlet observed at 1.66 ppm integrating for 9 protons is characteristic of the *tert*-butyl protons of the Boc-group. This is a clear indication of the addition of the Boc-group to the N-atom of isatin. The aromatic region contains four protons, a doublet at 8.08 ppm, doublet of doublets at 7.74 ppm, doublet of doublet of doublets at 7.70 ppm and a multiplet at 7.31 – 7.26 ppm corresponding to H-7, H-4, H-6 and H-5 respectively.

The ^{13}C NMR spectrum further confirmed formation of the product **40** by the presence of a signal due to the Boc methyl groups at 28.0 ppm and the quaternary carbon atom of the Boc-group visible as a signal at 85.6 ppm. The peak for the Boc carbonyl carbon atom overlaps with that for C-7a at 148.4 ppm, hence ten signals were observed in the ^{13}C NMR spectrum instead of eleven.[45] The two carbonyl carbon atoms of the oxindole moiety appeared at 180.3 ppm for C-3 and 155.7 ppm for C-2 respectively.

2.1.2 Synthesis of *tert*-butyl 3-hydroxy-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate and analogues



Scheme 9: Reagents and conditions: DCM, $MgSO_4$, rt, 22%

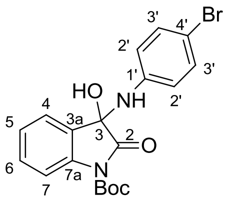
Having successfully synthesized *N*-Boc isatin **40**, we were ready to synthesize a series of Boc-protected aryl imines. The dehydration reaction was first tested with *p*-toluidine. The aniline was reacted with *N*-Boc isatin **40** in DCM at room temperature in the presence of magnesium sulfate ($MgSO_4$), added to remove water produced during formation of the imine. Under these conditions, *tert*-butyl 3-hydroxy-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **41** was isolated from the reaction mixture instead of the desired imine (**Scheme 9**). There is precedence in literature for the synthesis of these types of hydroxy compounds using the procedure described, as well as their subsequent reaction with a Grignard reagent.[37] These compounds can be regarded as a synthetic equivalent of the desired imine for the nucleophilic addition of the Grignard reagent in the subsequent step. Unreacted *N*-Boc isatin and *p*-toluidine were removed by silica gel column chromatography to afford **41** as a lime green solid in 22% yield.

The ^{1}H NMR spectrum confirmed the presence of **41** by two distinct singlets integrating for one proton each; one appearing at 10.20 ppm for NH and one at 8.70 ppm for the OH group. The presence of the NH and OH signals confirms that imine formation did not proceed to completion and a hydroxy-amino intermediate **41** was isolated instead. The most significant difference in the aromatic region was the presence of two doublets at 7.55 ppm and 7.20 ppm each integrating for two protons, they are characteristic of H-2’ and H-3’ respectively of the *p*-tolylamino group. Addition of the *p*-toluidine moiety to the carbonyl carbon at the C-3 position of *N*-Boc isatin resulted in a significant shift of the signals from the aromatic nucleus of the oxindole scaffold in the ^{1}H NMR spectrum (summarized in **Table 1** below). There are two distinct signals in the aliphatic region; one of them is characteristic of the Boc-group protons observed as a singlet at 1.54 ppm integrating for 9 protons, with the second one being a singlet integrating for 3 protons at 2.35 ppm characteristic of the aromatic methyl protons.

Table 1. ^{1}H NMR spectroscopic chemical shifts before and after addition of anilines to *N*-Boc isatin **40**

	H-4 (ppm)	H-5 (ppm)	H-6 (ppm)	H-7 (ppm)
40	7.74 (dd)	7.31-7.26 (m)	7.70 (ddd)	8.08 (d)
41	8.57 (dd)	7.07 (td)	7.60 (ddd)	8.47-8.42 (m)
42	8.51 (dd)	7.06 (ddd)	7.63-7.55 (m)	8.42 (dd)

The ^{13}C NMR spectrum of the product **41** further confirmed the nucleophilic addition of *p*-toluidine to C=O at the C-3 position of *N*-Boc isatin due to the presence of a new signal at 118.3 ppm for the chiral quaternary C-3. There was a significant shift in the two carbonyl carbon signals from 155.7 ppm to 190.3 ppm for C-2, and from 148.4 ppm to 159.7 ppm for the Boc C=O. The CH_3 peak of the *p*-tolylamino group appeared as a signal at 28.3 ppm, while its aromatic ring gave rise to 4 new signals in the aromatic region. In total, 16 carbon signals were observed in the ^{13}C NMR spectrum as expected. The IR spectrum further confirmed formation of the product due to the presence of the NH and OH signals at 3270 and 3130 cm^{-1} respectively. The molecular ion was confirmed by HRMS to be $[M+H]^{+}$ 355.1652 which was consistent with the mass calculated for $C_{20}H_{23}N_2O_4$ of 355.1658.

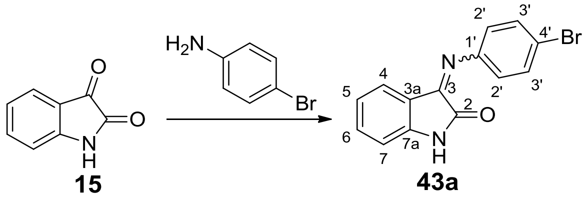


42

tert-Butyl 3-((4-bromophenyl)amino)-3-hydroxy-2-oxoindoline-1-carboxylate **42** was synthesized using the same method described for **41**. The product was isolated as a yellow solid in 43% yield. Formation of the product was also confirmed by ^{1}H NMR spectroscopy due to the presence of the two singlets integrating for 1 proton each; one at 10.15 ppm was assigned to the NH and the one at 8.51 pppm was assigned to the OH group, although these assignments were not confirmed. Another distinct feature was the two additional peaks observed in the aromatic region owing to the presence of H-2’ and H-3’; the peaks appear as multiplets at 7.63 – 7.55 ppm and 7.54 – 7.46 ppm respectively. In the ^{13}C NMR spectrum, the chiral quaternary carbon C-3 gave rise to a signal at 118.2 ppm, one of the distinct signals confirming formation of the product **42**. All of the 15 expected signals for **42** were observed in the ^{13}C NMR spectrum. The IR spectrum provided supporting information to conclude that we had isolated the product due to the presence of a strong signal at 3269 cm^{-1} corresponding to the N-H stretch and another at 3127 cm^{-1} due to the O-H stretch.

With two of the hydroxy amino compounds in hand, we decided to proceed to the subsequent step and subjected each of them to a Grignard reaction using vinylmagnesium bromide. Unfortunately, the nucleophilic addition reaction attempted with vinylmagnesium bromide was unsuccessful. Owing to this, and the fact that the hydroxy amino compounds were formed in low yields, we decided to find an alternative method to synthesize the desired aryl-imines.

2.1.3 Synthesis of 3-((4-bromophenyl)imino)indolin-2-one and analogues



Scheme 10: Reagents and conditions: Gl. acetic acid, Ab. ethanol, reflux, 66%

In order to prepare the desired isatin-3-arylimines, we changed the reaction conditions from those used previously. The first imine prepared, **43a**, was synthesized in a condensation reaction between isatin and 4-bromoaniline in the presence of glacial acetic acid as a catalyst (**Scheme 10**). As the Boc-protecting group is readily cleaved under acidic conditions, isatin was used in the imine formation reaction instead of *N*-Boc-protected isatin **40**. Isatin-3-arylimines are commonly known to exist as mixtures of *E* and *Z* isomers,[37,46,47] and we also found this to be the case. Product **43a** was isolated as 4:1 mixture of *E:Z* isomers in 66% yield. As expected, the thermodynamic product formed as the major isomer. Formation of 3-((4-bromophenyl)imino)indolin-2-one **43a** was confirmed by ^{1}H NMR spectroscopy. However, due to the presence of both the *E* and *Z* isomers, each signal in the ^{1}H NMR and ^{13}C NMR spectra was doubled up. NMR spectroscopic 2D experiments including COSY, NOESY and HSQC were utilized to determine the stereochemistry of the aryl-imine and assign each isomer separately. Theoretically, the *E* isomer is more thermodynamically favoured than the *Z* isomer and therefore we expected the *E* isomer to be the major isomer. The NOESY experiment proved that the *E* isomer was the major isomer in this case, because of the correlation between H-4 and H-2' (**Figure 11**). This was also reported by González *et al.* who synthesized a series of 3-arylimino-2-indolones derived from isatin.[46]

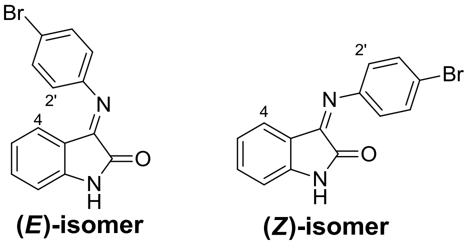


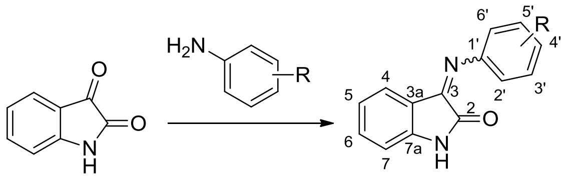
Figure 11. Structural representation of the *E* and *Z* isomers of 3-((4-bromophenyl)imino) indolin-2-one **43a**

A key signal in the ^{1}H NMR spectrum of the product is the singlet at 10.97 ppm integrating for 1 proton, characteristic of the NH group on the oxindole core. The chemical shifts of signals due to H-7, H-6, H-5 and H-4 were observed at δ 6.89 (d), 7.35 (td), 6.77 (td) and 6.43 (dd) respectively for the *E*-isomer. Interestingly, the signal for H-4 was shifted upfield by ~1 ppm relative to the *Z*-isomer. The signal for H-2' appeared at 6.97 ppm as a doublet integrating for 2 protons and H-3' appeared as a multiplet integrating for two protons at 7.69 – 7.61 ppm. The ^{13}C NMR spectrum further supports formation of the aryl-imine by the disappearance of the C-3 (C=O) signal at 184.6 ppm of the isatin starting material and the appearance of a new signal due to C=N at 155.3 ppm for the *E* isomer and 153.5 ppm for the *Z* isomer as summarized in **Table 2**. The signals at 163.3 ppm and 158.5 ppm are characteristic of carbonyl carbon atoms of the oxindole core. The molecular ion determined by HRMS was confirmed to be $[M+H]^+$ 300.9969 which was consistent with the mass calculated for $C_{14}H_{10}{}^{79}BrN_2O$ of 300.9977.

Table 2. Summary of the ^{13}C NMR spectroscopic chemical shifts for isatin and *Z* and *E*-isomers of 3-((4-bromophenyl)imino)indolin-2-one **43a**

Comp.	C-2 (ppm)	C-3 (ppm)	C-4 (ppm)	C-5 (ppm)	C-6 (ppm)	C-7 (ppm)	C-2' (ppm)	C-3' (ppm)
Isatin	159.5	184.6	124.9	122.9	138.5	112.4		
(E)-43a	155.3	163.3	125.4	121.8	134.7	111.6	119.7	132.4
(Z)-43a	153.5	158.5	122.9	122.3	134.4	110.8	121.2	131.1

Seven other aryl-imine analogues were prepared using the same method described (**Scheme 11**). In each case, the imine was characterised by ^1H and ^{13}C NMR spectroscopy and HRMS. As observed for **43a**, each of the imines was isolated as a mixture of *E* and *Z* isomers, with the *E* isomer being the major isomer. The key spectroscopic signals are summarized in **Table 3** and **4**.



15

43b: R = 4-Cl, 72%
43c: = 4-Me, 61%
43d: = 4-OMe, 94%
43e: = 3,4-diMe, 91%
43f: = 2,4-diMe, 51%
43g: = 2-Me,4-Cl, 84%
43h: = 2,3,4-triCl, 42%

Scheme 11. Reagents and conditions: Gl. acetic acid, Ab. Ethanol, reflux

Table 3. Key signals in the ^{1}H NMR spectra of aryl-imines **43b-43h** .

Comp.	NH (ppm)	Oxindole-core (ppm)	Aromatic region (ppm)	Aliphatic region (ppm)
(*E*)-43b	10.98 (s)	7.36 (td, H-6), 6.90 (d, H-7), 6.77 (td, H-5), 6.44 (dd, H-4)	7.56 – 7.47 (m, H-3’), 7.09 – 7.00 (m, H-2’)	
(*Z*)-43b	10.98 (s)	7.58 (dd, H-4), 7.45 (td, H-6), 7.11 – 7.00 (m, H-5), 6.88 (d,H-7)	7.37 (d, H-3’), 7.11 – 7.00 (m, H-2’)	
(*E*)-43c	10.97 (s)	7.38 – 7.22 (m, H-6), 6.93 – 6.85 (m, H-7), 6.72 (td, H-5), 6.49 (dd, H-4)	7.38 – 7.22 (m, H-3’), 6.93 – 6.85 (m, H-2’)	2.35 (s, CH_3)
(*Z*)-43c	10.85 (s)	7.57 (dd, H-4), 7.43 (td, H-6), 7.05 (td, H-5), 6.91 – 6.81 (m, H-7)	7.11 (d, H-3’), 6.99 – 6.92 (m, H-2’)	2.30 (s, CH_3)
(*E*)-43d	10.95 (s)	7.33 (td, H-6), 6.94 – 6.86 (m, H-7), 6.75 (td, H-5), 6.66 (dd, H-4)	7.10 – 6.94 (m, H-3’ and H-2’)	3.80 (s, OCH_3)
(*Z*)-43d	10.87 (s)	7.55 (dd, H-4), 7.41 (td, H-6), 7.11 – 6.94 (m, H-5), 6.85 (d, H-7)	7.23 – 7.15 (m, H-3’), 7.11 – 6.94 (m, H-2’)	3.77(s, OCH_3)
(*E*)-43e	10.96 (s)	7.32 (td, H-6), 6.89 (d, H-7), 6.82 – 6.65 (m, H-5), 6.52 (dd, H-4)	7.21 (d, H-5’), 6.82 – 6.65 (H-2’ and H-6’)	2.26 (s, CH_3), 2.24 (s, CH_3)
(*Z*)-43e	10.84 (s)	7.56 (dd, H-4), 7.43 (td, H-6), 6.86 – 6.81 (m, H-5), 6.82 – 6.65 (m, H-7)	7.06-7.04 (m, H-2’ and H-5’), 6.82 – 6.65 (H-6’)	2.20 (s, CH_3), 2.19 (s, CH_3)
(*E*)-43f	10.97 (s)	7.34 (td, H-6), 6.89 (d, H-7), 6.79 – 6.69 (m, H-5), 6.37 (dd, H-4)	7.15 (d, H-3’), 7.06 (dd, H-5’), 6.79 – 6.69 (m, H-6’)	2.32 (s, CH_3), 1.99 (s, CH_3)
(*Z*)-43f	10.97	7.61 (dd, H-4), 7.45 (td,	7.01 (d, H-3’), 6.94	2.26 (s, CH_3),

	(s)	H-6), 7.11– 6.98 (m, H-5), 6.94– 6.82 (m, H-7)	– 6.82 (H-5’ and H-6’)	2.06 (s, CH_3)
***(E)*-43g**	11.02	7.41 – 7.24 (m, H-6), 6.97 – 6.82 (m, H-7), 6.77 (td, H-5), 6.36 (dd, H-4)	7.48 – 7.41 (m, H-3’), 7.41 – 7.24 (m, H-5’), 6.97 – 6.82 (m, H-6’)	2.03 (s, CH_3),
***(Z)*-43g**	10.90	7.63 (dd, H-4), 7.18 – 7.03 (m, H-5 and H-6), 6.97– 6.82 (m, H-7)	7.01 (d, H-3’), 6.94 – 6.82 (H-5’ and H-6’)	2.07 (s, CH_3),
***(E)*-43h**	11.07	7.42 (td, H-6), 6.92 (dd, H-7), 6.83 (td, H-5), 6.46 (d, H-4)	7.73 (d, H-5’), 7.18 (d, H-6’)	
***(Z)*-43h**	11.07	7.63 – 7.47 (m, H-4 and H-6), 7.15 – 7.05 (m, H-5), 6.92 (dd, H-7)	7.71 – 7.65 (m, H-5’), 7.15 – 7.05 (m, H-6’)	

Table 4. Key signals in the ^{13}C NMR spectra of aryl-imines **43b-43h**.

Comp.	C-2 (ppm)	C-3 (ppm)	C-4 (ppm)	C-5 (ppm)	C-6 (ppm)	C-7 (ppm)	Aromatic region (ppm)	Aliphatic region (ppm)
(*E*)-43b	163.3	155.4	125.4	121.8	134.7	111.6	129.6 (C-3’), 119.4 (C-2’)	
(*Z*)-43b	158.5	153.6	122.9	122.3	134.4	110.8	128.2 (C-3’), 120.9 (C-2’)	
(*E*)-43c	163.5	154.7	125.2	121.6	134.2	111.4	129.9 (C-3’), 117.4 (C-2’)	20.5 (CH_3)
(*Z*)-43c	158.4	152.5	122.5	122.2	133.9	110.6	128.7 (C-3’), 119.6 (C-2’)	20.5 (CH_3)
(*E*)-43d	163.6	154.5	124.9	121.6	134.1	111.4	119.4 (C-2’), 114.7 (C-3’)	55.3 (OCH_3)
(*Z*)-43d	158.6	151.6	122.3	122.1	133.5	110.5	122.7 (C-2’),	55.2

							113.4 (C-3’)	(OCH_3)
(*E*)-43e	163.5	154.5	125.2	121.6	134.2	111.4	130.3 (C-5’), 118.5 (C-2’), 114.6 (C-6’)	19.4 (CH_3), 18.9 (CH_3)
(*Z*)-43e	158.4	152.2	122.5	122.2	133.8	110.6	129.5 (C-5’), 120.7 (C-2’), 111.5 (C-6’)	19.4 (CH_3), 18.9 (CH_3)
(*E*)-43f	163.4	154.8	125.2	121.9	134.3	111.4	131.3 (C-3’), 127.3 (C-5’), 116.3 (C-6’)	20.5 (CH_3), 17.1 (CH_3)
(*Z*)-43f	158.5	152.4	122.5	122.2	133.4	110.7	130.4 (C-3’), 126.8 (C-5’), 117.8 (C-6’)	20.5 (CH_3), 17.5 (CH_3)
(*E*)-43g	163.2	155.4	125.0	122.0	134.7	111.5	130.4 (C-3’), 126.8 (C-5’), 118.2 (C-6’)	16.9 (CH_3)
(*Z*)-43g	159.3	150.7	123.1	122.7	134.4	112.2	129.3 (C-3’), 124.6 (C-5’), 119.3 (C-6’)	17.3 (CH_3)
(*E*)-43h	163.0	157.1	125.5	122.4	135.6	111.8	129.7 (C-5’), 118.1 (C-6’)	
(*Z*)-43h	158.4	155.9	123.7	122.7	135.5	111.3	128.3 (C-5’), 118.9 (C-6’)	

The stereochemistry of the aryl-imines gave rise to doubling of the signals observed in both the ^{1}H NMR and ^{13}C NMR spectra. However, the ^{13}C NMR spectrum for **43g**, when run at 300K, was more complex than expected (a, **Figure 12**). This is a consequence of the presence of rotamers caused by restricted rotation around the C1’-N bond for one of the isomers. Variable temperature ^{13}C NMR spectroscopy was performed to resolve and simplify the ^{13}C NMR spectrum of **43g**. Simplification of the ^{13}C NMR spectrum was observed when run at 345K, as highlighted in Figure 12 below. At higher temperatures, the barrier to rotation about the C1’-N bond for one of the isomers is reduced, resulting in signals in the ^{13}C NMR spectrum for the two geometric isomers only.

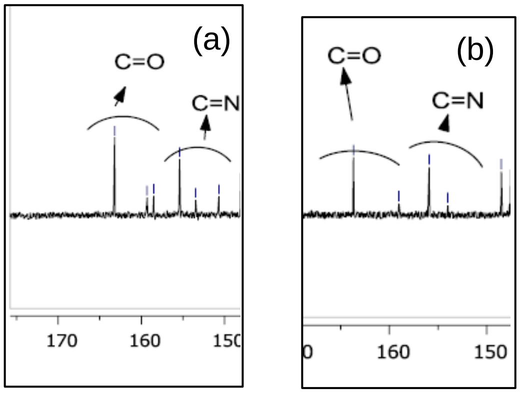
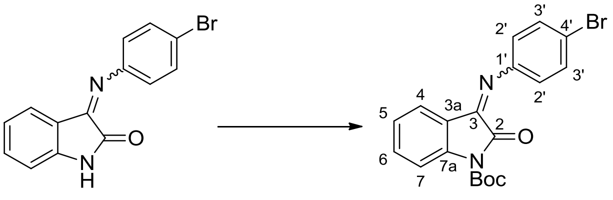


Figure 12. a) ^{13}C NMR spectrum of **43g** before variable temperature ^{13}C NMR spectroscopy, b) ^{13}C NMR spectrum of **43g** after variable temperature ^{13}C NMR spectroscopy

2.1.4 Synthesis of *tert*-butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate and analogues



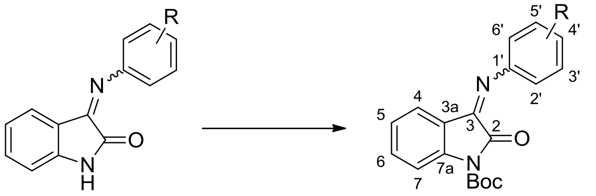
43a **44a**

Scheme 12. Reagents and conditions: Boc$_2$O, THF, DMAP, rt, 98%

The next step in our synthesis was the *N*-Boc protection of **43a** which was conducted in a similar manner to that described for *tert*-butyl 2,3-dioxoindoline-1-carboxylate **40**. The starting material **43a** was treated with di-*tert*-butyl dicarbonate in THF and the Boc-protection was facilitated by DMAP as a catalyst (**Scheme 12**). The Boc-protected product was observed as one spot on TLC analysis and no spot for the starting material was observed. The product was isolated and purified by recrystallization from EtOAc/hexane as a yellow solid in 98% yield. *tert*-Butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a** was isolated as a mixture of *E* and *Z* isomers in a ratio of 3:1.

The ^{1}H NMR spectra of the starting material and the Boc-protected aryl-imine were similar apart from the appearance of the notably new signal in the aliphatic region. The singlet at 1.67 ppm for the *E* isomer integrating for 9 protons is characteristic of the *tert*-butyl protons of the Boc group. Success of the Boc-protection reaction was also confirmed by ^{13}C NMR spectroscopy by the presence of a peak due to the Boc C=O at 149.0 ppm for the *E* isomer and 151.0 ppm for the *Z* isomer. The quaternary carbon atom of the Boc-group appears as a signal at 85.2 ppm, while the Boc methyl groups appear as a signal at 28.1 ppm. The IR spectrum displayed two strong signals at 1783 cm^{-1} and 1731 cm^{-1} characteristic of the carbonyl carbon atom of the oxindole core and Boc C=O, which was further evidence for the formation of the product. The molecular ion was confirmed by HRMS to be [M+H]$^{+}$ 401.0492, which was consistent with the mass calculated for $C_{19}H_{18}{}^{79}BrN_2O_3$ of 401.0501.

Analogues **44b-44e** were prepared in a similar manner to **44a** (**Scheme 13**). The ^{1}H NMR and ^{13}C NMR spectra of the prepared analogues are similar, as expected. Key spectroscopic signals for the compounds are described in **Table 5** below, along with melting points for the *E*/*Z* mixtures and HRMS for the *N*-Boc protected imines prepared.



44b: R = 4-Cl, 74%
44c: = 4-Me, 97%
44d: = 4-OMe, 89%
44e: = 3,4-diMe, 82%

Scheme 13. Reagents and conditions: Boc_2O, THF, DMAP, rt

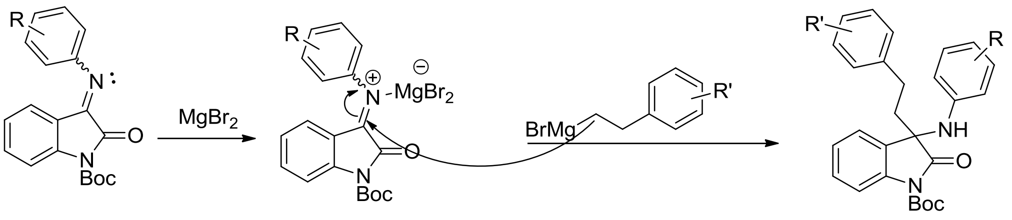
Table 5. Signals arising due to the Boc-group in ^{1}H NMR and ^{13}C NMR spectra of the Boc-protected aryl-imines **44b-e** and their respective melting points and molecular masses determined by HRMS.

Comp.	^{1}H NMR	^{13}C NMR			Melting points (^{o}C)	Molecular ion (HRMS) (calc.)	Molecular ion (HRMS) (found)
	$C(CH_3)_3$ (ppm)	Boc C=O (ppm)	$\underline{C}(CH_3)_3$ (ppm)	$C(\underline{C}H_3)_3$ (ppm)			
44b	1.67 (s)	148.4	85.2	28.1	267	357.1006	357.1012
(*E*)-44c	1.67 (s)	148.8	85.0	28.1	132	337.1552	337.1554
(*Z*)-44c	1.60 (s)	147.7	84.8	28.1			
(*E*)-44d	1.67 (s)	148.8	85.0	28.1	110-111	353.1501	353.1502
(*Z*)-44d	1.62 (s)	147.9	84.8	28.1			
44e	1.67 (s)	148.8	85.0	28.1	149-150	351.1709	351.1710

In general, Boc-protected aryl-imines were isolated as a mixture of *Z* and *E* isomers. This was evident by the observed doubling of the signals in the ^{1}H NMR spectra of the compounds. Doubling of signals was not observed for **44b** and **44e** in the ^{13}C NMR spectra of the compounds, although this was observed to some extent in the ^{1}H NMR spectra. From **Table 5** above it can be inferred that the signals which are missing are of the minor structural *Z*-isomer. This was deduced because signals of the minor isomer occur slightly more upfield than the major *E*-isomer. As these products were purified by crystallization, we suspect that the *E*-isomer may have been isolated in preference to the *Z*-isomer during this process, resulting in a larger *E*:*Z* ratio.

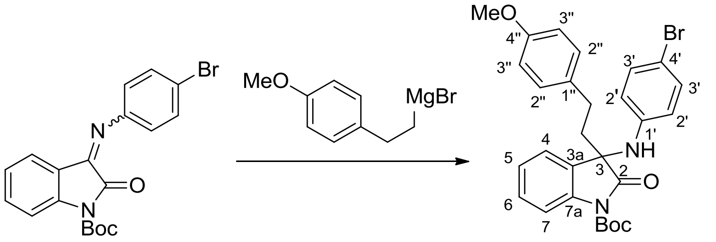
2.1.5 Synthesis of *tert*-butyl-3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate and analogues

The nucleophilic addition of a Grignard reagent to the imine carbon atom (C=N) at the C-3 position is a crucial step for the synthesis of the ring-opened analogues. As mentioned above, the imine carbon atom is a relatively weak electrophile and requires the presence of a Lewis acid to activate it. Magnesium bromide ($MgBr_2$) was used as the Lewis acid of choice because it was shown to be more efficient in promoting imine activation in comparison to other Lewis acids such as Boron trifluoride diethyl etherate ($BF_3.OEt_2$) and was compatible with the Boc-protecting group.[39] $MgBr_2$ facilitated activation of the C=N bond by coordination to the imine nitrogen and the oxindole oxygen for the nucleophilic addition of the Grignard reagent (represented in **scheme 14**).



Scheme 14. Schematic representation for the activation of C=N using $MgBr_2$

The nucleophilic addition reaction was first tested on *tert*-butyl 3-((4-bromophenyl)-imino)-2-oxoindoline-1-carboxylate **44a** by reacting it with 4-methoxyphenethyl magnesium bromide, a Grignard reagent made prior to use from 4-methoxyphenethyl bromide and magnesium turnings (**Scheme 15**). The reaction was conducted in dry THF in the presence of $MgBr_2$. After stirring overnight under an atmosphere of N_2, TLC analysis showed several spots. The product was purified by silica gel column chromatography and isolated in a 45% yield as a racemic mixture. At this stage, we decided to assess the racemic mixture for biological activity, however, each enantiomer might display different properties and may behave differently in biological systems. Stereoselective synthesis will be considered for future work on any active compounds identified.



44a

45

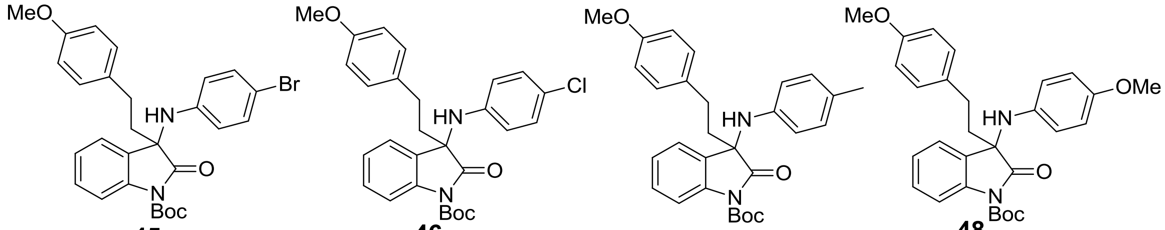
Scheme 15. Reagents and conditions: $MgBr_2$, THF, -40 °C – rt, 45%

Not surprisingly, one of the by-products isolated from the reaction was 3-((4-bromophenyl)- imino)indolin-2-one **43a**, implying attack by the Grignard reagent on the Boc-group of the starting material **44a**. The presence of the other products visible by TLC analysis could arise from competing reactions such as β-hydride elimination from 4-methoxyphenethyl magnesium bromide, a complication of Grignard reagents bearing H atoms β to the Mg atom. However, we did not isolate any products from the β-hydride elimination reaction.

^{1}H NMR spectroscopy provided sufficient evidence to confirm the successful addition of the nucleophilic Grignard reagent to the *N*-Boc-protected aryl-imine **44a** forming *tert*-butyl-3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate **45**. Several new signals resulting from the 4-methoxyphenethyl group were observed in the ^{1}H NMR spectrum. The two CH_2 groups are responsible for the multiplet at 3.01-2.33 ppm integrating for 4 protons, while the distinct methoxy peak was observed at 3.76 ppm as a singlet integrating for 3 protons. The aromatic region was carefully characterized with the assistance of 2D NMR spectroscopy experiments (COSY and HSQC). The multiplet observed at 7.02 - 6.89 ppm integrating for 2 protons results from H-3'', while that at 6.84 – 6.69 ppm integrating for 5 protons is a result of H-2'', H-2' and H-5 signals overlapping. The signal for H-3' was observed at 7.57 – 7.44 ppm as a multiplet integrating for 2 protons. The oxindole core protons H-7, H-6, H-5 and H-4 were observed at δ 7.83 (d), 7.39 (ddd), 6.84 – 6.69 (m) and 6.66 – 6.58 ppm (m) respectively. Another distinguishing feature of the product is the presence of a singlet integrating for 1 proton at 4.15 ppm resulting from the NH-group. The characteristic Boc-group protons were observed at 1.64 ppm as a singlet integrating for 9 protons.

The success of the nucleophilic addition reaction was evident by the appearance of several new signals including the signal for the quaternary carbon (C-3) observed at 91.9 ppm and signals for the 4-methoxyphenethyl group. The two signals occurring in the aliphatic region at 41.1 ppm and 29.5 ppm are characteristic of the two CH_2 groups and the methoxy group is observed at 55.3 ppm. C-3'' and C-2'' displayed distinct signals in the aromatic region, assigned to signals appearing at 129.2 ppm and 113.8 ppm respectively using ^{13}C NMR spectroscopy and HSQC. The ^{13}C NMR spectrum further confirmed formation of the product **45** by the disappearance of the C=N signal of the starting material **44a** at 152.7 ppm. Two carbonyl carbon signals were observed at 166.1 ppm and 151.3 ppm for C-2 and Boc C=O respectively. The quaternary carbon signal observed at 157.9 ppm was determined to be due to C-4'' using HMBC. IR spectroscopy also provided further evidence to confirm success of the addition reaction because of the strong N-H stretch observed at 3302 cm^{-1}. The molecular ion was confirmed by HRMS to be $[M+H]^+$ 537.1379, which was consistent with the mass calculated for $C_{28}H_{30}{}^{79}BrN_2O_4$ of 537.1389.

After the successful synthesis of **45**, a set of three other analogues were prepared by the Grignard reaction between 4-methoxyphenethylmagnesium bromide and Boc-protected aryl-imines **44b-d** to produce compounds **46-48** (**Figure 13**). This was confirmed by the key signals observed in the ^{1}H NMR, ^{13}C NMR and IR spectra tabulated below.



45 45% **46** 50% **47** 35% **48** 32%

Figure 13. Boc-protected ring-opened analogues derived from Boc-protected aryl-imines **44a-44d**.

Table 6. Key signals in the ^{1}H NMR spectra of the ring-opened analogues **46-48**.

Comp.	H-2'' and H-3'' (ppm)	NH (ppm)	OMe (ppm)	2×(CH$_2$) (ppm)	Boc-group protons (ppm)	CH$_3$ (ppm)
46	6.99 – 6.89 (m, H-3'') 6.86 – 6.70 (m, H-2'')	4.49 (s)	3.76 (s)	3.07 – 2.26 (4H, m)	1.63 (s)	

47	6.97 (d, H-3’’) 6.84 – 6.68 (m, H-2’’)	4.49 (s)	3.75 (s)	3.06 – 2.41 (4H, m)	1.63 (s)	2.38 (s)
48	7.00 – 6.90 (m, H-3’’) 6.78 – 6.71 (m, H-2’’)	4.66 (s)	3.76 (s) 3.85 (s)	2.91 – 2.38 (4H, m)	1.64 (s)	

Table 7. Key signals in the ^{13}C NMR spectra of the ring-opened analogues **46-48**.

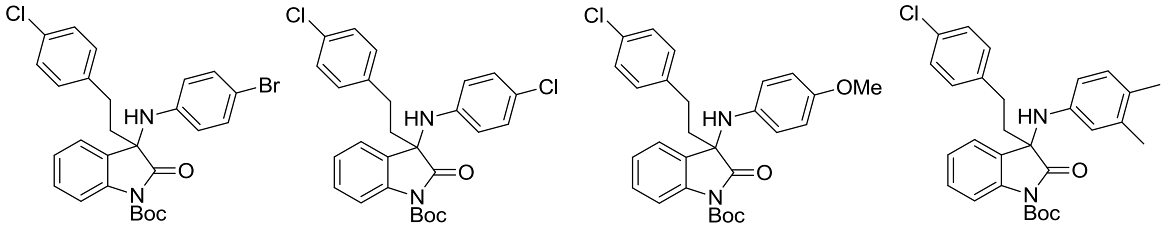
Comp.	(C-2) (ppm)	C-2’’, C-3’’ & C-4’’ (ppm)	C-3 (ppm)	OMe (ppm)	2×(CH_2) (ppm)	Boc-group (ppm)	CH_3 (ppm)
46	166.2	157.9 (C-4’’) 129.2 (C-3’’) 113.8 (C-2’’)	91.9	55.3	41.1 29.5	151.4 (C=O) 83.6 ($\underline{C}(CH_3)_3$) 28.5 ($C(\underline{C}H_3)_3$)	
47	165.6	157.9 (C-4’’) 129.2 (C-3’’) 113.8 (C-2’’)	91.8	55.2	41.2 29.6	151.4 (C=O) 83.3 ($\underline{C}(CH_3)_3$) 28.5 ($C(\underline{C}H_3)_3$)	21.0
48	171.1	157.9 (C-4’’) 129.2 (C-3’’) 113.8 (C-2’’)	91.9	55.5 60.4	41.3 29.6	165.9 (C=O) 83.4 ($\underline{C}(CH_3)_3$) 28.5 ($C(\underline{C}H_3)_3$)	

Table 8. IR spectroscopic N-H stretch signal for the ring-opened analogues **46-48**.

Comp.	**46**	**47**	**48**
cm^{-1} (N-H)	3118	3179	3127

The Grignard reaction between 4-methoxyphenethylmagnesium bromide and Boc-protected aryl-imines **44a-d** was successful despite low yields obtained for the reactions ranging from 32% - 50%. This was not unexpected as the imine carbon atom (C=N) is a relatively poor electrophile and there were competing side reactions taking place. Using 2 equivalents of $MgBr_2$ as an imine activator ensured success for the addition of the 4-methoxyphenethyl to the imine carbon atom (C=N). The success of the reaction was also confirmed by the IR spectra of the products due to the appearance of a strong stretching band at 3118 – 3179 cm^{-1} (**Table 8**), indicative of the N-H signal. The sp^3 chiral carbon (C-3) formed during the Grignard reaction resulted in a signal at ~91 ppm observed in the ^{13}C NMR spectra of the products. The signals due to the carbonyl carbon atoms, C-2 and Boc C=O, appear at 166-165 ppm and 151.4 ppm respectively for the majority of products. Notably, a shift in the signals arising due to the carbonyl carbons of **48** was observed at 171.1 ppm for C-2 and 165.9 for the Boc C=O.

After the successful synthesis of the ring-opened analogues using 4-methoxyphenethylmagnesium bromide, other Grignard reagents derived from commercially available phenethyl bromides and magnesium turnings were used. 4-Chlorophenethylmagnesium bromide was used in a Grignard reaction along with Boc-protected aryl-imines **44a, 44b, 44d and 44e** to produce **49-52**. Characterization data is summarized in **Table 9, 10 and 11** below.



49 34%

50 53%

51 39%

52 15%

Figure 14. Boc-protected ring-opened analogues **49-52**

Table 9. Key signals in the ^{1}H NMR spectra of the ring-opened analogues **49-52**

Comp.	H-2'' & H-3'' (ppm)	NH (ppm)	2×(CH$_2$)	C(CH$_3$)$_3$
49	7.24 – 7.08 (m, H-3'') 6.93 (d, H-2'')	4.58 (s)	3.14 – 2.27 (4H, m)	1.62 (s)
50	7.14 (d, H-3'') 6.91 (d, H-2'')	4.68 (s)	3.02 – 2.33 (4H, m)	1.61 (s)
51	7.18 – 7.13 (m, H-3'') 6.82 – 6.78 (m, H-2'')	4.75 (s)	2.94 – 2.41 (4H, m)	1.61 (s)
52	7.19 – 7.14 (m, H-3'') 6.99 – 6.92 (m, H-2'')	4.60 (s)	3.00 – 2.35 (4H, m)	1.62 (s)

Table 10. Key signals in the ^{13}C NMR spectra of the ring-opened analogues **49-52**

Comp.	C-2 (ppm)	C-3'' & C-2'' (ppm)	C-3 (ppm)	2×(CH_2) (ppm)	Boc-group (ppm)	Aniline subst. (ppm)
49	166.0	129.6 (C-2'') 128.4 (C-3'')	91.8	40.5 29.8	151.4 (C=O) 83.7 $\underline{C}(CH_3)_3$ 28.4 $C(\underline{C}H_3)_3$	
50	166.4	129.7 (C-2'') 128.4 (C-3'')	91.9	40.5 29.9	151.3 (C=O) 83.5 $\underline{C}(CH_3)_3$ 28.4 $C(\underline{C}H_3)_3$	
51	166.0	129.6 (C-2'') 128.4 (C-3'')	91.8	38.4 29.9	151.4 (C=O) 83.4 $\underline{C}(CH_3)_3$ 28.4 $C(\underline{C}H_3)_3$	55.5 (OMe)
52	165.2	129.7 (C-2'') 128.4 (C-3'')	91.7	40.6 29.9	151.4 (C=O) 83.4 $\underline{C}(CH_3)_3$ 28.4 $C(\underline{C}H_3)_3$	19.9 (CH_3) 19.3 (CH_3)

Table 11. Wavenumber of N-H signal in IR spectra, Melting points and HRMS results for the ring-opened analogues **49-52**

comp	cm^{-1} (N-H)	Melting point (^{o}C)	Molecular ion (HRMS) (calc)	Molecular ion (HRMS) (found)
49	3177	129-130	541.0894	541.0901
50	3196	122-123	497.1399	497.1400
51	3172	138-139	493.1894	493.1895
52	3171	138	491.2101	491.2108

In each of the products, the newly formed NH gave rise to a singlet integrating for one proton at ~4.6 ppm in the ^{1}H NMR spectra. ^{13}C NMR spectra further support formation of the products with a new signal appearing at 91.7 – 91.9 ppm due to the newly formed quaternary chiral carbon at the C-3 position. The disappearance of the signal expected at 155 ppm due to the imine carbon atoms (C=N) in the ^{13}C NMR spectra is another indication of the success of the addition reaction. HRMS results of the products were as expected and their melting points were found to be moderately high ranging from 122 – 139 °C.

The Grignard addition reaction was attempted on the Boc-protected aryl-imine **44c** using the same method described for the preparation of **45** (**Scheme 15**), unfortunately the reaction was not successful. The deprotected aryl-imine **43c** was isolated in 51% instead of the desired ring-opened analogue. Isolation of the deprotected aryl-imines was common during most of the Grignard reactions carried out. Another side product isolated is shown in **Figure 15** below and it is similar to that isolated during the test reaction on **44a** to produce **45** (**Scheme 15**). It is worth mentioning that the side products were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy only. The product formation could be explained by the presence of unreacted 4-methoxyphenethyl bromide during preparation of the Grignard reagent. Imine hydrolysis would afford a substituted aniline, which could react with the alkyl bromide to afford these substituted amines. In both cases they were isolated in relatively low yield.

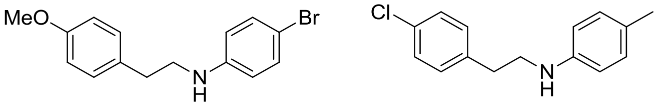


Figure 15. Side products produced during Grignard addition reaction of Boc-protected aryl-imine **44a** and **44c** respectively.

Ring-opened analogues were also prepared by nucleophilic addition reactions involving Boc-protected imines **44a-e** and the Grignard reagent derived from 3-chlorophenethyl bromide and magnesium turnings. These products (**53-57**, **Figure 16**) were characterised by IR, ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectra of these compounds gave adequate evidence to prove their formation. Addition of the 3-chlorophenethyl group resulted in the appearance of new signals in the aromatic region. H-4'' and H-6'' gave rise to a multiplet observed at ~7.2 – 7.1 ppm integrating for two protons, whereas H-2'' is responsible for a signal at ~6.9 ppm integrating for one proton. This signal either appears as a singlet or a doublet with coupling constant ranging from 2.1 – 1.5 Hz indicating *meta* coupling between H-2'' and H-4''. The multiplet at ~6.9 ppm integrating for one proton is due to H-5''. The NH formed during the reaction was observed at ~4.6 ppm in the ^{1}H NMR spectra. Key signals from the ^{1}H NMR, ^{13}C NMR and IR spectra are summarized in the **Table 12,13** and **14** below.

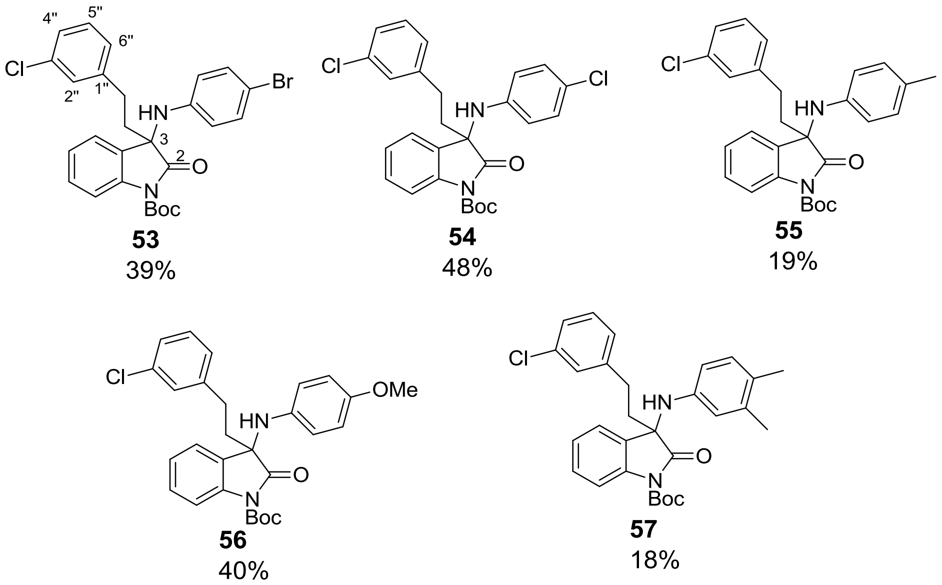


Figure 16. Boc-protected ring-opened analogues derived from Boc-protected aryl-imines **44a-44e**.

Table 12. Key signals in the ^{1}H NMR spectra of the ring-opened analogues **53-57**

Comp.	Phenethyl protons (ppm)	NH (ppm)	2×(CH$_2$) (ppm)	C(CH$_3$)$_3$ (ppm)	Aniline subst. (ppm)
53	7.15 – 7.10 (m, H-4’’ and H-6’’), 6.90 (d, *J* = 1.5 Hz, H-2’’), 6.93 – 6.86 (m, H-5’’)	4.61 (s)	2.98 – 2.35 (m)	1.62 (s)	
54	7.16 – 7.08 (m, H-4’’ and H-6’’), 6.95 (d, *J* = 1.6 Hz, H-2’’), 6.94 – 6.86 (m, H-5’’)	4.63 (s)	2.98 – 2.41 (m)	1.62 (s)	
55	7.15 – 7.08 (m,	4.53 (s)	3.03 – 2.43	1.62 (s)	2.39 (s, CH$_3$)

	H-4’’ and H-6’’), 6.98 (s, H-2’’), 6.96 – 6.90 (m, H-5’’)		(m)		
56	7.15 – 7.09 (m, H-4’’ and H-6’’), 7.00 – 6.89 (m, H-2’’ and H-5’’)	4.59 (s)	2.98 – 2.43 (m)	1.62 (s)	3.83 (s, OCH$_3$)
57	7.18 – 7.10 (m, H-4’’ and H-6’’), 6.99 (d, *J* = 2.1 Hz, H-2’’), 6.94 – 6.86 (m, H-5’’)	4.65 (s)	2.95 – 2.43 (m)	1.62 (s)	2.29 (s, CH$_3$) 2.26 (s, CH$_3$)

Table 13. Key signals in the ^{13}C NMR spectra of the ring-opened analogues **53-57**

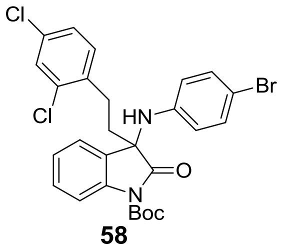
Comp.	C-2 (ppm)	Phenethyl C-signals (ppm)	C-3 (ppm)	2×(CH$_2$) (ppm)	Boc C-signals (ppm)	Aniline subst. (ppm)
53	166.0	129.6 (C-4’’), 128.5 (C-2’’), 126.3 (C-5’’), 126.2 (C-6’’)	91.8	40.3, 30.2	151.3 (C=O), 83.7 ($\underline{C}(CH_3)_3$), 28.4 ($C(\underline{C}H_3)_3$)	
54	166.1	129.6 (C-4’’), 128.5 (C-2’’), 126.5 (C-5’’), 126.3 (C-6’’)	91.8	40.3, 30.2	151.3 (C=O), 83.7 ($\underline{C}(CH_3)_3$), 28.4 ($C(\underline{C}H_3)_3$)	
55	165.4	129.6 (C-4’’), 128.5 (C-2’’), 126.4 (C-5’’), 126.2 (C-6’’)	91.7	40.4, 30.2	151.4 (C=O), 83.5 ($\underline{C}(CH_3)_3$), 28.5 ($C(\underline{C}H_3)_3$)	21.0 (CH$_3$)
56	165.9	129.6 (C-4’’),	91.8	38.4,	151.3 (C=O),	55.5 (OCH$_3$)

		128.5 (C-2’’), 126.5 (C-5’’), 126.1 (C-6’’)		29.9	83.4 ($\underline{C}(CH_3)_3$), 28.4 ($C(\underline{C}H_3)_3$)	
57	165.2	129.6 (C-4’’), 128.5 (C-2’’), 126.5 (C-5’’), 126.1 (C-6’’)	91.7	40.3, 30.2	151.4 (C=O), 83.7 ($\underline{C}(CH_3)_3$), 28.4 ($C(\underline{C}H_3)_3$)	19.9 (CH_3), 19.3 (CH_3)

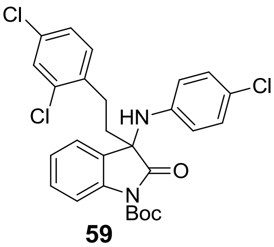
Table 14. Wavenumber of N-H signal in IR spectra, Melting points and HRMS results for the ring-opened analogues **53-57**

comp	cm^{-1} (N-H)	Melting point (°C)	Molecular ion (HRMS) (calc)	Molecular ion (HRMS) (found)
53	3191	108	541.0894	541.0897
54	3177	119	497.1399	497.1398
55	3293	98	477.1945	477.1929
56	3140	132-133	493.1894	493.1896
57	3166	129	491.2101	491.2113

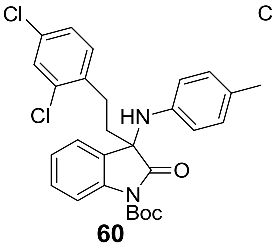
After the successful synthesis of the ring-opened analogues using mono-substituted phenethyl groups, the same method was used to synthesize a series of compounds using 2,4-dichlorophenethylmagnesium bromide in the Grignard reaction. The presence of the extra halide on the phenethyl group resulted in the production of oils instead of solids that had typically been isolated from the Grignard reaction. The yields of the products **58-61** (**Figure 17**) were low, ranging from 10% to 33%. Key signals in the ^{1}H NMR and ^{13}C NMR spectra are described in **Tables 15** and **16** below.



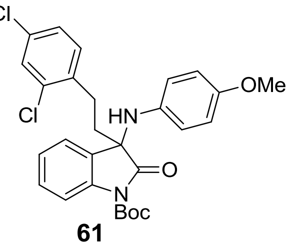
58
27%



59
15%



60
10%



61
33%

Figure 17. Boc-protected ring-opened analogues derived from Boc-protected aryl-imines **44a-44d**.

Table 15. Key signals in the ^{1}H NMR spectra of the ring-opened analogues **58-61**

Comp.	Phenethyl protons (ppm)	NH (ppm)	2×(CH$_2$) (ppm)	C(CH$_3$)$_3$ (ppm)	Aniline subst. (ppm)
58	7.36 (d, *J* = 2.1 Hz, H-3’’), 7.11 – 7.02 (m, H-5’’), 6.95 (d, H-6’’)	4.73 (s)	2.93 – 2.53 (m)	1.64 (s)	
59	7.30 (d, *J* = 2.1 Hz, H-3’’), 7.07 (dd, *J* = 8.2, 2.1 Hz, H-5’’), 6.96 (d, H-6’’)	4.79 (s)	2.90 – 2.52 (m)	1.64 (s)	
60	7.32 (d, *J* = 2.1 Hz, H-3’’), 7.09 (dd, *J* = 8.2, 2.2 Hz, H-5’’), 6.99 (d, H-6’’)	4.53 (s)	2.94 – 2.52 (m)	1.64 (s)	2.40 (CH$_3$)
61	7.30 (d, *J* = 2.1 Hz, H-3’’), 7.07 (dd, *J* = 8.2, 2.2 Hz, H-5’’), 7.00-6.91 (m, H-6’’)	4.70 (s)	2.91 – 2.51 (m)	1.63 (s)	3.83 (OCH$_3$)

Table 16. Key signals in the ^{13}C NMR spectra of the ring-opened analogues **58-61**

Comp.	C-2 (ppm)	Phenethyl C-signals (ppm)	C-3 (ppm)	2×(CH$_2$) (ppm)	Boc C-signals (ppm)	Aniline subst. (ppm)
58	165.9	131.2 (C-6’’), 129.3 (C-3’’), 127.1 (C-5’’)	91.7	38.8 28.0	151.3 (C=O), 83.7 ($\underline{C}$(CH$_3$)$_3$), 28.4 (C($\underline{C}$H$_3$)$_3$)	
59	166.0	131.2 (C-6’’), 129.2 (C-3’’),	91.7	38.8 28.0	152.8 (C=O), 83.7 ($\underline{C}$(CH$_3$)$_3$),	

		127.1 (C-5’’)			28.4 (C($\underline{C}H_3)_3$)	
60	165.1	131.2 (C-6’’), 129.2 (C-3’’), 127.1 (C-5’’)	91.7	39.0 28.0	151.4 (C=O), 83.6 ($\underline{C}(CH_3)_3$), 28.4(C($\underline{C}H_3)_3$)	21.0 (CH_3)
61	165.7	131.3 (C-6’’), 129.2 (C-3’’), 127.1 (C-5’’)	91.7	38.9 28.0	151.3 (C=O), 83.5 ($\underline{C}(CH_3)_3$), 28.4 (C($\underline{C}H_3)_3$)	55.5 (OCH_3)

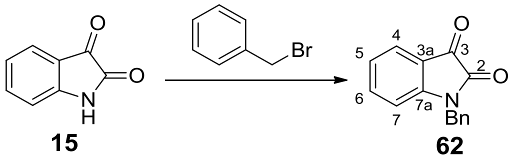
The ^{1}H NMR spectra of the newly synthesised analogues all contain signals arising due to the 2,4-dichlorophenethyl group. Three new signals were observed in the aromatic region; two doublets at 7.30 ppm (*J* = 2.1 Hz) and 6.96 ppm (*J* = 8.2 Hz) integrating for one proton each and a doublet of doublets at 7.07 ppm (*J* = 8.2, 2.2 Hz) also integrating for one proton. The two doublets are due to H-2’’ and H-6’’ respectively, while the doublet of doublets is due to H-5’’. The NH signal of **59** and **61** gave rise to broad singlets observed between 4.70 ppm and 4.79 ppm. In the ^{13}C NMR spectra, the signal due to the quaternary spiro carbon atoms appeared at 91.7 ppm as expected.

After the successful synthesis of the ring-opened analogues, we were now in a position to remove the Boc-protecting group. Conventionally, the Boc-group is readily cleaved in acidic environments. Hydrolysis of the Boc-group was first attempted with trifluoroacetic acid. Unfortunately the deprotection was unsuccessful, even after attempts to optimize the reaction. In each case, only starting material was recovered. Several other acids were also tested, including acetic acid and hydrochloric acid, but either the starting material was isolated or decomposition occured. Having many failed reactions using acids, unconventional experimental methods had to be tested including adsorbing the product onto silica and subjecting it to microwave irradiation. Again, the starting material was recovered. The use of strong and weak bases was also attempted. However, all the methods attempted were not successful in removing the Boc-group to give the desired unprotected ring-opened analogues. It is possible that the deprotected product decomposed under the reaction conditions required to facilitate removal of the Boc protecting group.

Considering that the Grignard addition to the Boc protected aryl imines was generally low yielding and removing the Boc-protecting group was unsuccessful, we thought to explore other protecting groups for this series of compounds. Benzyl and *p*-methoxybenzyl protecting groups were chosen since they are widely used for protection of the indole nitrogen.

2.2 Synthesis of ring-opened analogues using benzyl and *p*-methoxy benzyl protecting groups

2.2.1 Synthesis of 1-benzylindoline-2,3-dione

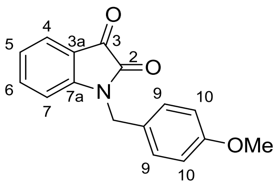


Scheme 16. Reagents and conditions: K_2CO_3, KI, DMF, 80 °C, 75%

The benzyl protection was carried out by dissolving commercially available isatin **15** and K_2CO_3 in DMF (**Scheme 16**). Then KI and benzyl bromide were added to the mixture and the reaction temperature was raised to 80 °C for 5 hours. After complete consumption of isatin, indicated by one spot observed using TLC analysis, the benzyl-protected product **62** was purified by recrystallization and isolated as a bright orange crystalline solid in 75% yield.

Success of the benzyl protection was confirmed by ^1H NMR spectroscopy due to the presence of a multiplet at 7.40 – 7.29 ppm integrating for five protons characteristic of the benzylic protons. The oxindole protons gave rise to four signals in the aromatic region, with each signal integrating for one proton; the doublet of doublets at 7.61 ppm and the doublet at 6.78 ppm are due to H-7 and H-4 respectively, while the two triplet of doublets at 7.48 ppm and 7.09 ppm are due to H-5 and H-4 respectively. The ^{13}C NMR spectrum further supports formation of benzyl-isatin **62** as thirteen signals were observed for the product as expected. The signal appearing at 44.1 ppm is due to the benzylic CH_2. The melting point obtained (134-135 °C) was comparable with the literature reported value of 136 °C.[61]

2.2.2 Synthesis of 1-(4-methoxybenzyl)indoline-2,3-dione

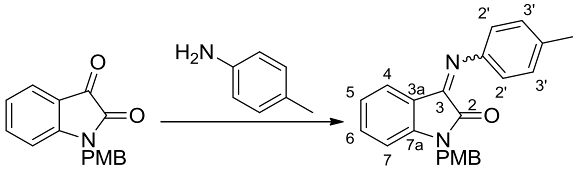


63

1-(4-Methoxybenzyl)indoline-2,3-dione **63** was synthesized using the same method described for 1-benzylindoline-2,3-dione **62** and the product was isolated as an orange crystalline solid in 72% yield. Formation of the product was confirmed by ^{1}H NMR spectroscopy by the presence of two singlets; one at 4.86 ppm integrating for two protons due to the benzylic CH_2 and the other at 3.78 ppm characteristic of the methoxy group. H-9 and H-10 are responsible for the multiplets observed in the aromatic region at 6.91 – 6.84 ppm and 7.32 – 7.24 ppm respectively, and each signal integrates for two protons. The two carbonyl carbon atoms of the oxindole core gave rise to signals at 183.4 ppm and 158.2 ppm in the ^{13}C NMR spectrum. The methoxy group appears as a signal at 55.3 ppm, while the benzylic CH_2 gave rise to a signal at 43.5 ppm.

2.3 Synthesis of aryl-imines using Bn and PMB-protecting groups

2.3.1 Synthesis of 1-(4-methoxybenzyl)-3-(*p*-tolylimino)indolin-2-one



63 **64**

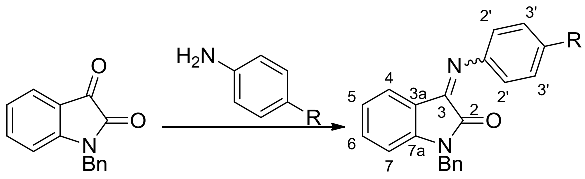
Scheme 17: Reagents and conditions: Gl. acetic acid, Ab. Ethanol, reflux, 55%

The imine condensation reaction was carried out between PMB-isatin **63** and *p*-toluidine in ethanol, using acetic acid as a catalyst (**Scheme 17**). The product **64** was purified by recrystallization and isolated as a yellow solid in 55% yield. In the ^{1}H NMR spectrum, H-3' is responsible for the multiplet at 7.24 – 7.221 ppm overlapping with the signal assigned to H-6, while the multiplet at 6.96 – 6.92 ppm integrating for 2 protons is due to H-2’. The methyl group gave rise to the new singlet in the aliphatic region at 2.40 ppm integrating for three protons.

Success of the imine condensation reaction was also confirmed by the appearance of a signal due to the C=N group at 154.0 ppm in the ^{13}C NMR spectrum. The functional group conversion consequently results in the disappearance of the signal at 183.4 ppm owing to the carbonyl carbon atom (C-3) of the starting material. Another notable difference is the significant shift from 158.2 ppm to 163.4 ppm of the signal due to the carbonyl carbon atom assigned as C-2.

The IR spectrum further confirmed formation of the product with the C=N stretch observed at 1653 cm^{-1}. The molecular ion determined by HRMS was confirmed to be $[M+H]^+$ 357.1603, which was consistent with the mass calculated for $C_{23}H_{21}N_2O_2$ of 357.1610.

Analogues **65a** and **65b** were synthesized in a similar manner starting from Bn-isatin **62** (**Scheme 18**). Key signals in the ^{1}H NMR and ^{13}C NMR spectra for **65a** and **65b** are represented in **Table 17** and **18** below.



62

65a: R = Cl, 67%
65b: R = Br, 90%

Scheme 18: Reagents and conditions: Gl. acetic acid, Ab. Ethanol, reflux

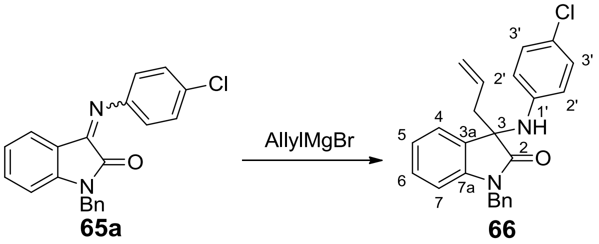
Table 17. Key signals in the ^{1}H NMR spectra of Bn-protected aryl-imines **65a and 65b**

Comp.	Imino protons (ppm)	Oxindole protons (ppm)	Bn protons (ppm)	Bn (CH_2) (ppm)
65a	7.47 – 7.28 (m, H-3’), 7.04 – 6.95 (m, H-2’)	7.47 – 7.28 (m, H-6), 6.82 – 6.69 (H-4, H-5 & H-7)	7.47 – 7.28 (m)	5.01 (s)
65b	7.60 -7.52 (m, H-3’), 6.99 – 6.89 (m, H-2’)	7.42 – 7.28 (m, H-6), 6.81 – 6.68 (H-4, H-5 & H-7)	7.42 – 7.28 (m)	5.01 (s)

Table 18. Key signals in the ^{13}C NMR spectra of Bn-protected aryl-imines **65a and 65b**

Comp.	C=O (ppm)	C=N (ppm)	Aromatic region (ppm)	Bn CH_2 (ppm)
65a	163.2	154.6	129.6 (C-3'), 119.4 (C-2')	44.1
65b	163.1	149.2	132.6 (C-3'), 119.8 (C-2')	44.1

2.3.2 Synthesis of 3-allyl-1-benzyl-3-((4-chlorophenyl)amino)indolin-2-one



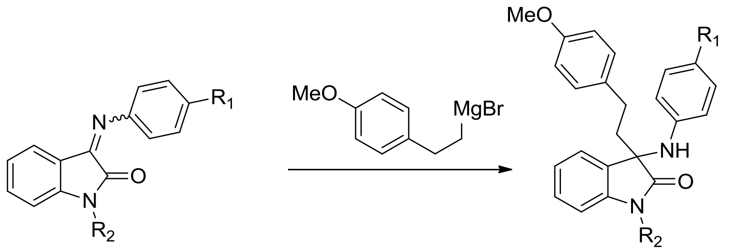
Scheme 19: Reagents and conditions: $MgBr_2$, DCM, -40°C- rt, 52%

The most crucial step in the synthesis of our ring opened analogues is the nucleophilic addition of Grignard reagents to the imine carbon atom (C=N). The reaction was first tested on **65a** dissolved in DCM and reacted with commercially available allylmagnesium bromide in the presence of $MgBr_2$ (**Scheme 19**). DCM is a rather unconventional solvent to be used in a Grignard reaction, and the reaction is typically conducted in coordinating solvents such as THF or diethyl ether. However, we found precedent in literature for the nucleophilic addition of an allyl-group to an imine derived from isatin in the presence of $MgBr_2$ and DCM as a solvent.[37] Using our system, the Grignard reaction gave **66** as a yellow solid in 52% yield.

Formation of the product **66** was confirmed by ^{1}H NMR spectroscopy due to the two multiplets observed in the alkene region; one at 5.82 – 5.63 ppm integrating for one proton, characteristic of the CH-group, and one at 5.28 – 5.13 ppm integrating for two protons, due to the terminal alkene CH_2 group. The allyl CH_2 is responsible for the multiplet in the aliphatic region at 2.82 – 2.55 ppm. Success of the reaction was further confirmed by the appearance of the singlet at 4.45 ppm integrating for one proton arising due to the NH formed during the addition reaction. The characteristic aromatic protons of the benzyl group gave rise to a multiplet at 7.35 – 7.16 ppm, overlapping with the signals due to H-6 and H-4. The multiplet at 5.10 – 4.71 ppm integrating for two protons is due to the benzylic CH_2 protons.

The nucleophilic addition reaction results in functional group conversion from an sp^2 C=N to an sp^3 quaternary spiro carbon atom at the C-3 position. The signal observed at 64.1 ppm in the ^{13}C NMR spectrum is due to the newly formed quaternary carbon atom. Disappearance of the signal at 154.6 ppm due to the C=N carbon atom indicates the success of the addition reaction. The carbonyl carbon atom gave rise to a signal at 177.3 ppm, which has shifted downfield by ~14 ppm relative to the C=O signal observed for the starting material. The signal at 130.2 ppm is due to the alkene CH, while the signal at 121.1 ppm arises from the terminal alkene CH_2 group. The two signals in the aliphatic region observed at 44.5 and 44.1 ppm are due to the allyl CH_2 and Bn CH_2 groups respectively.

The IR spectrum further provides evidence for the formation of the product. A strong sharp signal at 3317 cm^{-1} is due to the N-H stretch, while the =C-H stretch is visible as a signal observed at 3025 cm^{-1}, indicating the presence of the olefin in the product.



67: R_1 = Cl, R_2 = Bn
68: R_1 = Br, R_2 = Bn

Scheme 20: Reagents and conditions: Lewis acid, -40°C- rt

After the success of the Grignard reaction using a commercially available Grignard reagent, we were in a position to attempt the nucleophilic addition reaction on benzyl protected imines and PMB protected imines using *p*-methoxyphenethylmagnesium bromide, to give the desired ring opened analogues (**Scheme 20**). The method described for the synthesis of Boc-protected ring-opened analogues (**Scheme 15**) was initially attempted on substrates **64** and **65a** to produce the desired ring-opened analogues. Unfortunately, in each case the reaction was unsuccessful (entries 1 & 2, **Table 19**). When the Lewis acid was changed to $BF_3.OEt_2$, the nucleophilic addition of the *p*-methoxyphenethyl group was successful (entries 3 & 4). Despite the successful production of the desired ring-opened analogues **67** and **68**, the yields were disappointingly low, and significantly lower than the yields obtained for the Boc-protected analogues prepared. In an attempt to improve the yield, the reaction was carried out in DCM, and yield increased from 6% to 11% for **68** (entries 4 & 5). However, this route was deemed too low yielding to be pursued, with insufficient quantities of products isolated for deprotection strategies to be attempted. As a last resort, the Grignard addition reaction was attempted on an unprotected aryl-imine. However, the reaction was unsuccesful and most of the unprotected aryl-imine was recovered from the reaction.

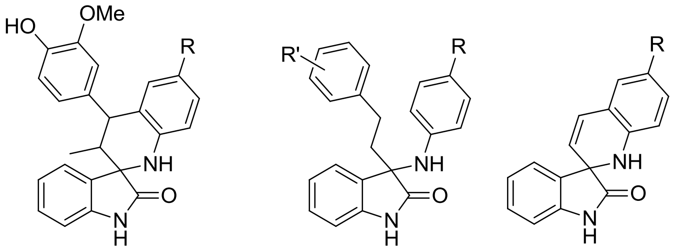
Table 19. Reaction of *p*-methoxyphenethylmagnesium bromide with substrate **64**, **65a** and **65b**

Entry	Imine intermediate	Lewis acid	Solvent	Product	Yield (%)
1.	**64**	$MgBr_2$	THF		-
2.	**65a**	$MgBr_2$	THF	**67**	-
3.	**65a**	$BF_3.OEt_2$	THF	**67**	12
4.	**65b**	$BF_3.OEt_2$	THF	**68**	6
5.	**65b**	$BF_3.OEt_2$	DCM	**68**	11

In summary, the ring-opened analogues were succesfully synthesised by a nucleophilic Grignard addition reaction to Boc-protected aryl-imines. Unfortunately, all attempts to remove the Boc-protecting group from the ring-opened analogues using conventional and unconventional methods, were unsuccessful. Considering that we were unable to isolate any deprotected products, we thought to use PMB and Bn-protecting groups as alternatives. The ring-opened analogues bearing either Bn or PMB protecting groups were isolated after changing to a stronger lewis acid, $BF_3.OEt_{2,}$ in the Grignard addition reaction. However, these compounds were isolated in such low yields we were not able to test the removal of the protecting group. This will be considered for future work.

2.4 Synthesis of ring closed analogues

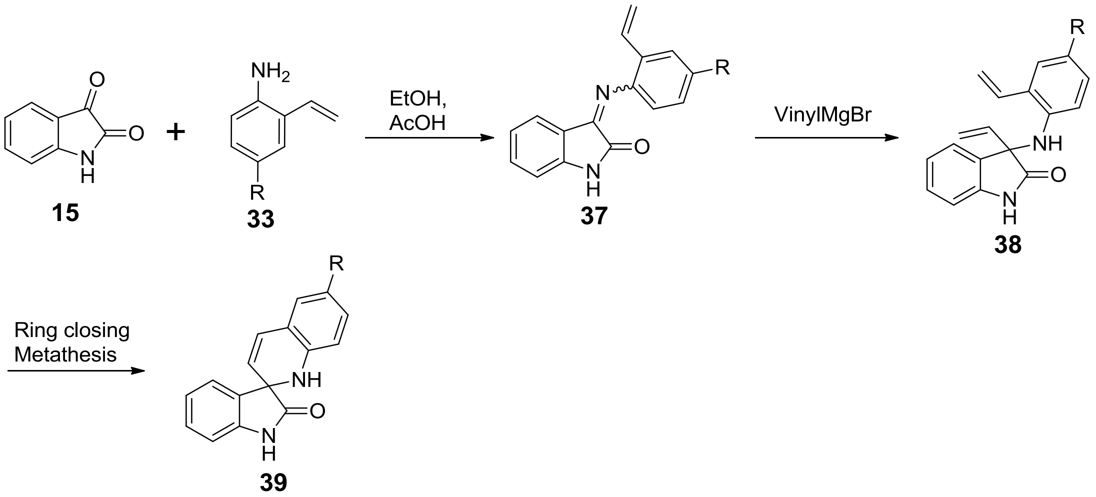
We aimed to synthesize ring-closed analogues of hit compound **29** as the second series of spirooxindoles (**39**, **Figure 18**). They are structurally similar to the series of compounds previously synthesized in our laboratory using the Povarov reaction, however, they lack the aryl-group derived from isoeugenol. The 6-membered spiro fused ring at the C-3 position will result in analogues that are more constrained and less flexible than the ring-opened analogues **31**. We hoped to assess the effect of these small variations in structure on the antimalarial activity of these compounds.



29 **31** **39**

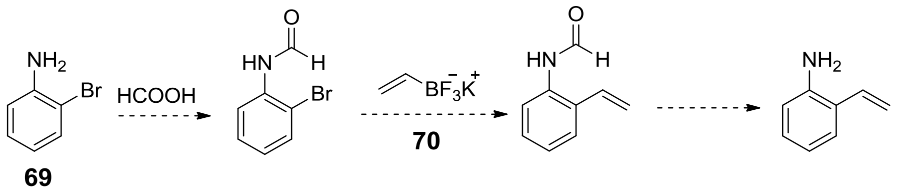
Figure 18. Spirooxindoles derived from the Povarov reaction **29**, ring-opened analogues **31** and the ring-closed analogues **39**

The proposed synthetic route towards synthesis of the ring closed analogues is represented in **Scheme 21**. Synthesis of the ring closed analogues would be initiated by formation of the aryl-imine intermediate **37** from reaction of commercially available isatin with *para*-substituted 2-vinylanilines **33**, the latter prepared from commercially available aniline. The aryl-imine intermediate will then be subjected to a nucleophilic addition reaction using commercially available vinylmagnesium bromide in the presence of a Lewis acid. This will be followed by the ring-closure step, which is the key step that will give rise to the 6-membered spiro fused compounds by means of ring-closing metathesis.



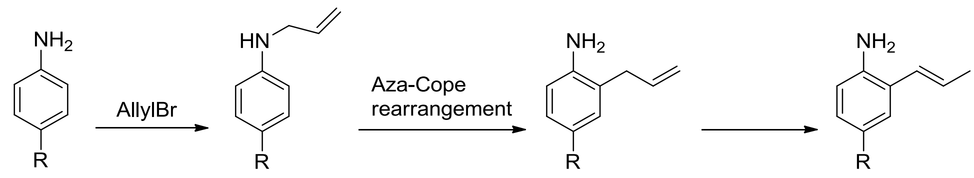
Scheme 21. Proposed synthetic route towards synthesis of the ring-closed analogues

The 2-vinylaniline could be prepared by a Suzuki cross coupling reaction between a terminal alkene and 2-bromoaniline **69**, which requires the formation of potassium vinyltrifluoroborate salt **70** (**Scheme 22**). Unfortunately we were unable to reproduce the salt as described by Molander and Rivero[40] and an alternative approach to 2-vinylanilines was considered.



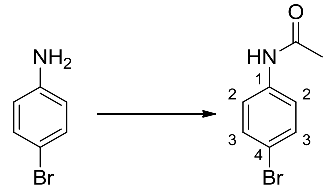
Scheme 22. Proposed synthetic route towards synthesis of the 2-vinylaniline using Suzuki cross coupling reaction

As an alternative synthesis of 2-vinylanilines, we considered using the aza-Cope rearrangement. The reaction allows for a [3,3]-sigmatropic shift, resulting in the rearrangement of an allylated aniline (**Scheme 23**).



Scheme 23. General synthesis of the 2-vinylanilines using Aza-Cope rearrangement

2.4.1 Synthesis of *N*-(4-bromophenyl)acetamide and analogues



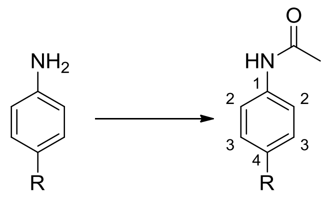
71a **72a**

Scheme 24. Reagents and conditions: Ac_2O, triethylamine, DCM, rt, 61%

Formation of the desired 2-allylaniline was initiated by acetylation of suitable anilines. Commercially available 4-bromoaniline **71a** was dissolved in DCM, followed by addition of acetic anhydride and triethylamine. The reaction was conducted at room temperature for 2 hours (**Scheme 24**). After this time TLC analysis showed the presence of a new product and no starting material. The desired product was isolated and purified by recrystallization in EtOAc/hexane as a white crystalline solid in 61% yield.

Four signals were observed in the ^1H NMR spectrum of **72a**, as expected. The singlet observed at 2.08 ppm integrating for three protons is due to the acetyl CH_3 group. Another singlet was observed downfield at 9.29 ppm integrating for one proton, characteristic of the amide NH. Finally, two doublets were observed in the aromatic region each integrating for two protons; one at 7.61 ppm due to H-2, and one at 7.44 ppm owing to H-3. The ^{13}C NMR spectrum gave further evidence of the formation of **72a** due to the presence of a signal in the carbonyl region at 168.2 ppm for the amide carbonyl carbon atom. The methyl of the amide group gave rise to the signal at 23.4 ppm. And lastly, 4 distinct signals were observed in the aromatic region owing to the phenyl ring.

Two other analogues were prepared in a similar manner (**Scheme 25**) and the ^1H NMR and ^{13}C NMR spectra of the products were similar. Key signals for the compounds are represented in **Table 20** and **21**.



71b: R = H
71c: R = Me

72b: R = H, 93%
72c: R = Me, 63%

Scheme 25. Reagents and conditions: Ac$_2$O, triethylamine, DCM, rt

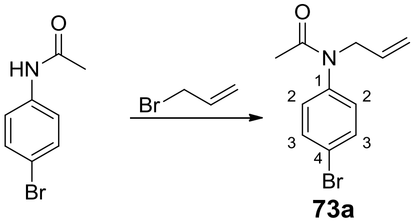
Table 20. Key signals in the ^{1}H NMR spectra for *N*-phenylacetamides **72b** and **72c**

Comp.	NH (ppm)	Aromatic region (ppm)	Ac- CH$_3$ (ppm)	R-group (ppm)
72b	8.33 (s)	7.51 (d, H-2), 7.48 – 7.39 (m, H-3), 7.07 (t, H-4)	2.12 (s)	
72c	7.55 (s)	7.40 – 7.34 (m, H-2) 7.10 (d, H-3)	2.14 (s)	2.30 (s)

Table 21. Key signals in the ^{13}C NMR spectra for *N*-phenylacetamides **72b** and **72c**

Comp.	C=O (ppm)	Aromatic region (ppm)	Ac- CH$_3$ (ppm)	R-group (ppm)
72b	169.1.2	138.8 (C-1), 131.5 (C-3), 120.9 (C-2), 114.9 (C-4)	24.3	
72c	168.5	135.4 (C-1), 133.9 (C-4), 129.4 (C-3), 120.1 (C-2)	24.4	20.8

2.4.2 Synthesis of *N*-allyl-*N*-(4-bromophenyl)acetamide and analogues



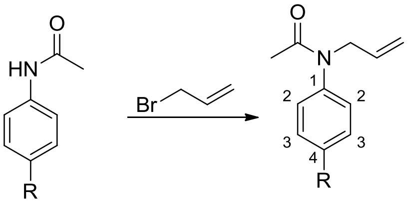
73a

Scheme 26. Reagents and conditions: NaH, TBAI, DMF, 0 °C-rt, 83%

Having prepared the acetyl-protected anilines we were in a position to introduce the allyl group. The reaction employs a base, NaH, which abstracts the amide proton from *N*-phenylacetamide to generate an anion. This attacks the electrophilic carbon atom of the allylbromide, thereby giving rise to **73a**. The starting material was dissolved in dry DMF, followed by addition of allylBr, TBAI and NaH (**Scheme 26**). After stirring the reaction mixture for 5 hours under N_2 atmosphere, the product was isolated and purified by silica gel column chromatography as a clear oil in 83% yield.

The ^1H NMR spectrum confirmed formation of **73a** due to the disappearance of the NH signal at 9.29 ppm. Furthermore, addition of the allyl group gave rise to two signals in the alkene region; one is a triplet of triplets at 5.83 ppm integrating for one proton due to the alkene CH and the other a multiplet at 5.17 – 5.01 ppm integrating for two protons owing to the alkene CH_2. The allyl CH_2 gave rise to the doublet of triplets in the aliphatic region at 4.27 ppm integrating for two protons. ^{13}C NMR spectroscopy gave further evidence for the introduction of the allyl group. The alkene CH_2 and CH carbons gave rise to signals at 132.9 ppm and 118.2 ppm respectively, while the allyl CH_2 was observed as a signal at 51.9 ppm.

Analogues **73b** and **73c** were prepared in a similar manner (**Scheme 27**). The ^1H NMR and ^{13}C NMR spectra of the products were similar as expected, and key signals are tabulated below (**Table 22** and **23**).



73b: R = H. 76%
73c: R = Me, 85%

Scheme 27. Reagents and conditions: NaH, TBAI, DMF, 0 °C-rt

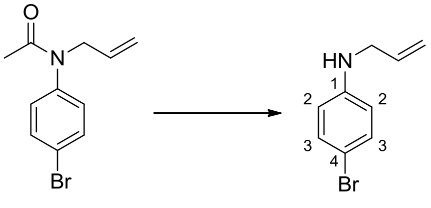
Table 22. Key signals in the ^1H NMR spectra for *N*-allyl-*N*-phenylacetamides **73b** and **73c**

Comp.	=CH (ppm)	=CH_2 (ppm)	Allyl CH_2 (ppm)
73b	5.97 – 5.75 (m)	5.17 – 4.96 (m)	4.37 – 4.20 (m)
73c	5.85 (ddt)	5.18 – 4.94 (m)	4.28 (dt)

Table 23. Key signals in the ^{13}C NMR spectra for *N*-allyl-*N*-phenylacetamides **73b** and **73c**

Comp.	$=CH_2$ (ppm)	=CH (ppm)	Allyl CH_2 (ppm)
73b	133.1	117.7	52.0
73c	133.3	117.6	52.0

2.4.3 Synthesis of *N*-allyl-4-bromoaniline and analogues



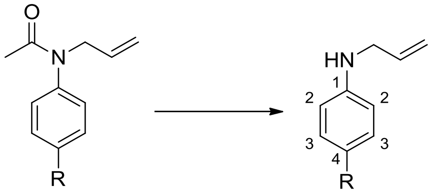
74a

Scheme 28. Reagents and conditions: NaOH, 10:1 ethanol/H_2O, reflux, 88%

Removal of the acetyl group was achieved by treating the starting material with NaOH in ethanol/water at reflux (**Scheme 28**). After stirring for 2 hours, TLC analysis showed the presence of a new product and no traces of the starting material. The product was purified by silica gel column chromatography and isolated as a clear yellow oil in 88% yield.

The most significant distinguishing feature in the ^1H NMR spectrum of the product is the disappearance of the signal observed due to the acetyl CH_3 in the aliphatic region. The singlet at 3.80 ppm integrating for one proton appeared as a result of the newly formed NH. Removal of the acetyl group is also confirmed by the ^{13}C NMR spectrum, again due to the disappearance of the amide carbonyl signal and the signal due to the acetyl CH_3 group.

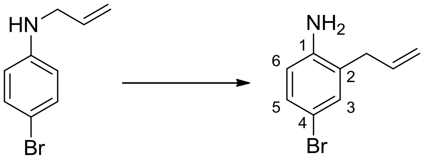
Analogues **74b** and **74c** were prepared using a similar method (**Scheme 29**). As mentioned above for **74a**, confirmation of the product is evident due to the disappearance of the acetyl-methyl group observed in the aliphatic region of the ^1H NMR spectra of the products. The newly formed NH group gave rise to signals observed at 3.83 – 3.46 ppm for **74c** and 4.10 – 3.78 ppm for **74b**. The NH signals appear as multiplets because they overlap with signals owing to the allyl CH_2 group.



74b: R = H, 90%
74c: R =Me, 92%

Scheme 29. Reagents and conditions: NaOH, 10:1 ethanol/H_2O, reflux

2.4.4 Synthesis of 2-allyl-4-bromoaniline



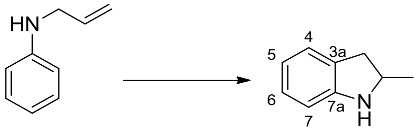
75

Scheme 30. Reagents and conditions: $BF_3.OEt_2$, xylene, 140 °C, 19 hours 10%

The Claisen rearrangement has been extensively studied due to the C-C bond formed via the 3,3-sigmatropic shift. In contrast, the nitrogen analogue of this reaction, known as the aza-Cope rearrangement, has received less attention.[48] This is because uncatalyzed aza-Cope rearrangements require high temperatures, longer reaction times and they are generally low yielding. Harsh conditions employed for these transformations result in the decomposition of the *N*-allylaniline starting material. The use of Lewis acids such as $BF_3.OEt_2$ has significantly improved the yield of the transformation.[48,49] The aza-Cope rearrangement was first tested on *N*-allyl-4-bromoaniline **74a**, dissolved in xylene and heated to 140 °C for 19 hours in the presence of $BF_3.OEt_2$ (**Scheme 30**). After purification by silica gel column chromatography, the product was isolated as a dark brown oil in 10% yield. The majority of the starting material was recovered in 69%.

The ^1H NMR spectrum of 2-allyl-4-bromoaniline **75** varies significantly from the starting material in the aromatic region because of the loss in symmetry after the rearrangement. H-3 and H-5 give rise to the multiplet observed at 7.20 – 7.10 ppm integrating for two protons, while H-6 is responsible for the doublet at 6.55 ppm integrating for one proton. The characteristic alkene signals observed due to the allyl group are observed at 5.91 ppm for the =CH and 5.23 – 5.01 ppm for the $=CH_2$. The doublet in the aliphatic region at 3.25 ppm is due to the allyl CH_2 group. The presence of the singlet observed at 3.66 ppm integrating for two protons is further evidence to prove that the aza-Cope rearrangement was successful because the signal is brought about by the NH_2 group formed during the reaction. Nine signals were observed in the ^{13}C NMR spectrum of the product as expected. The eight downfield signals are due to the six phenyl carbons and the two alkene carbons. The signal observed in the aliphatic region is due to the allyl CH_2 group.

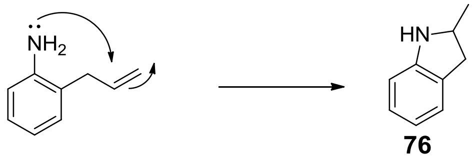
2.4.5 Synthesis of 2-methylindoline



76

Scheme 31. Reagents and conditions: $BF_3.OEt_2$, xylene, 170 °C, 3 days, 65%

Having successfully carried out the low yielding aza-Cope rearrangement, we attempted to improve the conversion of the reaction and thereby improve yields of the product. Initially, we did this by raising the reaction temperature and leaving the reaction for extended periods of time. As before, the starting material was dissolved in xylene and the reaction carried out in the presence of $BF_3.OEt_2$ (**Scheme 31**). The oil bath temperature was set at ~170 °C and the mixture was left stirring over three days. After this time, the product was purified by silica gel column chromatography and the newly formed product was isolated as a dark brown oil in 65% yield. To our surprise, the product isolated was not the expected 2-allylaniline, but rather 2-methylindoline **76**. Yadav *et al*. also reported similar ring-closure products when they attempted the aza-Cope rearrangement on a variety of *N*-allylanilines using a Zn^+ montmorillonite catalyst.[50] Formation of **76** seems to be favoured at higher temperatures and extended periods of time. The ring-closure reaction can be explained using Baldwin's rules of ring-closure, a set of guidelines for relative rates of ring-closure.[51,52] They represent the free energy of activation at the transition state necessary for formation of the ring. The activation energy depends on the size of the ring formed, hybridization state of the carbon atom undergoing the ring-closure and its interaction with the nucleophile to achieve advantageous orbital overlap and thereby forming new bonds.[51] According to Baldwin's rules, the ring system resulting from the *aza*-Cope reaction is 5-exo-trig favored. We propose that the ring-closure occurs after the aza-Cope rearrangement of the *N*-allylaniline to 2-allylaniline (**Scheme 32**). Lone pairs on the amino group are nucleophilic and therefore attack the alkene CH in the presence of $BF_3.OEt_2$ giving rise to **76**.

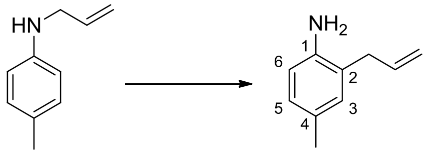


76

Scheme 32. Proposed mechanism for the ring-closure

The ^{1}H NMR spectrum validates the formation of **76** due to the four observed signals in the aromatic region, each integrating for one proton. The multiplet at 7.08 – 7.03 ppm is due to H-4, while the two triplet of doublets at 6.99 ppm and 6.66 ppm appear as a result of H-6 and H-5 respectively and H-7 is responsible for the doublet at 6.57 ppm. A doublet integrating for three protons at 1.23 ppm is observed due to the presence of the methyl group. The multiplet at 4.01 – 3.83 ppm integrating for one proton is due to the indoline CH group, and that observed at 3.16 – 2.54 ppm is due to the CH_2 group. Lastly, the NH group gave rise to the singlet observed at 3.69 ppm integrating for one proton. Nine signals were observed in the ^{13}C NMR spectrum of the product; six in the aromatic region as expected and three in the aliphatic region, owing to the CH group at 55.2, CH_2 at 37.7 ppm and the CH_3 at 22.2 ppm. The molecular ion was confirmed by HRMS to be $[M+H]^+$ 134.0987 which was consistent with the mass calculated for $C_9H_{12}N$ of 134.0970.

2.4.6 Synthesis of 2-allyl-4-methylaniline



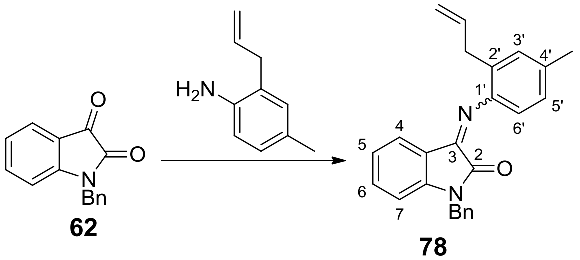
77

Scheme 33. Reagents and conditions: $BF_3.OEt_2$, Chlorobenzene, 120 °C, 3 days 18%

Other methods employed to improve the yield of the aza-Cope rearrangement included changing the solvent. Use of chlorobenzene gave the desired aza-Cope rearranged product in 18% yield. However, it must be noted that the reaction was left for 3 days and did not yield the ring-closure product as seen above. The reaction was carried out at 120 °C, so it is possible that the reaction temperature might have not been high enough to facilitate the ring-closure transformation.

The multiplet at 6.91 – 6.80 ppm, integrating for two protons, in the ^1H NMR spectrum was assigned to H-3 and H-5. The doublet visible at 6.56 ppm results from H-6 in the phenyl group. The ^1H NMR spectrum further confirmed success of the aza-Cope rearrangement due to the presence of the singlet at 3.57 ppm resulting from the amino group. Two multiplets were visible at 6.08 – 5.79 ppm and 5.27 – 4.94 ppm due to the presence of the alkene protons from the allyl group. The ^{13}C NMR spectrum also confirmed formation of the product due to the ten observed signals, as expected. The eight signals observed in the region 142.2 – 115.9 ppm were due to the phenyl ring and the two carbon atoms of the terminal alkene. The other two signals are observed in the aliphatic region owing to the presence of the allyl CH_2 and the methyl group at the 4-position of the aniline.

2.4.7 Synthesis of 3-((2-allyl-4-methylphenyl)imino)-1-benzylindolin-2-one



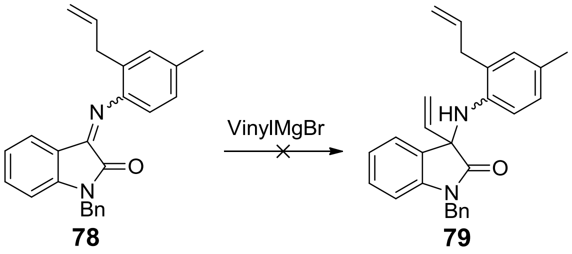
Scheme 34. Reagents and conditions: Gl. acetic acid, CH_3CN, reflux, 38%

With the aza-Cope product **77** in hand, we were ready to test the imine formation with our benzyl protected isatin. As already mentioned above, it would be preferable to avoid interaction with indole N-H during the nucleophilic addition step, hence benzyl-protected isatin was used as a starting material for the synthesis of aryl-imine **78** (**Scheme 34**). The benzyl protecting group was selected for this route because of the reasonable yield obtained during the allyl addition to 1-benzyl-3-((4-chlorophenyl)-imino)indolin-2-one

65a to produce **66** (**Scheme 16**). Bn-isatin **62** and 2-allyl-4-methylaniline **77** were dissolved in acetonitrile and acetic acid was added as a catalyst. The reaction mixture was stirred overnight under reflux. After extraction and purification by silica gel column chromatography, the product was isolated as a red oil in 38% yield.

Success of the imine condensation reaction was confirmed by ^{1}H NMR spectroscopy. The characteristic Bn-protons gave rise to a multiplet at 7.42 – 7.27 ppm integrating for five protons and the benzylic CH_2 is responsible for the singlet at 5.01 ppm. Two multiplets at 5.87 – 5.72 ppm and 4.98 – 4.81 ppm are due to the presence of the alkene CH and CH_2 groups respectively, while the doublet of doublets at 3.31 ppm, integrating for two protons is due to the allyl CH_2 group. The presence of the C=N signal at 154.2 ppm in the ^{13}C NMR spectrum further supports formation of the imine product. A strong C=N signal at 1638 cm^{-1} in the IR spectrum is also indicative of the desired imine functionality. The alkene =C-H stretch is observed as a signal at 3061 cm^{-1}. The molecular ion was confirmed by HRMS to be $[M+H]^+$ 367.1805 which was consistent with the mass calculated for $C_{25}H_{23}N_2O$ of 367.1810.

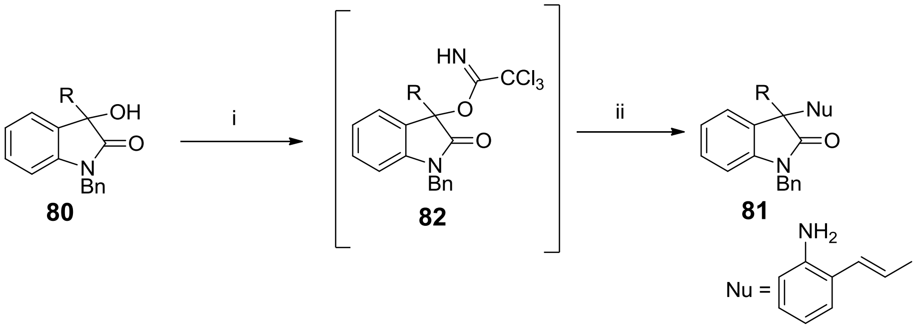
2.4.8 Synthesis of 3-((2-allyl-4-methylphenyl)amino)-1-benzyl-3-vinylindolin-2-one



Scheme 35. Reagents and conditions: $MgBr_2$, DCM, -40 °C – rt

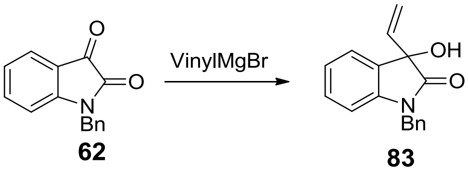
The next test reaction was the nucleophilic addition reaction of vinylmagnesium bromide to the imine **78** (**Scheme 35**). The reaction was carried out in a similar manner as described in **Scheme 19**. The nucleophilic addition of the vinyl group would have resulted in the production of the intermediate necessary for the ring-closure reaction using ring-closing metathesis. Unfortunately, the reaction was not successful and most of the starting material was recovered. Grignard reactions with vinylmagnesium bromide and imines are known to be difficult.

Since we were unable to synthesize the intermediate **79** required for ring-closing metathesis, other alternative routes towards the synthesis of the ring-closed analogues were investigated. A one-pot conversion of 3-hydroxyoxindoles **80** to 3,3-disubstituted oxindole **81** has been reported (**Scheme 36**)[53] and this sparked our interest because we could utilize this method to synthesize the ring-closure intermediate. The 3-hydroxyoxinole **80** can be synthesized by a Grignard reaction between protected isatin and vinylmagnesium bromide. The nucleophilic Grignard reagent will add to the carbonyl carbon atom at the C-3 position of the oxindole core. Since carbonyls are strong electrophiles, this will eliminate problems encountered during the nucleophilic addition reaction on the imine carbon atom. Formation of the 3,3-disubstituted oxindoles requires nucleophilic substitution of the hydroxyl group, which is not a good leaving group. The hydroxyl group is converted to a trichloroacetimidate prepared from trichloroacetonitrile and 1.8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DCM to afford **82**.



Scheme 36. Nucleophilic substitution of 3-hydroxyoxindole. Reagents and conditions: (i) DBU, Cl_3CCN, DCM, rt, (ii) TFA, Nu

2.4.9 Synthesis of 1-benzyl-3-hydroxy-3-vinylindolin-2-one

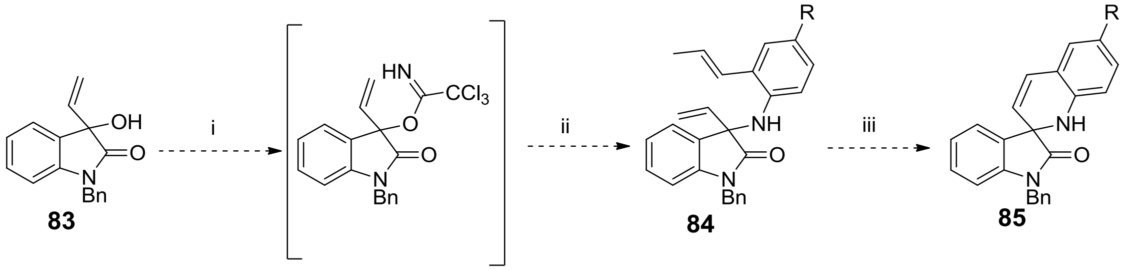


Scheme 37. Reagents and conditions: THF, -40 °C – rt, 82%

The 3-hydroxyoxindole **83** was prepared by reacting Bn-isatin **62** with vinylmagnesium bromide in THF (**Scheme 37**). The Grignard reagent was added to the Bn-isatin solution at -40 ^{o}C. After stirring the reaction mixture at this temperature for an hour, the reaction temperature was raised to room temperature overnight under a N_2 atmosphere. Bn-isatin impurities were removed by silica gel column chromatography and the product isolated as orange crystals in 82% yield.

Appearance of the signal arising due to the OH group at 5.02 – 4.63 ppm in the ^{1}H NMR spectrum indicates successful synthesis of the 3-hydroxyoxindole resulting from addition of the vinyl group. Two signals were observed due to the presence of the vinyl group; one at 6.09 ppm due to the CH group and the other at 5.47 – 5.21 ppm due to the terminal $=CH_2$. The characteristic benzylic protons were observed at 7.29 – 7.18 ppm as a multiplet integrating for five protons. The ^{13}C NMR spectrum confirmed formation of the product due to the presence of the signal at 77.2 ppm arising because of the newly formed quaternary carbon. A broad signal observed at 3314 cm^{-1} in the IR spectrum of the product confirmed the presence of an O-H group.

Due to unforeseen circumstances and time limitations, we were unable to carry out the nucleophilic 3,3-disubstition reaction on compound **83** in order to prepare **84** (**Scheme 38**). The reaction goes via an *in situ* trichloroacetimidate intermediate prepared from trichloroacetonitrile and DBU in DCM, which is then subjected to nucleophilic substitution with *para* substituted 2-(1-propenyl)anilines derived from the aza-Cope rearrangement. Successful synthesis of compound **84** could have enabled us to test the ring-closure reaction forming a 6-membered ring via ring-closing metathesis. This will be considered for future work.



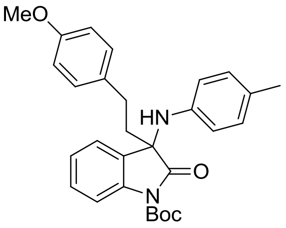
Scheme 38. Reagents and conditions: (i) DBU, Cl_3CCN, DCM, rt, (ii) TFA, *para* substituted 2-(1-propenyl)anilines, (iii) Grubbs G2 cat., Toluene, reflux

2.5 Antimalarial screening results for ring-opened analogues

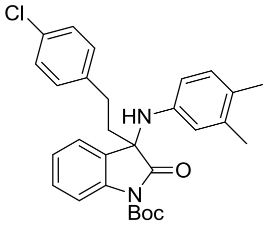
Ring-opened analogues **45-61** were tested for antimalarial activity in an *in vitro P. falciparum* screen with the Boc-group present. The screening was conducted by Prof R van Zyl at the Wits School of Pharmacy and Pharmacology. Dihydroartemisinin (DHA) and quinine were used in positive controls. The results for the biological screening are presented in **Table 24** below.

Table 24. Results for *in vitro* antimalarial screening against *P. falciparum* for **45-61**.

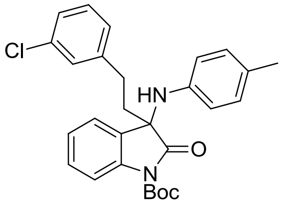
	% Parasitaemia				
Compounds	Average % growth	SD	% Kill	IC50 (µM)	SD
45	55.89	± 2.64	44.11		
46	54.70	± 5.31	45.30		
47	26.21	± 4.17	73.79	0.71	± 0.124
48	57.83	± 5.19	42.17		
49	50.28	± 5.80	49.72		
50	54.62	± 0.39	45.38		
51	54.18	± 6.01	45.82		
52	41.17	± 0.62	58.83	1.48	± 0.349
53	52.21	± 3.71	47.79		
54	50.88	± 10.98	49.12		
55	43.53	± 1.55	56.47	0.00472	± 0.00056
56	47.70	± 3.59	52.30		
57	34.80	± 3.58	65.20	1.73	± 0.287
58	39.93	± 0.18	60.07	1.97	± 0.386
59	66.67	± 8.27	33.33		
60	39.99	± 3.46	60.01	0.000602	± 0.0000229
61	49.08	± 3.62	50.92		
DHA	17.05	± 10.38	82.95	0.64	± 0.013
Quinine	14.06	± 2.58	85.94	0.01	± 0.00



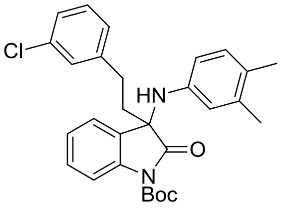
47
0.71 uM



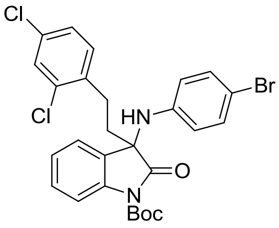
52
1.48 uM



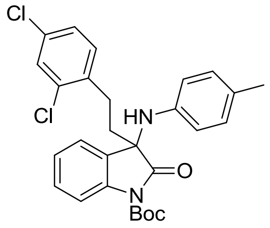
55
0.00472 uM



57
1.73 uM



58
1.97 uM



60
0.00060 uM

Figure 19. Hit compounds which display most potent antimalarial activity against *P. falciparum in vitro*.

The average percentage growth of the parasite was determined in the presence of the test compounds at an initial concentration of 5 µM, and IC_{50} values were determined for those compounds which displayed the highest percentage kill, of above 55%. Compound **60** was the most active with an IC_{50} value of 0.60 nM.

Six hit compounds were identified from the series of compounds tested and there is a clear structure-activity relationship observed on the active compounds. Those derived from *p*-toluidine, i.e. **47**, **55** and **60**, displayed the highest antimalarial activity with IC_{50} values of 0.71 µM, 0.00472 µM and 0.000602 µM respectively (**Figure 19**). Compound **57** varies from **55** structurally in that it contains an additional methyl group as it is derived from 3,4-dimethylaniline. The presence of the additional methylene substituent resulted in a significant decrease in biological activity with an IC_{50} value of 1.73 µM reported for **57**. Despite this, the flexibility of the ring-opened analogues synthesised in this project appears to have had a significant impact on antimalarial activity. The antimalarial activities of the six hit compounds described here are better in comparison to the previously synthesized spirooxindoles, upon which this project is based. The most potent compound prepared previously had an IC_{50} value of ~5 µM.

3 CONCLUSION AND FUTURE WORK

3.1 Conclusion

In conclusion, a synthetic route towards the synthesis of ring-opened spirooxindoles was developed and carried out successfully. The target compounds were synthesized by an initial condensation reaction between commercially available isatin and *para* substituted anilines. The aryl-imines were afforded as a mixture of *Z* and *E* isomers and this was evident due to doubling of signals observed in the ^{1}H NMR and ^{13}C NMR spectra of the products. The *E*-stereochemistry gave rise to the major isomer, as determined by 2D NOESY experiments showing strong correlation between H-4 and H-2' (**Figure 19**).

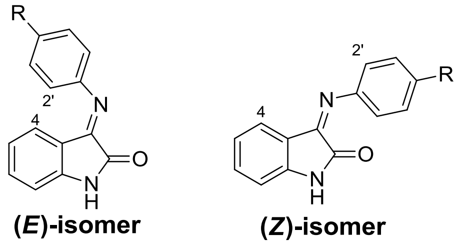


Figure 19. Structural representation of the *E* and *Z* isomers of the aryl-imines

To prevent interference by the oxindole NH during the subsequent nucleophilic addition step, the NH-group was protected using a *tert*-butoxycarbonyl group. The Boc-protected aryl-imines **44a – 44e** were isolated in high yields ranging from 74-98%.

With these results in hand, the nucleophilic addition reaction to the Boc-protected aryl imines was conducted with various Grignard reagents derived from substituted phenethyl bromides. This gave rise to the desired *N*-Boc-protected ring-opened analogues **45-61**. As imines are relatively weak electrophiles, a mild Lewis acid ($MgBr_2$) was used to activate the imine in this step. The flexible ring-opened analogues were isolated as a racemic mixture in yields ranging from 10-53%. The last step of the synthesis was removal of the Boc protecting group, which proved to be more challenging than expected. Several conventional and unconventional methods were attempted to remove the Boc group, including, hydrolysis by weak and strong acids. However, we were unsuccessful in isolating any deprotected products.

Considering that the nucleophilic addition step in the synthesis of the ring-opened analogues was low yielding in general, and removing the Boc-protecting group was unsuccessful, we thought to explore the use of other protecting groups. Benzyl and *p*-methoxybenzyl protecting groups were chosen as they are widely used for the protection of the indole nitrogen. The nucleophilic Grignard reaction was first tested on 1-benzyl-3-((4-chlorophenyl)-imino)indolin-2-one **65a** using commercially available allylMgBr in the presence of $MgBr_2$, and gave rise to the desired product 3-allyl-1-benzyl-3-((4-chlorophenyl)amino)indolin-2-one **66** in 52% yield. Having successfully conducted the nucleophilic addition reaction using a commercially available Grignard reagent, we were optimistic to attempt the Grignard reaction on Benzyl and PMB-protected aryl-imines using a Grignard reagent derived from 4-methoxyphenethyl bromide and magnesium; unfortunately the Grignard reaction was unsuccessful when $MgBr_2$ was used as a Lewis acid. Introducing a stronger Lewis acid, $BF_3.OEt_2$, resulted in formation of the desired ring-opened analogues 1-benzyl-3-((4-chlorophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one **67** and 1-benzyl-3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one **68**, however the yields were very low ranging from 6-12%. Changing the solvent from THF to DCM slightly increased the yield of 1-benzyl-3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one **68** from 6% to 11%. Therefore, although we were able to synthesize the ring-opened analogues using Bn-protected aryl imines, the nucleophilic addition step was low yielding and other alternative routes should be explored to synthesize ring-opened analogues by employing a protecting group that will not affect the efficiency of the nucleophilic addition step and readily be removed.

The second series of compounds we aimed to synthesize were the ring closed analogues. The route towards synthesis of the ring closed analogues was initiated by chemical conversion of substituted anilines to 2-allylanilines using the aza-Cope rearrangement. The aza-Cope transformation was first tested on *N*-allyl-4-bromoaniline **74a**, which was treated with $BF_3.OEt_2$ in xylene at 140 ^{o}C and the product **75** was isolated in 10 % yield. Unfortunately, increasing the temperature of the reaction to170 ^{o}C led to the formation of 2-methylindoline **76** in 65% yield. Another method employed in an attempt to improve the yield of the aza-Cope rearrangement included changing the solvent to chlorobenzene. The desired aza-Cope rearrangement product **77** was afforded in 18% yield. Compound **77** was subjected to an imine condensation reaction with benzyl-isatin to yield the aryl-imine **78**. This in turn was reacted with vinylMgBr to synthesize the intermediate required for the ring-closing metathesis step, but the reaction was unsuccessful. Since we were unable to synthesize the intermediate required for ring-closing metathesis other alternative routes towards the synthesis of the ring-closed analogues were investigated. One of the methods was a nucleophilic 3,3-disubstition reaction involving Grignard addition to isatin; conversion to a trichloroacetimidate displaced by *N* nucleophilic 2-(1-propenyl)anilines. Due to time limitations we were unable to test this route. Although we were unable to reach the final ring closure step for the synthesis, significant progress has been made towards the synthesis of the target compounds.

The antimalarial activity of the prepared ring-opened analogues was evaluated in an *in vitro* screen against *P. falciparum* using DHA and quinine as positive controls. Six hit compounds were identified from the study, with 3-(2,4-dichlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **60** being the most active with an IC_{50} value 0.6 nM against the FCR 3 strain. From the 6 hit compounds, those derived from *p*-toluidine were the most potent, with IC_{50} values reported in the nanomolar range for *tert*-butyl 3-(3-chlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **55** and 3-(2,4-dichlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **60**. *tert*-Butyl 3-(3-chlorophenethyl)-3-((3,4-dimethylphenyl)amino)-2-oxoindoline-1-carboxylate **57** varies from *tert*-butyl 3-(3-chlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **55** structurally in that it contains an additional methyl group as it is derived from 3,4-dimethylaniline. Having the additional methyl group resulted in significant reduction in activity. In conclusion, we have synthesised compounds with activity in the nanomolar range against *P. falciparum in vitro* by adding an element of flexibility to the hit compounds on which this project is based, with some compounds several orders of magnitude more potent than the original hit compounds (IC_{50} values ~ 5 µM).

3.2 Future work

The Grignard reaction was successful in the course of synthesizing the ring-opened analogues; however we were unable to remove the Boc protecting group from the final products. Therefore, for future purposes it would be essential to try other protecting groups which will be removed more easily without affecting efficiency of the nucleophilic addition reaction. As, the synthesised compounds are obtained as a racemic mixture; methodology for the enantioselective synthesis of the compounds should be considered. Second generation analogues should also be developed from the active hit compound **60** derived from *p*-toluidine.

We plan to complete the synthetic route towards the ring-closed analogues using either the Grignard reaction or the 3,3-disubstitution reaction for preparation of the intermediate required for ring-closing metathesis. Then the ring-closure reaction could then be facilitated by ring-closing metathesis, thereby forming the desired ring-closed compounds.

4 EXPERIMENTAL

4.1 General Procedures

4.1.1 Purification of solvents and reagents

All reagents were purchased from Sigma-Aldrich (South Africa) and used as received. Solvents used for reactions were distilled over their respective appropriate drying agents under inert environments. THF was dried over sodium wire in the presence of an indicator, benzophenone. DCM and DMF were distilled from calcium hydride. DMF was later stored over activated molecular sieves (4Å) under N_2 atmosphere. Absolute ethanol was obtained from commercial sources without any further purification. Solvents for column chromatography (ethyl acetate and hexane) were purified by distillation.

Chromatography

Thin Layer Chromatography (TLC) was carried out using Merck aluminium foil F_{254} backed plates coated with silica gel 60. Normal column chromatography was performed on Macherey-Nagel silica gel 60 particle size 0.063 – 0.200 mm and Merck silica gel of particle size 0.035 – 0.070 mm was employed for flash columns.

Spectroscopic and physical data

All melting points were determined using a Stuart SMP10 melting point apparatus and are uncorrected.

^1H and ^{13}C Nuclear Magnetic Resonance data were recorded on Bruker 300 or 500 MHz spectrometer using specified deuterated solvents. For those compounds soluble in deuterated chloroform ($CDCl_3$), chemical shifts were referenced against the internal standard tetramethyl silane (TMS) which occurs at zero parts per million. Chemical shifts are recorded in ppm, while coupling constants are recorded in Hertz.

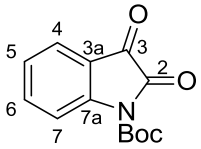
Infrared spectra were acquired from a Bruker Tensor-27 Fourier Transform spectrometer. Measurements are made by directly loading samples to the diamond cell. Signals are reported on the wavenumber scale (v, cm^{-1}).

High Resolution Mass Spectra (HRMS) were obtained on a SYNAPT G2 HDMS mass spectrometer (ESI) at Stellenbosch University.

4.2 Synthesis of the ring-opened analogues

4.2.1 Synthesis of *tert*-butyl 2,3-dioxoindoline-1-carboxylate 40[54,55]

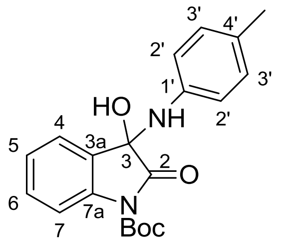
DMAP (0.120 g, 1.02 mmol) was added to a solution of isatin (3.00 g, 20.7 mmol) in anhydrous THF (100 ml), followed by a slow addition of di-*tert*-butyl dicarbonate (4.90 g, 23.1 mmol) to the heterogeneous mixture. After stirring the yellow solution at room temperature for 6 hours under an argon atmosphere, water (50 ml) was added and the product precipitated out. The yellow paste-like residue afforded after filtration was further purified by recrystallization in DCM/hexane yielding *tert*-butyl 2,3-dioxoindoline-1-carboxylate **40** as a yellow solid (2.54 g, 50%)



R_f = 0.81 (3:7 EtOAc/Hex); **m.p.**: 144-145 °C (lit. m.p. 132 °C)[55]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.08 (1H, d, *J* = 8.4 Hz, H-7), 7.74 (1H, dd, *J*=7.6, 1.4 Hz, H-4), 7.70 (1H, ddd, *J*=8.5, 7.6, 1.5 Hz, H-6), 7.31 – 7.26 (1H, m, H-5), 1.66 (9H, s, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 180.3 (C-3), 155.7 (C-2), 148.4 (Boc C=O and C-7a), 138.8 (C-6), 125.4 (C-5), 125.3 (C-4), 118.7 (C-3a), 116.8 (C-7), 85.6 ($(CH_3)_3\underline{C}$), 28.0 ($(\underline{C}H_3)_3C$).

4.2.2 General synthesis of *tert*-butyl 3-hydroxy-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate 41[37]

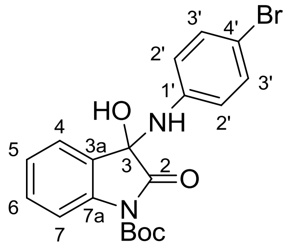
tert-Butyl 2,3-dioxoindoline-1-carboxylate **40** (0.500 g, 2.00 mmol) and *p*-toluidine (0.260 g, 2.43 mmol) were dissolved in dry CH_2Cl_2 (10 ml) in a round bottom flask equipped with a stirrer bar. Then $MgSO_4$ (1.95 g, 16.2 mmol) was added to absorb water produced during imine formation. The orange heterogeneous mixture was stirred overnight under argon at room temperature and filtered through a pad of Celite. The filtrate was concentrated using a rotary evaporator and the resulting residue was further purified by silica gel column chromatography (20% EtOAc/Hex) to afford *tert*-butyl 3-hydroxy-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **41** as a lime green solid (0.160 g, 22%).



R$_f$ = 0.67 (2:8 EtOAc/Hex); **m.p.** 143-145 ^{o}C; **IR** ν$_{max}$ (cm^{-1}): 3270 (NH), 3130 (OH), 2935 (C-H), 1702 (C=O); **^{1}H NMR** (500 MHz, CDCl$_3$) δ 10.20 (1H, s, NH), 8.70 (1H, s, OH), 8.57 (1H, dd, *J* = 8.1, 1.6 Hz, H-4), 8.47 – 8.42 (1H, m, H-7), 7.60 (1H, ddd, *J* = 8.7, 7.2, 1.6 Hz, H-6), 7.55 (2H, d, *J* = 8.1 Hz, H-3'), 7.20 (2H, d, *J* = 8.1 Hz, H-2'), 7.07 (1H, td, *J* = 7.6, 1.2 Hz, H-5), 2.35 (3H, s, CH$_3$), 1.54 (9H, s, (CH$_3$)$_3$C); **^{13}C NMR** (126 MHz, CDCl$_3$) δ 190.3 (C-2), 159.7 (Boc C=O), 152.7 (Ar-C), 143.3 (Ar-C), 136.5 (C-6), 135.2 (Ar-C), 134.5 (C-4), 134.1 (Ar-C), 129.8 (C-2'), 121.2 (C-5), 120.0 (C-3'), 119.3 (C-7), 118.3 (C-3), 81.1 ((CH$_3$)$_3$$\underline{C}$), 28.3 (($\underline{C}H_3$)$_3$C), 21.0 ($\underline{C}H_3$); **HRMS** (m/z), calculated for $C_{20}H_{23}N_2O_4$: 355.1658, found (M + H)$^+$: 355.1652

4.2.3 Synthesis of *tert*-butyl 3-((4-bromophenyl)amino)-3-hydroxy-2-oxoindoline-1-carboxylate 42

tert-Butyl 3-((4-bromophenyl)amino)-3-hydroxy-2-oxoindoline-1-carboxylate **42** was synthesized following the procedure described above for compound **41**. *tert*-Butyl 2,3-dioxoindoline-1-carboxylate **40** (1.00 g, 4.05 mmol) was dissolved in DCM (20 ml) followed by addition of *p*-bromoaniline (0.840 g, 4.85 mmol) and MgSO$_4$ (3.90 g, 32.4 mmol) to yield a bright yellow solid (0.760 g, 43%).

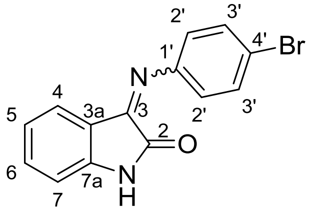


R$_f$ = 0.72 (2:8 EtOAc/Hex); **m.p.** 154 ^{o}C; **IR** ν$_{max}$ (cm^{-1}): 3269 (NH), 3127 (OH), 2938 (C-H), 1701 (C=O); **^{1}H NMR** (500 MHz, CDCl$_3$) δ 10.15 (1H, s, NH), 8.84 (1H, s, OH), 8.51 (1H, dd, *J* = 8.2, 1.7 Hz, H-4), 8.42 (1H, dd, *J* = 8.6, 1.2 Hz, H-7), 7.63 – 7.55 (3H, m, H-6, H-2'), 7.54 – 7.46 (2H, m, H-3'), 7.06 (1H, ddd, *J* = 8.3, 7.2, 1.2 Hz, H-5), 1.54 (9H, s, (CH$_3$)$_3$C); **^{13}C NMR** (126 MHz, CDCl$_3$) δ 189.7 (C-2), 159.7 (Boc C=O), 152.7 (Ar-C), 143.4 (Ar-C), 136.7 (C-6), 135.7 (Ar-C), 134.4 (C-4), 132.3 (C-3'), 121.5 (C-2'), 121.3 (C-5), 119.3 (C-7), 118.2 (C-3), 118.1 (Ar-C), 81.2 ((CH$_3$)$_3$$\underline{C}$), 28.3 (($\underline{C}H_3$)$_3$C). **HRMS** (m/z), calculated for $C_{19}H_{20}{}^{79}BrN_2O_4$: 419.0606, found (M + H)$^+$: 419.0608

4.3 General procedure for the synthesis of aryl-imines

4.3.1 Synthesis of 3-((4-bromophenyl)imino)indolin-2-one 43a[56,57]

Isatin (3.00g, 20.4 mmol) and *p*-bromoaniline (3.50 g, 20.4 mmol) were dissolved in absolute ethanol (120 ml), then glacial acetic acid (0.82 ml) was added as a catalyst. The resulting bright orange heterogeneous mixture was stirred at reflux (100 °C) under a nitrogen atmosphere for 5 hours. The reaction was then concentrated by removal of the solvent to allow for the product to precipitate out of solution. The mixture was filtered and the filter cake was further purified by recrystallization in ethanol to afford 3-((4-bromophenyl)imino)indolin-2-one **43a** as an orange solid (4.05 g, 66%) as a mixture of isomers (*E:Z* = 4:1).



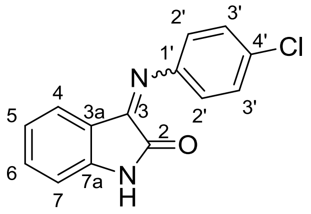
R_f = 0.12 (3:7 EtOAc/Hex); **m.p.** 278-279 °C (lit. m.p. 270 °C)[57]; **^{1}H NMR** (300 MHz, DMSO-d_6) ***E* Isomer** δ 10.97 (1H, s, NH), 7.69 – 7.61 (2H, m, H-3’), 7.35 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 6.97 (2H, d, *J* = 9.4 Hz, H-2’), 6.89 (1H, d, *J* = 7.9 Hz, H-7), 6.77 (1H, td, *J* = 7.7, 1.1 Hz, H-5), 6.43 (1H, dd, *J* = 7.8, 1.2 Hz, H-4); ***Z* Isomer** δ 10.97 (1H, s, N-H), 7.61 – 7.55 (1H, m, H-4), 7.51 – 7.41 (3H, m, H-3’ and H-6), 7.06 (1H, td, *J* = 7.6, 1.0 Hz, H-5), 6.97 (2H, d, *J* = 9.4Hz, H-2’), 6.95 – 6.82 (1H, m, H-7); **^{13}C NMR** (75 MHz, DMSO-d_6) ***E* Isomer** δ 163.3 (C=O), 155.3 (C=N), 149.6 (Ar-C), 147.1 (Ar-C), 134.7 (C-6), 132.4 (C-3’), 125.4 (C-4), 121.8 (C-5), 119.7 (C-2’), 117.1 (Ar-C), 115.6 (Ar-C), 111.6 (C-7); ***Z* Isomer** δ 158.5 (C=O), 153.5 (C=N), 148.4 (Ar-C), 145.8 (Ar-C), 134.4 (C-6), 131.1 (C-3’), 122.9 (C-4), 122.3 (C-5), 121.2 (C-2’), 116.6 (Ar-C), 115.6 (Ar-C), 110.8 (C-7); **HRMS** (m/z), calculated for $C_{14}H_{10}{}^{79}BrN_2O$: 300.9977, found $(M + H)^+$: 300.9969.

The following compounds were synthesised using the procedure described above for 3-((4-bromophenyl)imino)indolin-2-one **43a**.

4.3.2 Synthesis of 3-((4-chlorophenyl)imino)indolin-2-one 43b[58]

Isatin (2.00 g, 13.6 mmol) and *p*-chloroaniline (1.73 g, 13.6 mmol) were treated with gl. acetic acid (0.52 ml) in ethanol (120 ml) to afford an isomeric mixture of 3-((4-

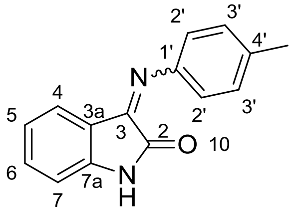
chlorophenyl)imino)indolin-2-one **43b** as an orange solid (2.54 g, 72%), with isomeric ratio of *E:Z* = 2.3:1.



R*f* = 0.22 (3:7 EtOAc/Hex); **m.p.** 268-279 °C (lit. m.p. 240 °C)[58]; **^{1}H NMR** (300 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 10.98 (1H, s, NH), 7.56 – 7.47 (2H, m, H-3'), 7.36 (1H, td, *J* = 7.6, 1.2 Hz, H-6), 7.09 – 7.00 (2H, m, H-2'), 6.90 (1H, d, *J* = 7.9 Hz, H-7), 6.77 (1H, td, *J* = 7.7, 1.0 Hz, H-5), 6.44 (1H, dd, *J* = 7.8, 1.2 Hz, H-4); ***Z* Isomer** δ 10.98 (1H, s, NH), 7.58 (1H, dd, *J* = 7.6, 1.2 Hz, H-4), 7.45 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.37 (2H,d, *J* = 7.8 Hz, H-3' overlapped with H-6 of *E* isomer), 7.11 – 7.00 (3H, m, H-5 and H-2' overlapped with H-2' of *E* isomer), 6.88 (1H, d, *J* = 9.9 Hz, H-7); **^{13}C NMR** (75 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 163.3 (C=O), 155.4 (C=N), 149.2 (Ar-C), 147.1 (Ar-C), 134.7 (C-6), 129.6 (C-3'), 129.0 (Ar-C), 125.4 (C-4), 121.8 (5), 119.4 (C-2'), 115.6 (Ar-C), 111.6 (C-7); ***Z* Isomer** δ 158.5 (C=O), 153.6 (C=N), 148.0 (Ar-C), 145.8 (Ar-C), 134.4 (C-6), 128.5 (Ar-C), 128.2 (C-3'), 122.9 (C-4), 122.3 (C-5), 121.2 (Ar-C), 120.9 (C-2'), 110.8 (C-7); **HRMS** (m/z), calculated for $C_{14}H_{10}{}^{35}ClN_2O$: 257.0482, found $(M + H)^+$: 257.0472

4.3.3 Synthesis of 3-(*p*-tolylimino)indolin-2-one 43c[58]

Synthesis of 3-(*p*-tolylimino)indolin-2-one **43c** was carried out by treating isatin (3.00 g, 20.4 mmol) and *p*-toluidine (2.19 g, 20.4 mmol) with gl. acetic acid (0.780 ml) in ethanol (130 ml). 3-(*p*-tolylimino)indolin-2-one **43c** was isolated as a mixture of *E:Z* isomers in a ratio of 3:1 as a yellow solid (2.94 g, 61%).

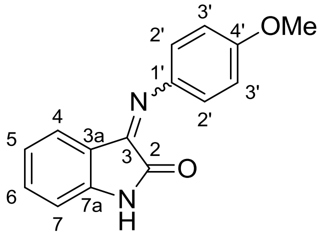


R*f* = 0.47 (3:7 EtOAc/Hex); **m.p.** 232-233 °C (lit. m.p. 222 °C)[58]; **^{1}H NMR** (300 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 10.97 (1H, s, NH), 7.38 – 7.22 (3H, m, H-6 and H-3'), 6.93 – 6.85 (3H, m, H-7 and H-2'), 6.72 (1H, td, *J* = 7.7, 1.0 Hz, H-5), 6.49 (1H, dd, *J* = 7.8, 1.2 Hz, H-4), 2.35 (3H, s, CH_3); ***Z* isomer** δ 10.85 (1H, s, NH), 7.57 (1H, dd, *J* = 7.5, 1.2 Hz, H-4), 7.43 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.11 (2H, d, *J* = 8.0 Hz, H-3'), 7.05 (1H, td, *J* = 7.6, 0.9 Hz, H-5), 6.99 – 6.92 (2H, m, H-2'), 6.91 – 6.81 (1H, m, H-7 overlapped with H-2' of E-isomer), 2.30 (3H, s, CH_3); **^{13}C NMR** (75 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 163.5

(C=O), 154.7 (C=N), 147.8 (Ar-C), 146.9 (Ar-C), 134.2 (C-6), 134.1 (Ar-C), 129.9 (C-3'), 125.2 (C-4), 121.6 (C-5), 117.4 (C-2'), 115.7 (Ar-C), 111.4 (C-7), 20.5 (CH_3); ***Z* Isomer** δ 158.4 (C=O), 152.5 (C=N), 146.1 (Ar-C), 145.4 (Ar-C), 133.9 (C-6), 133.9 (Ar-C), 128.7 (C-3'), 122.5 (C-4), 122.2 (C-5), 119.6 (C-2'), 115.7 (Ar-C), 110.6 (C-7), 20.5 (CH_3), **HRMS** (m/z), calculated for $C_{15}H_{13}N_2O$: 237.1028, found $(M + H)^+$: 237.1022

4.3.4 Synthesis of 3-((4-methoxyphenyl)imino)indolin-2-one 43d[57]

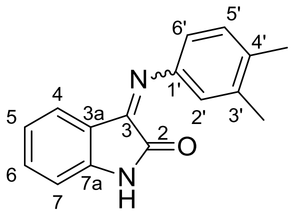
Isatin (5.00 g, 34.0 mmol) and *p*-anisidine (4.18 g, 34.0 mmol) in the presence of gl. acetic acid (1.30 ml) and ethanol (150 ml) afforded 3-((4-methoxyphenyl)imino)indolin-2-one **43d**, isolated as an orange solid (8.06 g, 94%) in an isomeric mixture of ratio *E:Z* = 4:1.



R_f = 0.16 (3:7 EtOAc/Hex); **m.p.** 241-242 °C (lit. m.p. 230 °C)[57]; **^{1}H NMR** (300 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 10.95 (1H, s, NH), 7.33 (1H, td, *J* = 7.7, 1.4 Hz, H-6), 7.10 – 6.94 (4H, m, H-2' and H-3'), 6.94 – 6.86 (1H, m, H-7), 6.75 (1H, td, *J* = 7.6, 1.0 Hz, H-5), 6.66 (1H, dd, *J* = 7.8, 1.3 Hz, H-4), 3.80 (3H, s, OCH_3); ***Z* Isomer** δ 10.87 (1H, s, NH), 7.55 (1H, dd, *J* = 7.6, 1.2 Hz, H-4), 7.41 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.23 – 7.15 (2H, m, H-3'), 7.11 – 6.94 (3H, m, H-2' and H-5), 6.85 (1H, d, *J* = 7.8 Hz, H-7), 3.77 (3H, s, OCH_3); **^{13}C NMR** (75 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 163.6 (C=O), 157.1 (C=N), 154.5 (Ar-C), 146.8 (Ar-C), 143.0 (Ar-C), 134.1 (C-6), 124.9 (C-4), 121.6 (C-5), 119.4 (C-2'), 115.8 (Ar-C), 114.7 (C-3'), 111.4 (C-7), 55.3 (OCH_3); ***Z* Isomer** δ 158.6 (C=O), 157.5 (C-4'), 151.6 (C=N), 145.0 (Ar-C), 141.0 (Ar-C), 133.5 (C-6), 122.7 (C-2'), 122.3 (C-4), 122.2 (Ar-C), 122.1 (C-5), 113.4 (C-3'), 110.5 (C-7), 55.2 (OCH_3); **HRMS** (m/z), calculated for $C_{15}H_{13}N_2O_2$: 253.0977, found $(M + H)^+$: 253.0968.

4.3.5 Synthesis of 3-((3,4-dimethylphenyl)imino)indolin-2-one 43e

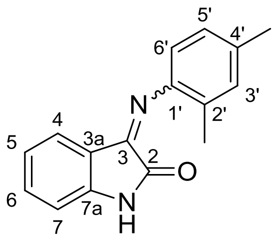
Isatin (3.00g, 20.4 mmol) and 3,4-xylidine (2.47 g, 20.4 mmol) were dissolved in ethanol (130 ml) in the presence of gl. acetic acid (0.780 ml) to yield an olive green solid (4.65 g, 91%). 3-((3,4-dimethylphenyl)imino)indolin-2-one **43e** was isolated as a mixture of *E:Z* isomers in a ratio of 5.7:1



R_f = 0.26 (3:7 EtOAc/Hex); **m.p.** 202-203 $^{\circ}$C; **IR** ν_{max} (cm^{-1}): 3192 (NH), 2919 (C-H), 1743 (C=O), 1650 (C=N); **^{1}H NMR** (DMSO-d_6, 300 MHz) ***E* Isomer** δ 10.96 (1H, s, NH), 7.32 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.21 (1H, d, *J* = 7.9 Hz, H-5'), 6.89 (1H, d, *J* = 7.8 Hz, H-7), 6.82 – 6.65 (3H, m, H-5, H-2' and H-6'), 6.52 (1H, dd, *J* = 7.8, 1.1 Hz, H-4), 2.26 (3H, s, CH_3), 2.24 (3H, s, CH_3); ***Z* Isomer** δ 10.84 (1H, s, NH), 7.56 (1H, dd, *J* = 7.6, 1.2 Hz, H-4), 7.43 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.06 – 7.04 (2H, m, H-5' and H-2'), 6.86 – 6.81 (1H, m, H-5), 6.82 – 6.65 (2H, m, H-7 and H-6'), 2.20 (3H, s, CH_3), 2.19 (3H, s, CH_3); **^{13}C NMR** (75 MHz, DMSO-d_6) ***E* Isomer** δ 163.5 (C=O), 154.5 (C=N), 148.1 (Ar-C), 146.8 (Ar-C), 137.5 (Ar-C), 134.2 (C-6), 132.8 (Ar-C), 130.3 (C-5'), 125.2 (C-4), 121.6 (C-5), 118.5 (C-2'), 115.7 (Ar-C), 114.6 (C-6'), 111.4 (C-7), 19.4 (CH_3), 18.9 (CH_3); ***Z* Isomer** δ 158.4 (C=O), 152.2 (C=N), 146.5 (Ar-C), 145.3 (Ar-C), 135.9 (Ar-C), 133.8 (C-6), 132.7 (Ar-C), 129.2 (C-5'), 122.5 (C-4), 122.2 (C-5), 120.7 (C-2'), 116.9 (Ar-C), 111.5 (C-6'), 110.6 (C-7), 19.4 (CH_3), 18.9 (CH_3); **HRMS** (m/z), calculated for $C_{16}H_{15}N_2O$: 251.1184, found $(M + H)^+$: 251.1178.

4.3.6 Synthesis of 3-((2,4-dimethylphenyl)imino)indolin-2-one 43f[59]

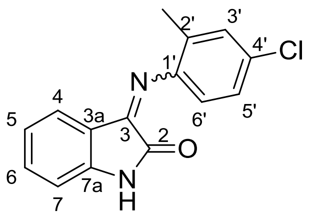
Isatin (5.00 g, 34.0 mmol) and 2,4-xylidine (4.20 ml, 34.0 mmol) were dissolved in ethanol (150 ml), in the presence of gl. acetic acid (1.30 ml) to afford 3-((2,4-dimethylphenyl)imino)indolin-2-one **43f** as an isomeric mixture in a ratio of *E:Z* = 9:1. The product was obtained as an orange solid (4.34 g, 51%).



R_f = 0.26 (3:7 EtOAc/Hex); **m.p.** 199-200 $^{\circ}$C (lit. m.p. 196-197 $^{\circ}$C)[57]; **^{1}H NMR** (300 MHz, DMSO-d_6) ***E* Isomer** δ 10.97 (1H, s, NH), 7.34 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.15 (1H, d, *J* = 1.8 Hz, H-3'), 7.06 (1H, dd, *J* = 7.8, 2.0 Hz, H-5'), 6.89 (1H, d, *J* = 7.9 Hz, H-7), 6.79-6.69 (2H, m, H-5 and H-6'), 6.37 (1H, dd, *J* = 7.8, 1.2 Hz, H-4), 2.32 (3H, s, CH_3), 1.99 (3H, s, CH_3); ***Z* Isomer** δ 10.97 (11H, s, NH), 7.61 (1H, dd, *J* = 7.6, 1.3 Hz, H-4), 7.45 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.11 – 6.98 (1H, m, H-5 overlapped with H-5' of *E*-isomer), 7.01 (1H, d, *J* = 1.8 Hz, H-3'), 6.94 – 6.82 (3H, m, H-5', H-6' and H-7 overlapped with H-7 of *E*-isomer), 2.26 (3H, s, CH_3), 2.06 (3H, s, CH_3); **^{13}C NMR** (75 MHz, DMSO-d_6) ***E* Isomer** δ 163.4 (C=O), 154.8 (C=N), 146.6 (Ar-C), 146.6 (Ar-C), 134.3 (C-6), 133.9 (Ar-C), 131.3 (C-3’), 127.3 (C-5’), 125.2 (C-4), 124.8 (Ar-C), 121.9 (C-5), 116.3 (C-6’), 116.0 (Ar-C), 111.4 (C-7), 20.5 (CH_3), 17.1 (CH_3); ***Z* Isomer** δ 158.5 (C=O), 152.4 (C=N), 145.4 (Ar- C), 145.4 (Ar- C), 133.4 (Ar-C and C-6), 132.0 (Ar-C), 130.4 (C-3’), 126.8 (C-5’), 126.1 (Ar-C), 122.5 (C-4), 122.2 (C-5), 117.8 (C-6’), 110.7 (C-7), 20.5 (CH_3),17.5 (CH_3); **HRMS** (m/z), calculated for $C_{16}H_{15}N_2O$: 251.1184, found $(M + H)^+$: 251.1181.

4.3.7 Synthesis of 3-((4-chloro-2-methylphenyl)imino)indolin-2-one 43g[60]

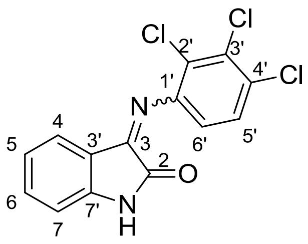
To a solution of isatin (5.00 g, 34.0 mmol) in ethanol (150 ml), was added 4-chloro-2-methylaniline (4.80 g, 34.0 mmol) and gl. acetic acid (1.30 ml) to afford 3-((4-chloro-2-methylphenyl)imino)indolin-2-one **43g** as a mixture of E:Z isomer, ratio =2.3:1. The product was isolated as a mustard solid (7.72 g, 84%).



R_f = 0.29 (3:7 EtOAc/Hex); **m.p.** 224-225 °C (lit. m.p. 175 °C)[60]; **^{1}H NMR** (300 MHz, DMSO-d_6) ***E* Isomer** δ 11.02 (1H, s, NH), 7.48 – 7.41 (1H, m, H-3’), 7.41 – 7.24 (2H, m, H-6 and H-5’), 6.97 – 6.82 (2H, m, H-7 and H-6’), 6.77 (1H, td, *J* = 7.6, 1.0 Hz, H-5), 6.36 (1H, dd, *J* = 7.7, 1.1 Hz, H-4), 2.03 (3H, s, CH_3); ***Z* Isomer** δ 10.90 (1H, s, NH), 7.63 (1H, dd, *J* = 7.5, 1.2 Hz, H-4), 7.54 – 7.23 (1H, m, H-3’), 7.18 – 7.03 (3H, m, H-5, H-6 and H-5’), 6.97 – 6.82 (2H, m, H-7 and H-6’ overlapped by H-7 and H-6’ of *E*-isomer), 2.07 (3H, s, CH_3); **^{13}C NMR** (75 MHz, DMSO-d_6) ***E* Isomer** δ 163.2 (C=O), 155.4 (C=N), 148.0 (Ar-C), 146.9 (Ar-C), 134.7 (C-6), 130.4 (C-3’), 128.7 (Ar-C), 128.0 (Ar-C), 126.8 (C-5’), 125.0 (C-4), 122.0 (C-5), 118.2 (C-6’), 115.9 (Ar-C), 111.5 (C-7), 16.9 (CH_3); ***Z* Isomer** δ 159.3 (C=O), 150.7 (C=N), 147.1 (Ar-C), 145.8 (Ar-C), 134.4 (C-6), 129.3 (C-12), 129.1 (Ar-C), 125.9 (Ar-C), 124.6 (C-10), 123.1 (C-4), 122.7 (C-5), 119.3 (C-6’), 115.0 (Ar-C), 112.2 (C-7), 17.3 (CH_3), **HRMS** (m/z), calculated for $C_{15}H_{12}{}^{35}ClN_2O$: 271.0638, found $(M + H)^+$: 271.0636

4.3.8 Synthesis of 3-((2,3,4-trichlorophenyl)imino)indolin-2-one 43h

3-((2,3,4-Trichlorophenyl)imino)indolin-2-one **43h** was successfully synthesized using isatin (5.00 g, 34.0 mmol) and 2,3,4-trichloroaniline (6.68 g, 34.0 mmol) in the presence of gl. acetic acid (1.30 ml) and ethanol (150 ml) to afford a blood red solid (4.65 g, 42%). The product was isolated as an isomeric mixture in a ratio *E:Z* = 1:1

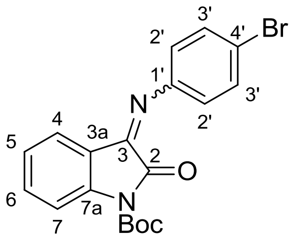


R*f* = 0.52 (3:7 EtOAc/Hex); **m.p.** 249-250 °C; **IR** ν_{max} (cm^{-1}): 3188 (NH), 1707 (C=O), 1615 (C=N); **^{1}H NMR** (300 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 11.07 (1H, s, NH), 7.73 (1H, d, *J* = 8.7 Hz, H-5’), 7.42 (1H, td, *J* = 7.7, 1.3 Hz, H-6), 7.18 (1H, d, *J* = 8.7 Hz, H-6’), 6.92 (2H, dd, *J*=7.8, 3.1 Hz, H-7); 6.83 (1H, td, *J* = 7.7, 1.0 Hz, H-5), 6.46 (1H, d, *J* = 7.7 Hz, H-4); **Z Isomer** δ 11.07 (1H, s, NH), 7.71 – 7.65 (1H, m, H-5’), 7.63 – 7.47 (2H, m, H-4 and H-6), 7.15 – 7.05 (2H, m, H-5 and H-6’), 6.92 (2H, dd, *J*=7.8, 3.1 Hz, H-7); **^{13}C NMR** (75 MHz, DMSO-*d*$_6$) ***E* Isomer** δ 163.0 (C=O), 157.1 (C=N), 147.7 (Ar-C), 147.3 (Ar-C), 135.6 (C-6), 129.7 (C-5’), 128.5 (Ar-C), 125.5 (C-4), 122.4 (C-5), 121.6 (Ar-C), 120.1 (Ar-C), 118.1 (C-6’), 115.6 (Ar-C), 111.8 (C-7); ***Z* Isomer** δ 158.4 (C=O), 155.9 (C=N), 147.8 (Ar-C), 146.6 (Ar-C), 135.5 (C-6), 131.2 (Ar-C), 130.0 (Ar-C), 128.3 (C-4), 126.9 (Ar-C), 123.7 (C-5), 122.7 (C-5’), 121.7 (Ar-C), 118.9 (C-6’), 111.3 (C-7); **HRMS** (m/z), calculated for $C_{14}H_8{}^{35}Cl_3N_2O$: 324.9702, found $(M + H)^+$: 324.9689.

4.4 General procedure for the Boc protection of Aryl-imines

4.4.1 Synthesis of *tert*-butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate 44a[54]

To a solution of 3-((4-bromophenyl)imino)indolin-2-one **43a** (1.50 g, 4.98 mmol) in dry THF (10 ml) was added DMAP (30.0 mg, 0.270 mmol), followed by slow addition of di-*tert*-butyl dicarbonate (1.19 g, 5.48 mmol). The reaction was stirred under a N_2 atmosphere at room temperature for 3 hours. After complete consumption of the starting material, water was added to the solution and a yellow solid precipitated. The filtered paste-like precipitate was then dissolved in EtOAc and dried over $MgSO_4$. The $MgSO_4$ was filtered off through a pad of Celite and the resulting filtrate was concentrated using a rotary evaporator. Further purification was achieved by recrystallization from EtOAc/hexane to afford *tert*-butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a** as a yellow solid (1.96 g, 98%) as an isomeric mixture in a ratio of *E:Z* = 3:1.

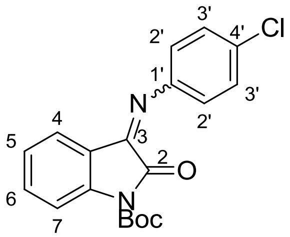


R$_f$ = 0.74 (3:7 EtOAc/Hex); **m.p.** 277-278 °C; **IR** ν$_{max}$ (cm^{-1}): 2981 (C-H), 1783 (C=O), 1731 (C-2), 1654 (C=N); **^{1}H NMR** (300 MHz, $CDCl_3$) ***E* Isomer** δ 8.02 (1H, d, *J* = 8.3 Hz, H-7), 7.63 – 7.52 (2H, m, H-3'), 7.50 – 7.41 (1H, m, H-6), 6.99 – 6.78 (4H, m, H-2', H-4 and H-5), 1.67 (9H, s, $C(CH_3)_3$); ***Z* isomer** δ 7.94 (1H, d, *J* = 8.2 Hz, H-7), 7.79 (1H, dd, *J* = 7.5, 1.4 Hz, H-4), 7.57 – 7.37 (3H, m, H-3' and H-6), 7.00 – 6.76 (3H, m, H-2' and H-5), 1.60 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) ***E* Isomer** δ 160.4 (C-2), 152.7 (C=N), 149.0 (Boc C=O), 148.7 (Ar-C), 147.8 (Ar-C), 144.6 (Ar-C), 134.7 (C-6), 132.7 (C-3'), 125.7 (C-4), 124.4 (C-5), 119.4 (C-2'), 118.5 (Ar-C), 116.3 (C-7), 85.2 ($\underline{C}(CH_3)_3$) , 28.1 ($C(\underline{C}H_3)_3$). ***Z* Isomer** ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.9 (C-2), 158.4 (C=N), 151.0 (Boc C=O), 149.0 (Ar-C), 147.4 (Ar-C), 143.3 (Ar-C), 134.4 (C-6), 131.8 (C-3'), 125.0 (C-4), 123.0 (C-5), 120.7 (C-2'), 118.6 (Ar-C), 115.7 (C-7), 85.1 ($\underline{C}(CH_3)_3$), 28.1 ($C(\underline{C}H_3)_3$). **HRMS** (m/z), calculated $C_{19}H_{18}{}^{79}BrN_2O_3$: 401.0501, found (M + H)$^+$: 401.0492.

The following *N*-Boc-protected imines were synthesized using the method described above for *tert*-butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a**

4.4.2 Synthesis of *tert*-butyl 3-((4-chlorophenyl)imino)-2-oxoindoline-1-carboxylate 44b

3-((4-Chlorophenyl)imino)indolin-2-one **43b** (1.00 g, 3.90 mmol) was dissolved in THF (10 ml), followed by the addition of DMAP (0.02 g, 0.19 mmol) and di-*tert*-butyl dicarbonate (0.940 g, 4.29 mmol) to afford *tert*-butyl 3-((4-chlorophenyl)imino)-2-oxoindoline-1-carboxylate **44b** as a yellow solid (1.03 g, 74%)

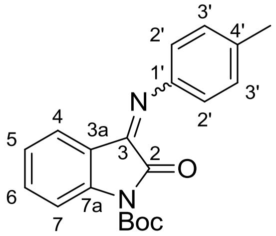


R$_f$ = 0.90 (3:7 EtOAc/Hex); **m.p.** 267 °C; **IR** ν$_{max}$ (cm^{-1}): 2983 (C-H), 1718 (C=O), 1731 (C=O), 1656 (C=N); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.01 (1H, dd, *J* = 8.3, 0.8 Hz, H-7), 7.50 – 7.37 (3H, m, H-3' and H-6), 6.98 – 6.89 (3H, m, H-2' and H-5), 6.85 – 6.79 (1H, m, H-4), 1.67 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 160.5 (C-2), 152.7 (C=N), 148.4 (Boc C=O), 144.5 (Ar-C), 134.7 (H-6), 130.9 (Ar-C), 129.7 (H-3'), 128.8 (Ar-C), 125.7 (C-4), 124.3 (C-5), 120.4 (Ar-C), 119.1 (C-2'), 116.3

(C-7), 85.2 ($\underline{C}(CH_3)_3$), 28.1($C(\underline{C}H_3)_3$); **HRMS** (m/z), calculated for $C_{19}H_{18}{}^{35}ClN_2O_3$: 357.1006, found $(M + H)^+$: 357.1012

4.4.3 Synthesis of *tert*-butyl 2-oxo-3-(*p*-tolylimino)indoline-1-carboxylate 44c

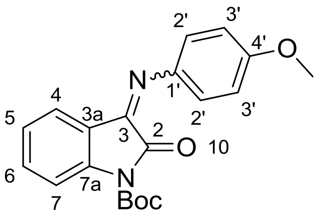
tert-Butyl 2-oxo-3-(*p*-tolylimino)indoline-1-carboxylate **44c** was synthesized by dissolving 3-(*p*-tolylimino)indolin-2-one **43c** (2.00 g, 8.47 mmol) in THF (20 ml), followed by treatment with di-*tert*-butyl dicarbonate (2.03 g, 9.31 mmol) in the presence of DMAP (0.05 g, 0.42 mmol). The product was isolated as a mixture of E:Z isomers in a ratio of 9:1, as a yellow solid (2.77 g, 97%).



R_f = 0.66 (3:7 EtOAc/Hex); **m.p.** 132 °C; **IR** $ν_{max}$ (cm^{-1}): 3031 (C-H), 1785 (C=O), 1733 (C=O),1653 (C=N); **^{1}H NMR** (300 MHz, $CDCl_3$) ***E* Isomer** δ 8.00 (1H, dd, *J* = 8.3, 0.7 Hz, H-7), 7.46 – 7.37 (1H, m, H-6), 7.29 – 7.20 (2H, m, H-3’), 6.92 – 6.84 (4H, m, H-2’, H-5, H-4), 2.41 (3H, s, CH_3), 1.70 – 1.65 (9H, m, $C(CH_3)_3$); ***Z* Isomer** δ 7.95 – 7.89 (1H, m, H-7), 7.84 – 7.77 (1H, m, H-4), 7.56 – 7.47 (1H, m, H-6), 7.29 – 7.13 (3H, m, H-3’ and H-5), 7.02 – 6.94 (2H, m, H-2’), 2.37 (3H, s, CH_3), 1.61 – 1.58 (9H, m, $C(CH_3)_3$)); **^{13}C NMR** (75 MHz, $CDCl_3$) ***E* Isomer** δ 160.8 (C-2), 152.1 (C=N), 148.8 (Boc C=O), 147.5 (Ar-C), 144.2 (Ar-C), 135.3 (Ar-C), 134.1 (C-6), 130.1 (C-3’), 125.6 (C-4), 124.2 (C-5), 117.7 (C-2’), 119.5 (Ar-C), 116.0 (C-7), 85.0 ($\underline{C}(CH_3)_3$), 28.1 ($C(\underline{C}H_3)_3$), 21.1 (CH_3); ***Z* Isomer** δ 156.9 (C-2), 151.1 (C=N), 147.7 (Boc C=O), 146.3 (Ar-C), 144.3 (Ar-C), 133.8 (C-6), 130.1 (Ar-C), 129.3 (C-3’), 123.3 (C-4), 123.2 (C-5), 116.7 (C-2’), 119.5 (Ar-C), 115.5 (C-7), 84.8 ($\underline{C}(CH_3)_3$), 28.1 ($C(\underline{C}H_3)_3$), 21.2.(CH_3). **HRMS** (m/z), calculated for $C_{20}H_{21}N_2O_3$: 337.1552, found $(M + H)^+$:337.1554.

4.4.4 Synthesis of *tert*-butyl 3-((4-methoxyphenyl)imino)-2-oxoindoline-1-carboxylate 44d

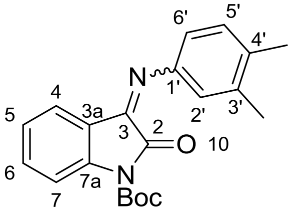
To a solution of 3-((4-methoxyphenyl)imino)indolin-2-one **43a** (2.00 g, 7.93 mmol) in dry THF (20 ml) was added DMAP (50.0 mg, 0.400 mmol), followed by slow addition of di-*tert*-butyl dicarbonate (1.90 g, 8.72 mmol). The reaction was stirred under N_2 atmosphere at room temperature for 3 hours. When the reaction had reached completion, the organic layer was washed with brine and extracted with EtOAc then dried over $MgSO_4$. $MgSO_4$ was filtered off through a pad of Celite and the resulting filtrate was concentrated using a rotary evaporator. Further purification was achieved by recrystallization from EtOAc/hexane to afford *tert*-butyl 3-((4-methoxyphenyl)imino)-2-oxoindoline-1-carboxylate **44d** as a yellow solid (2.49 g, 89%) as an isomeric mixture in a ratio of *E:Z* = 4:1.



R_f = 0.57 (3:7 EtOAc/Hex); **m.p.** 110-111 °C; **IR** ν_{max} (cm^{-1}): 2987.58 (C-H), 1733.19 (C=O, C-2), 1655.55 (Boc C=O), 1602.15 (C=N) **^{1}H NMR** (500 MHz, $CDCl_3$) ***E* Isomer** δ 8.00 (1H, dd, *J* = 8.3, 0.8 Hz, H-7), 7.42 (1H, ddd, *J* = 8.3, 7.6, 1.4 Hz, H-6), 7.06 (1H, dd, *J* = 7.8, 1.5 Hz, H-4), 7.03 – 6.95 (4H, m, H-2' and H-3'), 6.94 – 6.88 (1H, m, H-5), 3.86 (3H, s, OCH_3), 1.67 (9H, s, $C(CH_3)_3$); ***Z* Isomer** δ 7.92 – 7.87 (1H, m, H-7), 7.81 – 7.77 (1H, m, H-4), 7.49 (1H, ddd, *J* = 8.3, 7.5, 1.4 Hz, H-6), 7.26 – 7.20 (4H, m, H-2' and H-3'), 6.83 (1H, d, *J* = 8.7 Hz, H-5), 3.84 (3H, s, OCH_3), 1.62 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) ***E* Isomer** δ 160.9 (C-2), 157.9 (C-4'), 151.7 (C=N), 148.8 (Boc C=O), 144.2 (Ar-C), 142.8 (Ar-C), 134.0 (C-7), 125.1 (C-4), 124.1 (C-5), 119.9 (C-2'), 116.1 (C-3'), 114.7 (Ar-C), 113.8 (C-7), 85.0 ($\underline{C}(CH_3)_3$), 55.5 (OCH_3), 28.1 ($C(\underline{C}H_3)_3$). ***Z* Isomer** δ 158.6 (C-2), 155.0 (C-4'), 149.1 (C=N), 147.9 (Boc C=O), 142.4 (Ar-C), 141.0 (Ar-C), 133.3 (C-6), 124.8 (C-5), 123.2 (C-2'), 122.4 (C-4), 116.7 (C-3'), 115.4 (Ar-C), 114.4 (C-7), 84.8 ($\underline{C}(CH_3)_3$), 55.7 (OCH_3), 28.3 ($C(\underline{C}H_3)_3$). **HRMS** (m/z), calculated for $C_{20}H_{21}N_2O_4$: 353.1501, found $(M + H)^+$ 353.1502.

4.4.5 Synthesis of *tert*-butyl 3-((3,4-dimethylphenyl)imino)-2-oxoindoline-1-carboxylate 44e

To a solution of 3-((3,4-dimethylphenyl)imino)indolin-2-one **43e** (2.00 g, 8.32 mmol) in THF (20 ml) was added DMAP (50.0 mg, 0.420 mmol) and di-*tert*-butyl dicarbonate (2.00 g, 9.16 mmol) to afford *tert*-butyl 3-((3,4-dimethylphenyl)imino)-2-oxoindoline-1-carboxylate **44e** as an orange solid (2.39 g, 82%).



R_f = 0.87 (3:7 EtOAc/Hex); **m.p.** 149-150 °C; **IR** ν_{max} (cm^{-1}): 2982 (C-H), 1730 (C=O), 1655 (C=O), 1602 (C=N); **^{1}H NMR** (300

MHz, $CDCl_3$) δ 7.99 (1H, dd, *J* = 8.3, 0.9 Hz, H-7), 7.41 (1H, m, H-6), 7.17 (1H, d, *J* = 7.9 Hz, H-5'), 6.94 – 6.85 (2H, m, H-5 and H-6'), 6.78 (1H, d, *J* = 2.2 Hz, H-2'), 6.72 (1H, dd, *J* = 7.9, 2.2 Hz, H-4), 2.31 (3H, s, CH_3), 2.28 (3H, s, CH_3), 1.67 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 160.8 (C-2), 151.9 (C=O), 148.8 (Boc C=O), 147.8 (Ar-C), 144.2 (Ar-C), 137.9 (Ar-C), 134.0 (C-6), 133.9 (Ar-C), 130.5 (C-5'), 125.6 (C-4), 124.1 (C-5), 118.9 (C-2'), 116.7 (Ar-C), 116.0 (C-6'), 114.9 (C-7), 85.0 ($\underline{C}(CH_3)_3$), 28.1 ($C(\underline{C}H_3)_3$), 19.9 (CH_3), 19.4 (CH_3). **HRMS** (m/z), calculated for $C_{21}H_{23}N_2O_3$: 351.1709, found $(M + H)^+$ 351.1710.

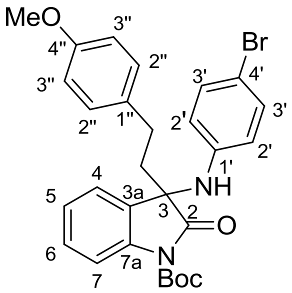
4.5 General procedure for Grignard addition reaction to *N*-Boc-protected aryl-imines

4.5.1 Synthesis of *tert*-butyl 3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate 45[37]

The Grignard reagent was prepared prior to use from a suitably substituted phenethyl bromide.[61] In this case 4-methoxyphenethyl bromide (1.00 eq., 0.7 ml, 4.98 mmol) was treated with magnesium turnings (1.10 eq., 0.120 g, 5.48 mmol) in THF (5 ml). Heat was applied to initiate the reaction. The mixture was then stirred at room temperature under a N_2 atmosphere for an hour. The Grignard reagent was used without any purification in the subsequent step.

tert-Butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a** (1.00 g, 2.49 mmol) was dissolved in THF (30 ml), treated with $MgBr_2$ (1.29 g, 4.98 mmol) and the heterogeneous mixture was cooled to -40 °C for 20 min. The previously prepared Grignard reagent (2.00 eq.) was then added slowly to the cooled solution. The reaction mixture was stirred at -40 °C for 1 hour and the temperature was raised to room temperature overnight under a N_2 atmosphere. The reaction was quenched with saturated aqueous NH_4Cl solution and the organic layer was partitioned with EtOAc. The organic layer was dried over $MgSO_4$ and filtered through a pad of Celite. The solvent was removed by evaporation under reduced pressure and the resulting residue was purified by flash silica gel column chromatography (5% EtOAc/Hex) to afford *tert*-

butyl 3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate **45** as a lime green solid (0.60 g, 45%).

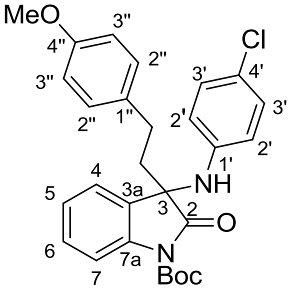


R*f* = 0.87 (3:7 EtOAc/Hex); **m.p.** 143-144 °C; **IR** ν_{max} (cm^{-1}): 3302 (N-H), 2980 (C-H), 2836 (C-H), 1710 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.83 (1H, d, *J* = 7.6 Hz, H-7), 7.57 – 7.44 (2H, m, H-3’), 7.39 (1H, ddd, *J* = 8.6, 7.3, 1.4 Hz, H-6), 7.02 – 6.89 (2H, m, H-3’’), 6.84 – 6.69 (5H, m, H-2’, H-2’’ and H-5), 6.66 – 6.58 (1H, m, H-4), 4.15 (1H, s, N-H), 3.76 (3H, s, OCH_3), 3.01 – 2.33 (4H, m, 2×(CH_2)), 1.64 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.1 (C-2), 157.9 (C-4’’), 151.3 (Boc C=O), 149.9 (Ar-C), 148.3 (Ar-C), 134.4 (C-6), 132.6 (Ar-C), 132.6 (C-3’), 129.2 (C-3’’), 126.3 (C-4), 122.5 (C-5), 120.0 (C-2’), 119.4 (Ar-C), 116.8 (Ar-C), 116.2 (C-7), 113.8 (C-2’’), 91.9 (C-3), 83.5 ($\underline{C}(CH_3)_3$), 55.3 (OCH_3), 41.1 (CH_2), 29.5 (CH_2), 28.5 ($C(\underline{C}H_3)_3$); **HRMS** (m/z), calculated for $C_{28}H_{30}{}^{79}BrN_2O_4$: 537.1389, found $(M + H)^+$: 537.1379.

The following compounds were prepared using the procedure described for *tert*-butyl 3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate **45** above.

4.5.2 Synthesis of *tert*-butyl 3-((4-chlorophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate 46

tert-Butyl 3-((4-chlorophenyl)amino)-3-(4-methoxyphenethyl)-2-oxoindoline-1-carboxylate **46** was successfully synthesised from a Grignard addition reaction of *tert*-butyl 3-((4-chlorophenyl)imino)-2-oxoindoline-1-carboxylate **44b** (0.466 g, 1.31 mmol) in THF (20 ml), followed by addition of $MgBr_2$ (0.120 g, 2.61 mmol) and the Grignard reagent, derived from 4-methoxyphenethyl bromide (0.41 ml, 2.62 mmol) and Mg(0) (70.1 mg, 2.88 mmol). The product was isolated as a cream solid (0.32 g, 50%)

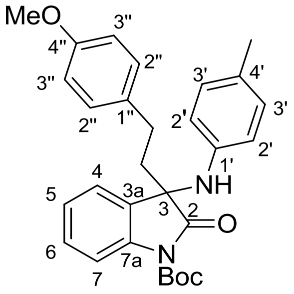


R*f* = 0.81 (3:7 EtOAc/Hex); **m.p.** 125 °C; **IR** ν_{max} (cm^{-1}): 3118 (N-H), 2980 (C-H), 2837 (C-H), 1708 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.83 (1H, d, *J* = 10.0 Hz, H-7), 7.45 – 7.30 (3H, m, H-3’ and H-6), 6.99 – 6.89 (2H, m, H-3’’), 6.86 – 6.70 (5H, m, H-2’, H-

2’’ and H-5), 6.62 (1H, dd, *J* = 8.0, 1.4 Hz, H-4), 4.49 (1H, s, NH), 3.76 (3H, s, OCH_3), 3.07 – 2.26 (4H, m, 2×(CH_2)), 1.63 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.2 (C-2), 157.9 (C-4’’), 151.4 (Boc C=O), 149.4 (Ar-C), 148.2 (Ar-C), 134.3 (C-6), 132.6 (Ar-C), 129.7 (C-3’), 129.2 (Ar-C), 129.2 (C-3’’), 126.3 (C-4), 122.5 (C-5), 119.6 (C-2’), 119.4 (Ar-C), 116.2 (C-7), 113.8 (C-2’’), 91.9 (C-3), 83.6 ($\underline{C}(CH_3)_3$), 55.3 (OCH_3), 41.1 (CH_2), 29.5 (CH_2), 28.5 ($C(\underline{C}H_3)_3$); **HRMS** (m/z), calculated for $C_{28}H_{40}{}^{35}ClN_2O_4$: 493.1894, found $(M + H)^+$: 493.1882.

4.5.3 Synthesis of *tert*-butyl 3-(4-methoxyphenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate 47

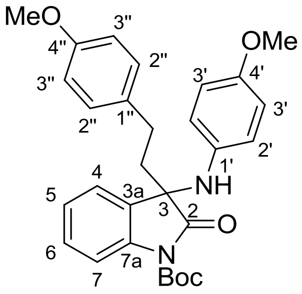
tert-Butyl 3-(4-methoxyphenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **47** was successfully synthesised by treating *tert*-butyl 2-oxo-3-(*p*-tolylimino)indoline-1-carboxylate **44c** (0.500 g, 1.49 mmol) with the Grignard reagent made from 4-methoxyphenethyl bromide (0.5 ml, 2.97 mmol) and Mg(0) (79.4 mg, 3.27 mmol). The reaction was carried out in THF (20 ml) in the presence of $MgBr_2$ (0.767 g, 2.97 mmol) to afford a yellow powder (0.25 g, 35 %).



R_f = 0.77 (3:7 EtOAc/Hex); **m.p.** 109-110 °C; **IR** ν_{max} (cm^{-1}): 3179 (N-H), 2966 (C-H), 2923 (C-H), 1699 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.98 – 7.78 (1H, m, H-7), 7.40 – 7.30 (1H, m, H-6), 7.18 (2H, d, *J* = 7.9 Hz, H-3’), 6.97 (2H, d, *J* = 8.6 Hz, H-3’’), 6.84 – 6.68 (5H, m, H-2’, H-2’’ and H-5), 6.67 – 6.60 (1H, m, H-4), 4.49 (1H, s, NH), 3.75 (3H, s, OCH_3), 3.06 – 2.41 (4H, m, 2×(CH_2)), 2.38 (3H,s, CH_3), 1.63 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 165.6 (C-2), 157.9 (C-4’’), 151.4 (Boc C=O), 148.3 (Ar-C), 148.1 (Ar-C), 133.8 (C-6), 133.4 (Ar-C), 132.9 (Ar-C), 130.1 (C-3’), 129.2 (C-3’’), 126.4 (C-4), 122.3 (C-5), 119.7 (Ar-C), 117.9 (C-2’), 116.0 (C-7), 113.8 (C-2’’), 91.8 (C-3), 83.3 ($\underline{C}(CH_3)_3$), 55.2 (OCH_3), 41.2 (CH_2), 29.6 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 21.0 (CH_3); **HRMS** (m/z), calculated for $C_{29}H_{33}N_2O_4$: 473.2440, found $(M + H)^+$: 473.2432.

4.5.4 Synthesis of *tert*-butyl 3-(4-methoxyphenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate 48

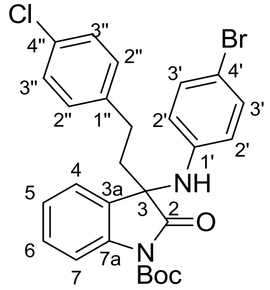
tert-Butyl 3-((4-methoxyphenyl)imino)-2-oxoindoline-1-carboxylate **44d** (0.500 g, 1.42 mmol) was subjected to a Grignard addition reaction in the presence of $MgBr_2$ (0.733 g, 2.84 mmol) and (4-methoxyphenethyl)magnesium bromide derived from 4-methoxyphenethyl bromide (0.44 ml, 2.84 mmol) and Mg (0) (75.9 mg, 3.12 mmol). The reaction was carried out in THF (20 ml) to afford *tert*-butyl 3-(4-methoxyphenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate **48** as a yellow solid (0.22 g, 32%).



R*f* = 0.52 (3:7 EtOAc/Hex); **m.p.** 145-146 °C; **IR** ν_{max} (cm^{-1}): 3127(N-H), 2983 (C-H), 1703 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.85 (1H, dt, *J* = 7.6, 1.1 Hz, H-7), 7.36 (1H, ddd, *J* = 8.5, 7.2, 1.5 Hz, H-6), 7.00 – 6.90 (4H, m, H-3' and H-3''), 6.86 – 6.80 (2H, m, H-2'), 6.78 – 6.71 (3H, m, H-2'' and H-5), 6.69 (1H, dd, *J* = 8.0, 1.5 Hz, H-4), 4.66 (1H, s, NH), 3.85 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 2.91 – 2.38 (4H, m, 2×(CH_2)), 1.64 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.1 (C-2), 165.9 (Boc C=O), 157.9 (C-4''), 156.5 (C-4'), 144.1 (Ar-C), 133.9 (C-6), 132.8 (Ar-C), 129.2 (C-3''), 129.0 (Ar-C), 126.3 (C-4), 122.4 (C-5), 119.7 (Ar-C), 119.3 (C-3'), 116.1 (C-7), 114.8 (C-2'), 113.8 (C-2''), 91.9 (C-3), 83.4 ($\underline{C}(CH_3)_3$), 60.4 (OCH_3), 55.5 (OCH_3), 41.3 (CH_2), 29.6 (CH_2), 28.5 ($C(\underline{C}H_3)_3$). **HRMS** (m/z), calculated for $C_{29}H_{33}N_2O_5$: 489.2389, found $(M + H)^+$: 489.2394.

4.5.5 Synthesis of *tert*-butyl 3-((4-bromophenyl)amino)-3-(4-chlorophenethyl)-2-oxoindoline-1-carboxylate 49

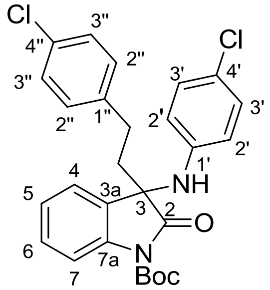
To a mixture of *tert*-butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a** (0.500 g, 1.25 mmol) and $MgBr_2$ (0.644 g, 2.49 mmol) in THF (20 ml) was added the Grignard reagent made from 4-chlorophenethyl bromide (0.40 ml, 2.49 mmol) and Mg(0) (66.6 mg, 2.74 mmol). This gave, using the procedure described above, *tert*-butyl 3-((4-bromophenyl)amino)-3-(4-chlorophenethyl)-2-oxoindoline-1-carboxylate **49** isolated as a yellow solid (0.23 g, 34%).



R$_f$ = 0.90 (3:7 EtOAc/Hex); **m.p.** 129-130 $^{\circ}$C; **IR** ν_{max} (cm^{-1}): 3177 (NH), 2976 (C-H), 2930 (C-H), 1720 (C=O); **^{1}H NMR** (300 MHz, CDCl$_3$) δ 7.81 (1H, m, H-7), 7.50 (2H, d, *J* = 8.2 Hz, H-3'), 7.46 – 7.34 (1H, m, H-6), 7.24 – 7.08 (2H, m, H-3''), 6.93 (2H, d, *J* = 8.0 Hz, H-2''), 6.75 (3H, m, H-2' and H-5), 6.67 – 6.57 (1H, m, H-4), 4.58 (1H, s, NH), 3.14 – 2.27 (4H, m, 2×(CH$_2$)), 1.62 (9H, s, C(CH$_3$)$_3$); **^{13}C NMR** (75 MHz, CDCl$_3$) δ 166.0 (C-2), 151.4 (Boc C=O), 149.7 (Ar-C), 148.1 (Ar-C), 139.0 (Ar-C), 134.4 (C-6), 132.7 (Ar-C), 132.6 (C-3'), 131.8 (Ar-C), 129.6 (C-2''), 128.4 (C-3''), 126.3 (C-4), 122.6 (C-5), 119.9 (C-2'), 119.3 (Ar-C), 116.9 (Ar-C), 116.3 (C-7), 91.8 (C-3), 83.7 ($\underline{C}$(CH$_3$)$_3$), 40.5 (CH$_2$), 29.8 (CH$_2$), 28.4 (C($\underline{C}$H$_3$)$_3$); **HRMS** (m/z), calculated for $C_{27}H_{27}{}^{79}Br^{35}ClN_2O_3$: 541.0894, found (M + H)$^+$: 541.0901.

4.5.6 Synthesis of *tert*-butyl 3-(4-chlorophenethyl)-3-((4-chlorophenyl)amino)-2-oxoindoline-1-carboxylate 50

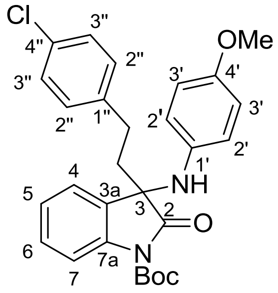
tert-Butyl 3-((4-chlorophenyl)imino)-2-oxoindoline-1-carboxylate **44b** (1.00 g, 2.80 mmol) was dissolved in THF (30 ml) and subjected to a Grignard reagent made from 4-chlorophenethyl bromide (0.8 ml, 5.61 mmol) and Mg (0) (0.150 g, 6.17 mmol) in the presence of MgBr$_2$ (0.257 g, 5.61 mmol). *t*-Butyl 3-(4-chlorophenethyl)-3-((4-chlorophenyl)amino)-2-oxoindoline-1-carboxylate **50** was afforded as a brown solid (0.74 g, 53%).



R$_f$ = 0.87 (3:7 EtOAc/Hex); **m.p.** 122 - 123 $^{\circ}$C; **IR** ν_{max} (cm^{-1}): 3196 (N-H), 2981 (C-H), 2931 (C-H), 1714 (C=O); **^{1}H NMR** (300 MHz, CDCl$_3$) δ 7.96 – 7.77 (1H, m, H-7), 7.44 – 7.28 (3H, m, H-3' and H-6), 7.14 (2H, d, *J* = 8.3 Hz, H-3''), 6.91 (2H, d, *J* = 8.3 Hz, H-2''), 6.82 – 6.69 (3H, m, H-2' and H-5), 6.61 (1H, d, *J* = 7.8 Hz, H-4), 4.68 (1H, s, NH), 3.02 – 2.33 (4H, m, 2×(CH$_2$)), 1.61 (9H, s, C(CH$_3$)$_3$); **^{13}C NMR** (75 MHz, CDCl$_3$) δ 166.4 (C-2), 151.3 (Boc C=O), 149.3 (Ar-C), 148.3 (Ar-C), 139.1 (Ar-C), 134.4 (C-6), 131.8 (Ar-C), 129.7 (C-3'), 129.7 (C-2''), 129.3 (Ar-C), 128.4 (C-3''), 126.3 (C-4), 122.5 (C-5), 119.6 (C-2'), 119.2 (Ar-C), 116.3 (C-7), 91.9 (C-3), 83.5 ($\underline{C}(CH_3)_3$), 40.5 (CH_2), 29.9 (CH_2), 28.4 ($C(\underline{C}H_3)_3$). **HRMS** (m/z), calculated for $C_{27}H_{27}{}^{35}Cl_2N_2O_3$: 497.1399, found $(M + H)^+$: 497.1400.

4.5.7 Synthesis of *tert*-butyl 3-(4-chlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate 51

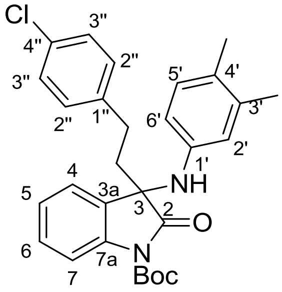
tert-Butyl 3-(4-chlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate **51** was successfully synthesised by dissolving the *tert*-butyl 3-((4-methoxyphenyl)imino)-2-oxoindoline-1-carboxylate **44d** (0.500 g, 1.42 mmol) in the presence of $MgBr_2$ (0.733 g, 2.84 mmol) in THF (20 ml) and adding the Grignard reagent derived from 4-chlorophenethyl bromide (0.41 ml, 2.84 mmol) and Mg(0) (75.9 mg, 3.12 mmol). *t*-Butyl 3-(4-chlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate **51** was isolated as a yellow powder (0.27 g, 39%).



R_f = 0.48 (3:7 EtOAc/Hex); **m.p.** 138-139 °C; **IR** ν_{max} (cm^{-1}): 3172 (N-H), 2965 (C-H), 1702 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.92 – 7.73 (1H, m, H-7), 7.36 (1H, ddd, *J* = 8.6, 7.2, 1.5 Hz, H-6), 7.18 – 7.13 (2H, m, H-3''), 6.98 – 6.88 (4H, m, H-2' and H-3'), 6.82 – 6.78 (2H, m, H-2''), 6.74 (1H, ddd, *J* = 8.2, 7.2, 1.0 Hz, H-5), 6.68 (1H, dd, *J* = 7.9, 1.4 Hz, H-4), 4.75 (1H, s, NH), 3.83 (3H, s, OCH_3), 2.94 – 2.41 (4H, m, 2×(CH_2)), 1.61 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.0 (C-2), 156.5 (C-4'), 151.4 (Boc C=O), 143.8 (Ar-C), 139.2 (Ar-C), 137.2 (Ar-C), 134.0 (C-6), 132.1 (Ar-C), 131.7 (Ar-C), 129.6 (C-2''), 128.4 (C-3''), 126.3 (C-4), 122.4 (C-5), 119.2 (C-3'), 116.1 (C-7), 114.8 (C-2'), 91.8 (C-3), 83.4 ($\underline{C}(CH_3)_3$), 55.5 (OCH_3), 38.4 (CH_2), 29.9 (CH_2), 28.4 ($C(\underline{C}H_3)_3$); **HRMS** (m/z), calculated for $C_{28}H_{30}{}^{35}ClN_2O_4$: 493.1894, found $(M + H)^+$: 493.1895.

4.5.8 Synthesis of *tert*-butyl 3-(4-chlorophenethyl)-3-((3,4-dimethylphenyl)amino)-2-oxoindoline-1-carboxylate 52

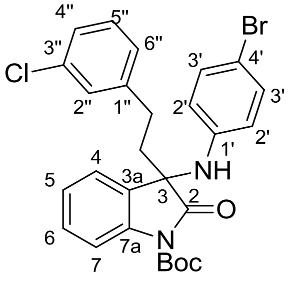
To a solution of *tert*-butyl 3-((3,4-dimethylphenyl)imino)-2-oxoindoline-1-carboxylate **44e** (0.500 g, 1.43 mmol) and $MgBr_2$ (0.736 g, 2.85 mmol) in THF (20 ml), was added ((4-chlorophenethyl)magnesium bromide) derived from 4-chlorophenethyl bromide (0.42 ml, 2.85 mmol) and Mg(0) (76.2 mg, 3.14 mmol) to afford *tert*-butyl 3-(4-chlorophenethyl)-3-((3,4-dimethylphenyl)amino)-2-oxoindoline-1-carboxylate **52** as a yellow solid (0.11 g, 15%).



R*f* = 0.90 (3:7 EtOAc/Hex); **m.p.** 138 °C; **IR** ν_{max} (cm^{-1}): 3171 (N-H), 2972 (C-H), 2920 (C-H), 1703 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.95 – 7.70 (1H, m, H-7), 7.35 (1H, ddd, *J* = 8.5, 7.2, 1.5 Hz, H-6), 7.19 – 7.14 (2H, m, H-3''), 7.13 (1H, d, *J* = 7.9 Hz, H-5'), 6.99 – 6.92 (2H, m, H-2''), 6.76 – 6.70 (1H, m, H-5), 6.69 – 6.62 (2H, m, H-2' and H-6'), 6.60 (1H, dd, *J* = 7.9, 2.2 Hz, H-4), 4.60 (1H, s, NH), 3.00 – 2.35 (4H, m, 2×(CH_2)), 2.29 (3H, s, CH_3), 2.26 (3H, s, CH_3), 1.62 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.2 (C-2), 151.4 (Boc C=O), 148.5 (Ar-C), 147.9 (Ar-C), 139.3 (Ar-C), 137.8 (Ar-C), 133.8 (C-6), 132.1 (Ar-C), 131.6 (Ar-C), 130.6 (C-5'), 129.7 (C-2''), 128.4 (C-3''), 126.5 (C-4), 122.4 (C-5), 119.7 (Ar-C), 119.1 (C-2'), 116.0 (C-7), 115.1 (C-6'), 91.7 (C-3), 83.4 ($\underline{C}(CH_3)_3$), 40.6 (CH_2), 29.9 (CH_2), 28.4 ($C(\underline{C}H_3)_3$), 19.9 (CH_3), 19.3 (CH_3); **HRMS** (m/z), calculated for $C_{29}H_{32}{}^{35}ClN_2O_3$: 491.2101, found $(M + H)^+$: 491.2108.

4.5.9 Synthesis of *tert*-butyl 3-((4-bromophenyl)amino)-3-(3-chlorophenethyl)-2-oxoindoline-1-carboxylate 53

tert-Butyl 3-((4-bromophenyl)amino)-3-(3-chlorophenethyl)-2-oxoindoline-1-carboxylate **53** was successfully synthesized from treatment of the *tert*-butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a** (0.500 g, 1.25 mmol) with the Grignard reagent made from 3-chlorophenethyl bromide (0.547 g, 2.49 mmol) and Mg(0) (66.6 mg, 2.74 mmol). The reaction was carried out in dry THF (20 ml) with $MgBr_2$ (0.644 g, 2.49 mmol) to afford a yellow solid (0.26 g, 39%).

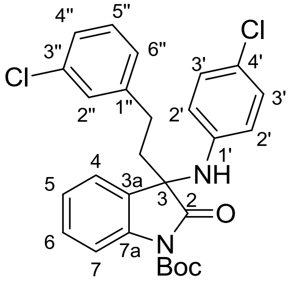


R*f* = 0.90 (3:7 EtOAc/Hex); **m.p.** 108 °C; **IR** ν_{max} (cm^{-1}): 3191 (NH), 2979 (C-H),1702 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.83 (1H, d, *J*=8.8 Hz, H-7), 7.53 – 7.47 (2H, m, H-3'), 7.40 (1H, ddd, *J* = 8.6, 7.2, 1.4 Hz, H-6), 7.15 – 7.10 (2H, m, H-4'' and H-6''), 6.96 (1H, d, *J*=1.5 Hz, H-2''), 6.93 – 6.86 (1H, m, H-5''), 6.82 – 6.71

(3H, m, H-2’ and H-5), 6.63 (1H, dd, *J* = 7.9, 1.3 Hz, H-4), 4.61 (1H, s, NH), 2.98 – 2.35 (4H, m, 2×(CH_2)), 1.62 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.0 (C-2), 151.3 (Boc C=O), 149.7 (Ar-C), 148.1 (Ar-C), 142.6 (Ar-C), 134.5 (C-6), 134.1 (Ar-C), 132.6 (C-3’), 129.6 (C-4’’), 128.5 (C-2’’), 126.5 (C-4), 126.3 (C-5’’), 126.2 (C-6’’), 122.7 (C-5), 120.0 (C-2’), 119.2 (Ar-C), 116.9 (Ar-C), 116.3 (C-7), 91.8 (C-3), 83.7 $\underline{C}(CH_3)_3$, 40.3 (CH_2), 30.2 (CH_2), 28.4 ($C(\underline{C}H_3)_3$); **HRMS** (m/z), calculated for $C_{27}H_{27}{}^{79}Br^{35}ClN_2O_3$: 541.0894, found $(M + H)^+$: 541.0897.

4.5.10 Synthesis of *tert*-butyl 3-(3-chlorophenethyl)-3-((4-chlorophenyl)amino)-2-oxoindoline-1-carboxylate 54

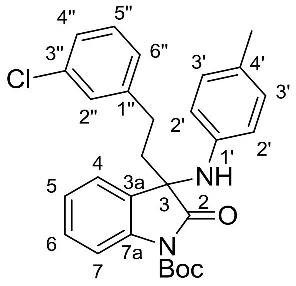
To a solution of *tert*-butyl 3-((4-chlorophenyl)imino)-2-oxoindoline-1-carboxylate **44b** (1.00 g, 2.80 mmol) in THF (30 ml), was added $MgBr_2$ (0.257 g, 5.61 mmol) and a Grignard reagent made from 2-chlorophenethyl bromide (1.23 g, 5.61 mmol) and Mg(0) (0.150 g, 6.17 mmol) to afford *tert*-butyl 3-(3-chlorophenethyl)-3-((4-chlorophenyl)amino)-2-oxoindoline-1-carboxylate **54** as a yellow solid (0.66 g, 48 %).



R_f = 0.87 (3:7 EtOAc/Hex); **m.p.** 119 °C; **IR** ν_{max} (cm^{-1}): 3177 (N-H), 2980 (C-H), 2930 (C-H), 1702 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.83 (1H, d, *J* = 9.2 Hz, H-7), 7.45 – 7.30 (3H, m, H-3’ and H-6), 7.16 – 7.08 (2H, m, H-4’’ and H-6’’), 6.95 (1H, d, *J* = 1.6 Hz, H-2’’), 6.94 – 6.86 (1H, m, H-5’’), 6.85 – 6.72 (3H, m, H-2’ and H-5), 6.62 (1H, dd, *J*=8.0, 1.3 Hz, H-4), 4.63 (1H, s, NH), 2.98 – 2.41 (4H, m, 2×(CH_2)), 1.62 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.1 (C-2), 151.3 (Boc C=O), 149.2 (Ar-C), 148.1 (Ar-C), 142.6 (Ar-C), 134.5 (C-6), 134.1 (Ar-C), 129.7 (C-3’), 129.6 (C-4’’), 129.3 (Ar-C), 128.5 (C-2’’), 126.5 (C-5’’), 126.3 (C-6’’), 126.2 (C-4), 122.6 (C-5), 119.6 (C-2’), 119.2 (Ar-C), 116.3 (C-7), 91.8 (C-3), 83.7 ($\underline{C}(CH_3)_3$), 40.3 (CH_2), 30.2 (CH_2), 28.4 ($C(\underline{C}H_3)_3$); **HRMS** (m/z), calculated for $C_{27}H_{27}{}^{35}Cl_2N_2O_3$: 497.1399, found $(M + H)^+$: 497.1398.

4.5.11 Synthesis of *tert*-butyl 3-(3-chlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate 55

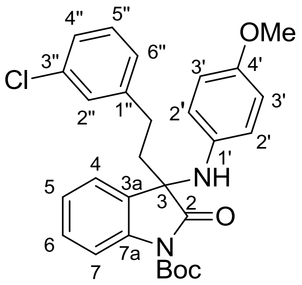
To a solution of *tert*-butyl 2-oxo-3-(*p*-tolylimino)indoline-1-carboxylate **44c** (0.500 g, 1.49 mmol) in THF (20 ml), was added $MgBr_2$ (0.765 g, 2.97 mmol) and the Grignard reagent made from 3-chlorophenethyl bromide (0.652 g, 2.97 mmol) and Mg(0) (79.4 mg, 3.27 mmol). The reaction carried out afforded *tert*-butyl 3-(3-chlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **55** as a yellow solid (0.14 g, 19%).



$\mathbf{R}_{\boldsymbol{f}}$ = 0.87 (3:7 EtOAc/Hex); **m.p.** 98 °C; **IR** ν_{max} (cm^{-1}): 3293 (N-H), 2972 (C-H), 2918 (C-H), 1703 (C=O); ^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.95 – 7.69 (1H, m, H-7), 7.36 (1H, ddd, *J* = 8.6, 7.2, 1.5 Hz, H-6), 7.24 – 7.14 (2H, m, H-3’), 7.15 – 7.08 (2H, m, H-4’’ and H-6’’), 6.98 (1H, s, H-2’’), 6.96 – 6.90 (1H, m, H-5’’), 6.84 – 6.69 (3H, m, H-2’ and H-5), 6.67 – 6.60 (1H, m, H-4), 4.53 (1H, s, NH), 3.03 – 2.43 (4H, m, 2×(CH_2)), 2.39 (3H, s, CH_3), 1.62 (9H, s, $C(CH_3)_3$); ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 165.4 (C-2), 151.4 (Boc C=O), 148.2 (Ar-C), 147.9 (Ar-C), 142.8 (Ar-C), 134.0 (Ar-C), 134.0 (C-6), 133.5 (Ar-C), 130.1 (C-3’), 129.6 (C-4’’), 128.5 (C-2’’), 126.5 (C-4), 126.4 (C-5’’), 126.2 (C-6’’), 122.5 (C-5), 119.6 (Ar-C), 117.9 (C-2’), 116.1 (C-7), 91.7 (C-3), 83.5 ($\underline{C}(CH_3)_3$), 40.4 (CH_2), 30.2 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 21.0 (CH_3); **HRMS** (m/z), calculated for $C_{28}H_{30}{}^{35}ClN_2O_3$: 477.1945, found $(M + H)^+$: 477.1929.

4.5.12 Synthesis of *tert*-butyl 3-(3-chlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate 56

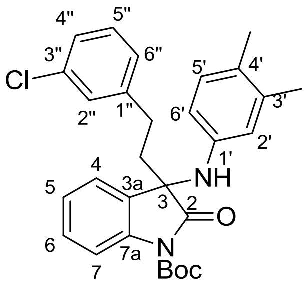
To a mixture of *tert*-butyl 3-((4-methoxyphenyl)imino)-2-oxoindoline-1-carboxylate **44d** (0.500 g, 1.42 mmol) and $MgBr_2$ (0.733 g, 2.84 mmol) in THF (20 ml), was added a Grignard reagent prepared from 3-chlorophenethyl bromide (0.620 g, 2.84 mmol) and Mg(0) (75.9 mg, 3.12 mmol) to afford *tert*-butyl 3-(3-chlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate **56** as a yellow solid (0.28 g, 40%).



R$_f$ = 0.61 (3:7 EtOAc/Hex); **m.p.** 132-133 $^{\circ}$C; **IR** ν_{max} (cm^{-1}): 3140 (NH), 2969 (C-H), 2931 (C-H), 1702 (C=O); **^{1}H NMR** (500 MHz, CDCl$_3$) δ 7.85 (1H, d, *J* = 8.0 Hz, H-7), 7.36 (1H, ddd, *J* = 8.6, 7.2, 1.5 Hz, H-6), 7.15 – 7.09 (2H, m, H-4'' and H-6''), 7.00 – 6.89 (4H, m, H-2'', H-3' and H-5''), 6.85 – 6.79 (2H, m, H-2'), 6.74 (1H, ddd, *J* = 8.2, 7.2, 1.0 Hz, H-5), 6.69 (1H, dd, *J* = 7.9, 1.4 Hz, H-4), 4.59 (1H, s, NH), 3.83 (3H, s, OCH$_3$), 2.98 – 2.43 (4H, m, 2×(CH$_2$)), 1.62 (9H, s, C(CH$_3$)$_3$); **^{13}C NMR** (126 MHz, CDCl$_3$) δ 165.9 (C-2), 156.5 (C-4'), 151.3 (Boc C=O), 147.9 (Ar-C), 143.8 (Ar-C), 142.8 (Ar-C), 134.2 (Ar-C), 134.0 (C-6), 129.6 (C-4''), 128.5 (C-2''), 126.5 (C-4), 126.2 (C-5''), 126.1 (C-6''), 122.5 (C-5), 119.5 (Ar-C), 119.3 (C-3'), 116.1 (C-7), 114.8 (C-2'), 91.8 (C-3), 83.4 ($\underline{C}$(CH$_3$)$_3$), 55.5 (OCH$_3$), 38.4 (CH$_2$), 29.9 (CH$_2$), 28.4 (C($\underline{C}$H$_3$)$_3$); **HRMS** (m/z), calculated for C$_{28}$H$_{30}$^{35}ClN$_2$O$_4$: 493.1894, found (M + H)$^+$: 493.1896.

4.5.13 Synthesis of *tert*-butyl 3-(3-chlorophenethyl)-3-((3,4-dimethylphenyl)amino)-2-oxoindoline-1-carboxylate 57

tert-Butyl 3-((3,4-dimethylphenyl)imino)-2-oxoindoline-1-carboxylate **44e** (0.500 g, 1.43 mmol) was dissolved in THF (20 ml) and subjected to a Grignard addition reaction in the presence of MgBr$_2$ (0.737 g, 2.85 mmol) and (3-chlorophenethyl)magnesium bromide derived from 3-chlorophenethyl bromide (0.626 g, 2.85 mmol) and Mg(0) (76.2 mg, 3.14 mmol). *tert*-Butyl 3-(3-chlorophenethyl)-3-((3,4-dimethylphenyl)amino)-2-oxoindoline-1-carboxylate **57** was isolated as a yellow solid (0.12 g, 18%).

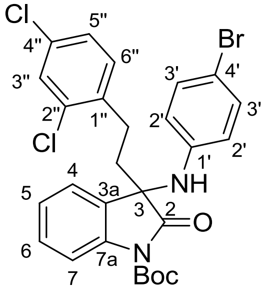


R$_f$ = 0.97 (3:7 EtOAc/Hex); **m.p.** 129 $^{\circ}$C; **IR** ν_{max} (cm^{-1}): 3166 (N-H), 2972 (C-H), 2928 (C-H), 1702 (C=O); **^{1}H NMR** (500 MHz, CDCl$_3$) δ 7.86 (1H, d, *J* = 7.5 Hz, H-7), 7.36 (1H, ddd, *J* = 8.6, 7.2, 1.5 Hz, H-6), 7.18 – 7.10 (3H, m, H-4'', H-5' and H-6''), 6.99 (1H, d, *J* = 2.1 Hz, H-2''), 6.96 – 6.90 (2H, m, H-5''), 6.74 (1H, ddd, *J* = 8.2, 7.3, 1.0 Hz, H-5), 6.69 – 6.65 (2H, m, H-2' and H-6'), 6.62 (1H, dd, *J* = 7.8, 2.3 Hz, H-4), 4.65 (1H, s, NH), 2.95 – 2.43 (4H, m, 2×(CH$_2$)), 2.29 (3H, s, CH$_3$), 2.26 (3H, s, CH$_3$), 1.62 (9H, s, C(CH$_3$)$_3$); **^{13}C NMR** (126 MHz, CDCl$_3$)

δ 165.2 (C-2), 151.4 (Boc C=O), 148.5 (Ar-C), 147.9 (Ar-C), 142.9 (Ar-C), 137.8 (Ar-C), 134.0 (Ar-C), 133.9 (C-6), 132.1 (Ar-C), 130.6 (C-5’), 129.6 (C-4’’), 128.5 (C-2’’), 126.5 (C-4), 126.5 (C-5’’), 126.1 (C-6’’), 122.4 (C-5), 119.7 (Ar-C), 119.1 (C-2’), 116.1 (C-7), 115.1 (C-6’), 91.7 (C-3), 83.4 ($\underline{C}(CH_3)_3$), 40.3 (CH_2), 30.2 (CH_2), 28.4 ($C(\underline{C}H_3)_3$), 19.9 (CH_3), 19.3 (CH_3); **HRMS** (m/z), calculated for $C_{29}H_{32}{}^{35}ClN_2O_3$: 491.2101, found $(M + H)^+$: 491.2113.

4.5.14 Synthesis of *tert*-butyl 3-((4-bromophenyl)amino)-3-(2,4-dichlorophenethyl)-2-oxoindoline-1-carboxylate 58

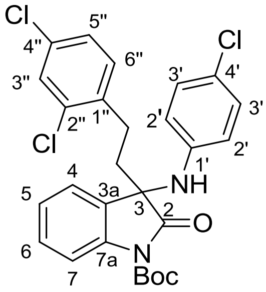
tert-Butyl 3-((4-bromophenyl)imino)-2-oxoindoline-1-carboxylate **44a** (0.500 g, 1.25 mmol) was subjected to a Grignard addition reaction using (2,4-dichlorophenethyl)magnesium bromide derived from 2,4-dichlorophenethyl bromide (0.40 ml, 2.49 mmol) and Mg(0) (66.6 mg, 2.74 mmol), in the presence of $MgBr_2$ (0.644 g, 2.49 mmol) and THF (20 ml) as a solvent to afford *tert*-butyl 3-((4-bromophenyl)amino)-3-(2,4-dichlorophenethyl)-2-oxoindoline-1-carboxylate **58** as a brown oil (0.19 g, 27%).



R_f = 0.90 (3:7 EtOAc/Hex); **IR** ν_{max} (cm^{-1}): 3304 (NH), 2976 (C-H), 2931 (C-H), 1702 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.88 (1H, m, H-7), 7.50 (2H, d, *J* = 8.6 Hz, H-3’), 7.39 (1H, ddd, *J*=8.6, 7.2, 1.4 Hz, H-6), 7.30 (1H, d, *J* = 2.1 Hz, H-3’’), 7.11 – 7.02 (1H, m, H-5’’), 6.95 (1H, d, *J* = 8.3 Hz, H-6’’), 6.87 – 6.72 (3H, m, H-2’ and H-5), 6.66 (1H, dd, *J* = 7.9, 1.4 Hz, H-4), 4.73 (1H, s, NH), 2.93 – 2.53 (4H, m, 2×$(CH)_2$), 1.64 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 165.9 (C-2), 151.3 (Boc C=O), 149.8 (Ar-C), 148.3 (Ar-C), 136.9 (Ar-C), 134.5 (C-6), 134.4 (Ar-C), 132.7 (C-3’), 132.6 (Ar-C), 131.2 (C-6’’), 129.3 (C-3’’), 127.1 (C-5’’), 126.4 (C-4), 122.6 (C-5), 120.0 (C-2’), 119.1 (Ar-C), 116.9 (Ar-C), 116.3 (C-7), 91.7 (C-3), 83.7 ($\underline{C}(CH_3)_3$)), 38.8 (CH_2), 28.4 ($C(\underline{C}H_3)_3$)), 28.0 (CH_2); **HRMS** (m/z), calculated for $C_{27}H_{26}{}^{79}Br^{35}Cl_2N_2O_3$: 575.0504, found $(M + H)^+$: 575.0504.

4.5.15 Synthesis of *tert*-butyl 3-((4-chlorophenyl)amino)-3-(2,4-dichlorophenethyl)-2-oxoindoline-1-carboxylate 59

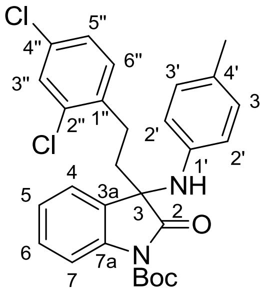
tert-Butyl 3-((4-chlorophenyl)imino)-2-oxoindoline-1-carboxylate **44b** (1.00 g, 2.80 mmol) was dissolved in THF (30 ml) and subjected to a Grignard addition reaction with (2,4-dichlorophenethyl)magnesium bromide derived from 2,4-dichlorophenethyl bromide (0.84 ml, 5.61 mmol) and Mg(0) (0.150 g, 6.17 mmol) in the presence of $MgBr_2$ (0.257 g, 5.61 mmol) to afford *tert*-butyl 3-((4-chlorophenyl)amino)-3-(2,4-dichlorophenethyl)-2-oxoindoline-1-carboxylate **59** as a brown oil (0.22 g, 15%).



R_f = 0.90 (3:7 EtOAc/Hex); **IR** ν_{max} (cm^{-1}): 3413 (N-H), 2976 (C-H), 2928 (C-H), 1710 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.00 – 7.79 (1H, m, H-7), 7.42 – 7.33 (3H, m, H-3’ and H-6), 7.30 (1H, d, *J* = 2.1 Hz, H-3’’), 7.07 (1H, dd, *J* = 8.2, 2.1 Hz, H-5’’), 6.96 (1H, d, *J* = 8.2 Hz, H-6’’), 6.86 (2H, d, *J* = 8.6 Hz, H-2’), 6.77 (1H, t, *J* = 8.0 Hz, H-5), 6.66 (1H, d, *J* = 7.3 Hz, H-4), 4.79 (1H, s, NH), 2.90 – 2.52 (4H, m, 2×(CH_2)), 1.64 (9H, s, $C(CH_3)_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.0 (C-2), 152.8 (Boc C=O), 151.2 (Ar-C), 149.2 (Ar-C), 148.2 (Ar-C), 136.8 (Ar-C), 134.5 (Ar-C), 134.4 (C-6), 132.6 (Ar-C), 131.2 (C-6’’), 129.7 (C-3’), 129.3 (Ar-C), 129.2 (C-3’’), 127.1 (C-5’’), 126.3 (C-4), 122.6 (C-5), 119.5 (C-2’), 116.2 (C-7), 91.7 (C-3), 83.7 ($\underline{C}(CH_3)_3$), 38.8 (CH_2), 28.4 ($C(\underline{C}H_3)_3$), 28.0 (CH_2); **HRMS** (m/z), calculated for $C_{27}H_{26}{}^{35}Cl_3N_2O_3$: 531.1009, found $(M + H)^+$: 531.0986.

4.5.16 Synthesis of *tert*-butyl 3-(2,4-dichlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate 60

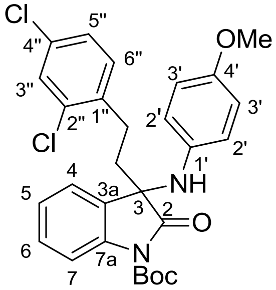
tert-Butyl 2-oxo-3-(*p*-tolylimino)indoline-1-carboxylate **44c** (0.517 g, 1.54 mmol) and $MgBr_2$ (0.793 g, 3.07 mmol) in THF (20 ml), were treated with the Grignard reagent prepared from 2,4-dichlorophenethyl bromide (0.5 ml, 3.07 mmol) and Mg(0) (82.1 g, 3.38 mmol). The reaction carried out resulted in successful isolation of *tert*-butyl 3-(2,4-dichlorophenethyl)-2-oxo-3-(*p*-tolylamino)indoline-1-carboxylate **60** as a brown oil (0.082 g, 10%).



R$_f$ = 0.90 (3:7 EtOAc/Hex); **IR** ν$_{max}$ (cm^{-1}): 3429 (N-H), 2976 (C-H), 2927 (C-H), 1709 (C=O); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.87 (1H, d, *J* = 7.7 Hz, H-7), 7.36 (1H, ddd, *J* = 8.5, 7.2, 1.4 Hz, H-6), 7.32 (1H, d, *J* = 2.1 Hz, H-3’’), 7.23 – 7.17 (2H, m, H-3’), 7.09 (1H, dd, *J* = 8.2, 2.2 Hz, H-5’’), 6.99 (1H, d, *J* = 8.2 Hz, H-6’’), 6.85 – 6.79 (2H, m, H-2’), 6.75 (1H, ddd, *J* = 8.2, 7.2, 1.0 Hz, H-5), 6.67 (1H, ddd, *J* = 7.8, 1.4, 0.7 Hz, H-4), 4.53 (1H, s, NH), 2.94 – 2.52 (4H, m, CH$_2$), 2.40 (3H, s, CH$_3$), 1.64 (9H, s, C(CH$_3$)$_3$); **^{13}C NMR** (126 MHz, CDCl$_3$) δ 165.1 (C-2), 151.4 (Boc C=O), 148.2 (Ar-C), 143.8 (Ar-C), 137.0 (Ar-C), 134.5 (Ar-C), 134.0 (C-6), 133.5 (Ar-C), 132.5 (Ar-C), 131.3 (C-3’’), 130.2 (C-3’), 129.3 (C-5’’), 127.1 (C-6’’), 126.5 (C-4), 122.5 (C-5), 119.6 (Ar-C), 117.9 (C-2’), 116.0 (C-7), 91.6 (C-3), 83.6 ($\underline{C}$(CH$_3$)$_3$), 39.0 (CH$_2$), 28.5 (C($\underline{C}$H$_3$)$_3$), 28.0 (CH$_2$), 21.0 (CH$_3$); **HRMS** (m/z), calculated for $C_{28}H_{29}{}^{35}Cl_2N_2O_3$: 511.1555, found (M + H)$^+$: 511.1558.

4.5.17 Synthesis of *tert*-butyl 3-(2,4-dichlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate 61

tert-Butyl 3-((4-methoxyphenyl)imino)-2-oxoindoline-1-carboxylate **44d** (0.500 g, 1.42 mmol) was dissolved in THF (20 ml) and subjected to a Grignard addition reaction in the presence of MgBr$_2$ (0.733 g, 2.84 mmol) and the Grignard reagent derived from 2,4-dichlorophenethyl bromide (0.46 ml, 2.84 mmol) and Mg(0) (75.9 mg, 3.12 mmol) to afford *tert*-butyl 3-(2,4-dichlorophenethyl)-3-((4-methoxyphenyl)amino)-2-oxoindoline-1-carboxylate **61** as a brown oil (0.23 g, 33%).



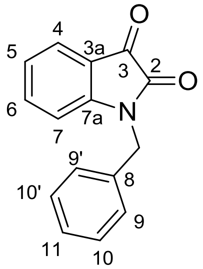
R$_f$ = 0.65 (3:7 EtOAc/Hex); **IR** ν$_{max}$ (cm^{-1}): 3330 (NH), 2966 (C-H), 2936 (C-H), 1706 (C=O); **^{1}H NMR** (500 MHz, CDCl$_3$) δ 8.03 – 7.73 (1H, m, H-7), 7.36 (1H, ddd, *J* = 8.6, 6.7, 2.0 Hz, H-6), 7.30 (1H, d, *J*=2.1 Hz, H-3’’), 7.07 (1H, dd, *J* = 8.2, 2.2 Hz, H-5’’), 7.00 – 6.91 (3H, m, H-3’ and H-6’’), 6.89 – 6.82 (2H, m, H-2’), 6.77 – 6.67 (2H, m, H-4 and H-5), 4.70 (1H, s, NH), 3.83 (3H, s, OCH$_3$), 2.91 – 2.51 (4H, m, 2×(CH$_2$)), 1.63 (9H, s, C(CH$_3$)$_3$); **^{13}C NMR** (75 MHz, CDCl$_3$) δ 165.7 (C-2), 156.6 (C-4’), 151.4 (Boc C=O), 148.0 (Ar-C), 143.9 (Ar-C), 137.1 (Ar-C), 134.5 (Ar-C), 134.0

(C-6), 132.5 (Ar-C), 131.3 (C-6’’), 129.2 (C-3’’), 127.1 (C-5’’), 126.3 (C-4), 122.5 (C-5), 119.5 (Ar-C), 119.2 (C-3’), 116.1 (C-7), 114.9 (C-2’), 91.7 (C-3), 83.5 ($\underline{C}(CH_3)_3$), 55.5 (OCH_3), 38.9 (CH_2), 28.4 ($C(\underline{C}H_3)_3$), 28.0 (CH_2); **HRMS** (m/z), calculated for $C_{28}H_{29}{}^{35}Cl_2N_2O_4$: 527.1504, found $(M + H)^+$: 527.1502.

4.6 General procedure for the synthesis of ring-opened analogues using *p*-methoxybenzyl and benzyl protecting groups

4.6.1 Synthesis of 1-benzylindoline-2,3-dione 62[62,63]

Isatin (3.00 g, 20.4 mmol) and K_2CO_3 (3.38 g, 24.5 mmol) in dry DMF (90 ml) were stirred for an hour at room temperature. Benzyl bromide (3 ml, 24.5 mmol) and KI (0.677 g, 4.08 mmol) were then added to the heterogeneous mixture. The reaction temperature was raised to 80 °C and left to stir for 5 hours under reflux. After this time, the reaction mixture was cooled to room temperature, and aqueous HCl (1M, 50 ml) was added to neutralise the excess base. The organic material was extracted into EtOAc, then washed with brine (6 × 30 ml) and dried over $MgSO_4$. The solvent was removed using a rotary evaporator and further purification was achieved by recrystallization from EtOAc/hexane. 1-Benzylindoline-2,3-dione **62** was isolated as a bright orange crystalline solid (3.6 g, 75%).

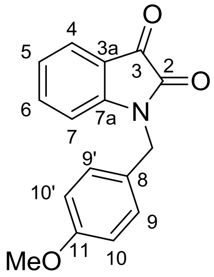


R_f = 0.24 (3:7 EtOAc/Hex); **m.p.**: 134-135 °C (lit. m.p. 136 °C)[63]; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.61 (1H, ddd, *J* = 7.5, 1.4, 0.6 Hz, H-7), 7.48 (1H, td, *J* = 7.8, 1.4 Hz, H-6), 7.40 – 7.29 (5H, m, Bn-H), 7.09 (1H, td, *J* = 7.5, 0.8 Hz, H-5), 6.78 (1H, d, *J* = 8.0 Hz, H-4), 4.93 (2H, s, CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 183.2 (C-3), 158.3 (C-2), 150.7 (C-7a), 138.3 (C-6), 134.5 (C-8), 129.1 (C-10 and C-10’), 128.2 (C-11), 127.4 (C-9 and C-9’), 125.4 (C-4), 123.8 (C-5), 117.7 (C-3a), 111.0 (C-7), 44.1 (CH_2).

4.6.2 Synthesis of 1-(4-methoxybenzyl)indoline-2,3-dione 63[64]

1-(4-Methoxybenzyl)indoline-2,3-dione **63** was synthesized by the procedure described above for 1-benzylindoline-2,3-dione **62**. Isatin (1.00 g, 6.80 mmol) was treated with

K_2CO_3 (1.03 g, 7.48 mmol), KI (0.226 g, 1.36 mmol) and 4-methoxybenzyl chloride (1 ml, 7.48 mmol) in DMF (30 ml) to yield an orange crystalline solid (1.3 g, 72%).

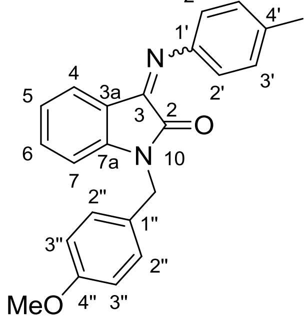


R*f* = 0.36 (3:7 EtOAc/Hex); **m.p.**: 169-170 °C (lit. m.p. 172 °C)[64]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (1H, dd, *J* = 7.4, 1.3 Hz, H-7), 7.48 (1H, td, *J* = 7.8, 1.4 Hz, H-6), 7.32 – 7.24 (2H, m, H-10 and H-10'), 7.08 (1H, td, *J* = 7.5, 0.9 Hz, H-5), 6.91 – 6.84 (2H, m, H-9 and H-9'), 6.81 (1H, d, *J* = 7.9 Hz, H-4), 4.86 (2H, s, CH_2), 3.78 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.4 (C-3), 159.5 (C-11), 158.2 (C-2), 150.8 (C-7a), 138.2 (C-6), 128.9 (C-9 and C-9'), 126.5 (C-8), 125.4 (C-4), 123.8 (C-5), 117.7 (C-3a), 114.4 (C-10 and C-10'), 111.0 (C-7), 55.3 (OCH_3), 43.5 (CH_2).

4.7 General procedure for the synthesis of PMB and Bn-protected arylimines

4.7.1 Synthesis of 1-(4-methoxybenzyl)-3-(*p*-tolylimino)indolin-2-one 64[56]

1-(4-Methoxybenzyl)-3-(*p*-tolylimino)indolin-2-one **64** was successfully synthesized using the procedure described for 3-((4-bromophenyl)imino)indolin-2-one **43a**[56] above. 1-(4-Methoxybenzyl)indoline-2,3-dione **63** (1.00 g, 3.74 mmol) and *p*-toluidine (0.400 g, 3.74 mmol) were dissolved in ethanol (50 ml), followed by addition of gl. acetic acid (150 µl) as a catalyst. The reaction mixture was heated under reflux at 100 °C for 5 hours. After cooling to room temperature, the solvent was removed under reduced pressure and the product precipitated out of solution. The yellow residue was purified by recrystallization from EtOAc/hexane and dried under vacuum. 1-(4-Methoxybenzyl)-3-(*p*-tolylimino)indolin-2-one **64** was isolated as a bright yellow solid (0.73 g, 55%).

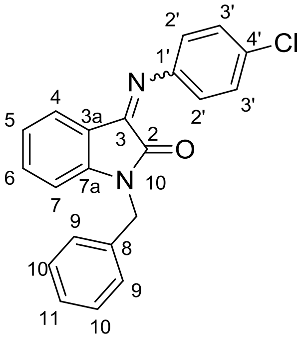


R*f* = 0.54 (3:7 EtOAc/Hex); **m.p.**: 156-157 °C; **IR** ν_{max} (cm^{-1}): 2927 (C-H), 2831 (C-H), 1727 (C=O), 1653 (C=N); ^{1}**H NMR** (500 MHz, $CDCl_3$) δ 7.34 – 7.29 (2H, m, H-3''), 7.24 – 7.21 (3H, m, H-3' and H-6), 6.96 – 6.92 (2H, m, H-2'), 6.89 – 6.85 (2H, m, H-2''), 6.76 – 6.73 (3H, m, H-4 H-5 and H-7), 4.94 (2H, s, Bn-CH_2), 3.78 (3H, s, OCH_3), 2.40 (3H, s, CH_3); ^{13}**C NMR** (126 MHz,

$CDCl_3$) δ 163.4 (C=O), 159.3 (C-4’’), 154.0 (C=N), 147.6 (Ar-C), 147.1 (Ar-C), 135.1 (Ar-C), 133.8 (C-6), 129.9 (C-3’), 128.9 (C-3’’), 127.2 (Ar-C), 126.0 (C-4), 122.5 (C-5), 118.0 (C-2’), 116.0 (Ar-C), 114.3 (C-2’’), 110.2 (C-7), 55.3 (OCH_3), 43.5 (Bn-CH_2), 21.1 (CH_3); **HRMS** (m/z), calculated for $C_{23}H_{21}N_2O_2$: 357.1610, found $(M + H)^+$: 357.1603.

4.7.2 Synthesis of 1-benzyl-3-((4-chlorophenyl)imino)indolin-2-one 65a

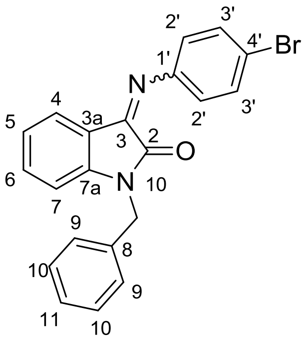
1-Benzylindoline-2,3-dione **62** (2.00 g, 8.43 mmol) and *p*-chloroaniline (1.08 g, 8.43 mmol) were dissolved in ethanol (100 ml) and treated with gl. acetic acid (0.5 ml) to afford 1-benzyl-3-((4-chlorophenyl)imino)indolin-2-one **65a** as a yellow solid (2.0 g, 67%).



R_f = 0.54 (3:7 EtOAc/Hex); **m.p.**: 199-200 °C; **IR** ν_{max} (cm^{-1}): 2930 (C-H), 1732 (C=O), 1655 (C=N); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.47 – 7.28 (8H, m, H-3’, H-6 and Bn-H), 7.04 – 6.95 (2H, m, H-2’), 6.82 – 6.69 (3H, m, H-4, H-5 and H-7), 5.01 (2H, s, CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 163.2 (C=O), 154.6 (C=N), 148.7 (Ar-C), 147.4 (Ar-C), 135.0 (Ar-C), 134.4 (C-6), 130.8 (Ar-C), 129.6 (C-3’), 129.0 (C-10), 128.0 (C-11), 127.5 (C-9), 126.2 (C-4), 122.8 (C-5), 119.4 (C-2’), 115.7 (Ar-C), 110.5 (C-7), 44.1 (CH_2); **HRMS** (m/z), calculated for $C_{21}H_{16}{}^{35}ClN_2O$: 347.0951, found $(M + H)^+$: 347.0943.

4.7.3 Synthesis of 1-benzyl-3-((4-bromophenyl)imino)indolin-2-one 65b

To a solution of 1-benzylindoline-2,3-dione **62** (2.00 g, 8.43 mmol) and *p*-bromoaniline (1.45 g, 8.43 mmol) in ethanol (100 ml), was added gl. acetic acid (0.5 ml) to yield 1-benzyl-3-((4-bromophenyl)imino)indolin-2-one **65b** as yellow solid (3.0 g, 90%).

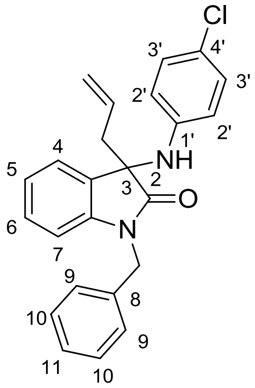


R_f = 0.52 (3:7 EtOAc/Hex); **m.p.**: 151 °C; **IR** ν_{max} (cm^{-1}): 2981 (C-H), 2919 (C-H), 1732 (C=O), 1650 (C=N); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.60 – 7.52 (2H, m, H-3‘), 7.42 – 7.28 (6H, m, Bn-H and H-6), 6.99 – 6.89 (2H, m, H-2’), 6.81 – 6.68 (3H, m, H-4, H-5 and H-7), 5.01 (2H, s, CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 163.1 (C=O), 149.2 (C=N), 147.4 (Ar-C), 135.0 (Ar-C), 134.4 (C-6), 132.6 (C-3’), 131.7 (Ar-C), 129.0 (C-10), 128.0 (C-11), 127.5 (C-9), 126.2 (C-4), 122.8 (C-5), 119.8 (C-2'), 118.5 (Ar-C), 115.7 (Ar-C), 110.5 (C-7), 44.1 (CH_2); **HRMS** (m/z), calculated for $C_{21}H_{16}{}^{79}BrN_2O$: 391.0446, found $(M + H)^+$: 391.0439.

4.8 General procedure for the Grignard addition reaction on PMB- and Bn-protected arylimines

4.8.1 Synthesis of 3-allyl-1-benzyl-3-((4-chlorophenyl)amino)indolin-2-one 66[37]

1-Benzyl-3-((4-chlorophenyl)imino)indolin-2-one **65a** (0.500 g, 1.44 mmol) and $MgBr_2$ (0.130 g, 2.88 mmol) were dissolved in DCM (20 ml) and the temperature of the reaction mixture was reduced to -40 °C for 20 min. AllylMgBr (1.59 ml, 1.59 mmol, 1M in diethyl ether) was then added and the temperature was kept at -40 °C for an hour. The temperature was raised to room temperature and the reaction mixture was left stirring overnight under a N_2 atmosphere. The reaction mixture was quenched with saturated aqueous NH_4Cl. The organic layer was extracted into DCM, dried over $MgSO_4$, and filtered through a pad of celite. After removal of solvent under reduced pressure the resulting residue was purified by silica gel column chromatography (5% EtOAc/Hex) to yield 3-allyl-1-benzyl-3-((4-chlorophenyl)amino)indolin-2-one **66** as yellow solid (0.29 g, 52%).

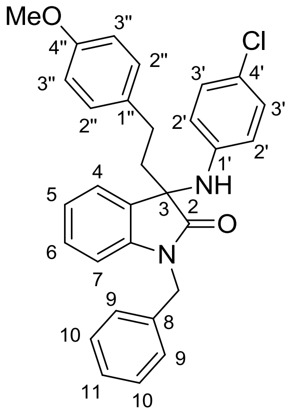


$\mathbf{R_f}$ = 0.52 (3:7 EtOAc/Hex); **m.p.**: 140 °C; **IR** ν_{max} (cm^{-1}): 3317 (NH), 3025 (=C-H), 2927 (C-H), 1705 (C=O), 1613 (C=C); ^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.35 – 7.16 (7H, m, Bn-H, H-4 and H-6), 7.03 (1H, td, *J* = 7.6, 1.1 Hz, H-5), 6.90 – 6.77 (3H, m, H-3' and H-7), 6.19 – 6.08 (2H, m, H-2'), 5.82 – 5.63 (1H, m, =CH), 5.28 – 5.13 (2H, m, =CH_2), 5.10 – 4.71 (2H, m, Bn-CH_2), 4.45 (1H, s, NH), 2.82 – 2.55 (2H, m, CH_2); ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 177.3 (C=O), 143.7 (Ar-C), 141.9 (Ar-C), 135.5 (C-6), 130.2 (=CH), 129.3 (Ar-C), 129.1 (Ar-C), 128.8 (C-3') 128.7 (C-10), 127.8 (C-11), 127.7 (C-9), 124.2, (Ar-C) 123.9 (C-4), 123.0 (C-5), 121.1 (=CH_2), 117.0 (C-2'), 109.7 (C-7), 64.1 (C-3), 44.5 (Allyl-CH_2), 44.1 (Bn-CH_2); **HRMS** (m/z), calculated for $C_{24}H_{22}{}^{35}ClN_2O$: 389.1421, found $(M + H)^+$: 389.1414.

4.8.2 Synthesis of 1-benzyl-3-((4-chlorophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one 67[37]

p-Methoxyphenethyl bromide (0.45 ml, 2.88 mmol) was added to a two necked round bottom flask equipped with a stirrer bar and a heterogeneous mixture of Mg turnings (77.0 mg, 3.17 mmol) in dry THF (3 ml). Heat was applied to initiate formation of the Grignard reagent, which was used immediately in the subsequent step.

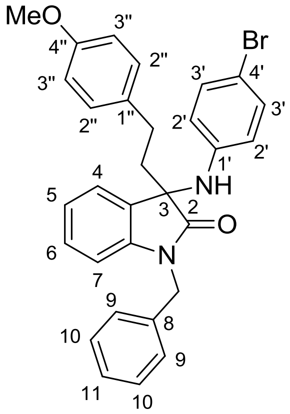
1-Benzyl-3-((4-chlorophenyl)imino)indolin-2-one **65a** (0.500 g, 1.44 mmol) was dissolved in THF (20 ml), then treated with $BF_3.OEt_2$ (0.360 ml, 2.88 mmol) and the reaction mixture was cooled to -40 °C. The Grignard reagent was added after cooling for 20 minutes and the temperature was raised to room temperature after stirring at -40 °C for an hour. The reaction mixture was left to stir at room temperature overnight under a N_2 atmosphere. The reaction was quenched with saturated aqueous NH_4Cl and the organic layer was extracted into EtOAc. The resulting mixture was dried over $MgSO_4$ and filtered through a pad of Celite. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (5% EtOAc/Hex). 1-Benzyl-3-((4-chlorophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one **67** was isolated as a cream solid (86 mg, 12%).



R_f = 0.68 (3:7 EtOAc/Hex); **m.p.**: 187 °C; **IR** ν_{max} (cm^{-1}): 3308 (NH), 2931 (C-H), 2838 (C-H), 1703(C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.37 (1H, dd, *J* = 7.3, 1.3 Hz, H-4), 7.33 – 7.15 (6H, m, H-6 and Bn-H), 7.14 – 7.05 (1H, m, H-5), 7.03 – 6.95 (2H, m, H-3''), 6.91 – 6.73 (5H, m, H-2'', H-3' and H-7), 6.18 – 6.11 (2H, m, H-2'), 5.09 (1H, d, *J* = 15.4 Hz, one of the Bn CH_2), 4.69 (1H, d, *J* = 15.4 Hz, one of the Bn CH_2) 3.77 (3H, s, OCH_3), 2.58 – 2.21 (3H, m, NH and 2×(CH_2)); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.4 (C=O), 158.1 (C-4''), 143.8 (Ar-C), 142.3 (Ar-C), 135.5 (Ar-C), 132.4 (C-6), 130.7 (Ar-C), 129.3 (Ar-C), 129.3 (H-3''), 128.9 (H-3'), 128.7 (H-10), 127.8 (H-11), 127.7 (H-9), 124.7 (Ar-C), 123.9 (C-4), 123.3 (C-5), 117.7 (H-2'), 113.9 (H-2''), 109.8 (C-7), 65.3 (C-3), 55.3 (OCH_3), 44.1 (Bn-CH_2), 42.2 (CH_2), 28.4 (CH_2); **HRMS** (m/z), calculated for $C_{30}H_{28}ClN_2O_2$: 483.1839, found $(M + H)^+$: 483.1840.

4.8.3 Synthesis of 1-benzyl-3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one 68

1-Benzyl-3-((4-bromophenyl)imino)indolin-2-one **65b** (0.500 g, 1.28 mmol) was dissolved in THF (20 ml) in the presence of $BF_3.OEt_2$ (0.320 ml, 2.56 mmol) and treated with (4-methoxyphenethyl) magnesium bromide to yield 1-benzyl-3-((4-bromophenyl)amino)-3-(4-methoxyphenethyl)indolin-2-one **68** as a yellow solid (41 mg, 6%). The reaction was repeated using DCM as a solvent, to afford the product in a slightly improved yield of 11%.

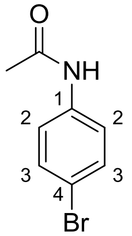


R_f = 0.68 (3:7 EtOAc/Hex); **m.p.**: 192-193 °C; **IR** ν_{max} (cm^{-1}): 3307 (NH), 2956 (C-H), 1703(C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.39 – 7.32 (1H, m, H-4), 7.32 – 7.17 (6H, m, H-6 and Bn-H), 7.13 – 7.05 (1H, m, H-5), 7.03 – 6.95 (4H, m, H-3' and H-3''), 6.87 – 6.75 (3H, m, H-2'' and H-7), 6.16 – 6.00 (2H, m, H-2'), 5.09 (1H, d, *J* = 15.4 Hz, one of the Bn-CH_2), 4.70 (1H, d, *J* = 15.4 Hz, one of the Bn-CH_2), 4.34 (1H, s, NH), 3.77 (3H, s, OCH_3), 2.58 – 2.18 (4H, m, 2×(CH_2)); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.4 (C=O), 158.1 (C-4''), 144.3 (Ar-C), 142.3 (Ar-C), 135.5 (Ar-C), 132.4 (C-6), 131.8 (H-3'), 129.3 (Ar-C), 129.3 (Ar-C), 129.3 (H-3''), 128.8 (C-10), 127.9 (C-11), 127.8 (C-9), 123.9 (C-4), 123.3 (C-5), 117.9 (H-2'), 113.9 (C-2''), 111.9 (Ar-C), 109.8 (C-7), 65.2 (C-3), 55.3 (CH_3), 44.2 (Bn-CH_2), 42.2 (CH_2), 28.4 (CH_2); **HRMS** (m/z), calculated for $C_{30}H_{28}{}^{79}BrN_2O_2$: 527.1334, found $(M + H)^+$: 527.1328.

4.9 Synthesis of the ring-closed analogues

4.9.1 Synthesis of *N*-(4-bromophenyl)acetamide 72a[28,65]

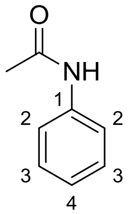
p-Bromoaniline (5.00 g, 29.1 mmol) was dissolved in DCM (60 ml) at 0 °C. (7.34 ml, 72.7 mmol) and triethylamine (6.08 ml, 43.6 mmol) were then added and the mixture warmed to room temperature. After stirring the solution at room temperature for 2 hours under a N_2 atmosphere, $NaHCO_3$ was added to neutralize the solution. The solution was transferred to a separating funnel and the organic mixture was partitioned between DCM and water. The organic layer was dried over Na_2SO_4 and the solvent was removed by evaporation under reduced pressure. The resulting residue was purified by recrystallization from EtOAc/hexane to give compound **72a** as a white crystalline solid (3.8 g, 61%).



R_f = 0.13 (3:7 EtOAc/Hex); **m.p.**: 172 °C (lit. m.p. 174-178 °C)[65]; **^{1}H NMR** (500 MHz, Acetone) δ 9.29 (1H, s, NH), 7.61 (2H, d, J = 8.9 Hz, H-2), 7.44 (2H, d, J = 8.9 Hz, H-3), 2.08 (3H, s, CH_3); **^{13}C NMR** (126 MHz, Acetone) δ 168.2 (C=O), 138.9 (C-1), 131.5 (C-3), 120.9 (C-2), 114.9 (C-4), 23.4 (CH_3).

4.9.2 Synthesis of *N*-phenylacetamide 72b[65]

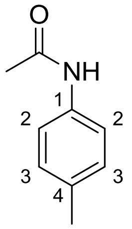
To a solution of aniline (5.00 ml, 54.8 mmol) in DCM (60 ml), was added Ac_2O (13.0 ml, 0.137 mmol) and triethylamine (11.5 ml, 82.2 mmol). The above described procedure was used to synthesize *N*-(4-bromophenyl)acetamide **72b**, isolated as a white crystalline solid (6.9 g, 93%).



R_f = 0.16, 0.63 (3:7 EtOAc/Hex); **m.p.**: 118 °C (lit. m.p. 118-123 °C)[65]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.33 (1H, s, NH), 7.51 (2H, d, J = 7.7 Hz, H-2), 7.31 – 7.23 (2H, m, H-3), 7.07 (1H, t, J = 7.4 Hz, H-4), 2.12 (3H, s, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.1 (C=O), 138.1 (C-1), 128.9 (C-3), 124.3 (C-4), 120.2 (C-2), 24.3 (CH_3).

4.9.3 Synthesis of *N*-(*p*-tolyl)acetamide 72c[66]

p-Toluidine (10.0 g, 93.3 mmol) was dissolved in DCM (100 ml), then Ac_2O (23.5 ml, 0.233 ml) and triethylamine (19.5 ml, 0.140 mol) were added to yield *N*-(*p*-tolyl)acetamide **72c** as a white crystalline solid (8.8 g, 63%).

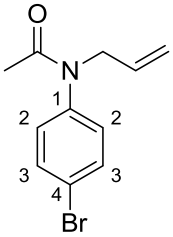


R_f = 0.12 (3:7 EtOAc/Hex); **m.p.**: 153-154 °C (lit. m.p. 152-154 °C)[66]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.55 (1H, s, NH), 7.40 – 7.34 (2H, m, H-2), 7.10 (2H, d, J = 8.0 Hz, H-3), 2.30 (3H, s, CH_3), 2.14 (3H, s, Ac-CH_3); **^{13}C NMR** (126 MHz,

$CDCl_3$) δ 168.5 (C=0), 135.4 (C-1), 133.9 (C-4), 129.4 (C-3), 120.1 (C-2), 24.4 (Ac-CH_3), 20.8 (CH_3).

4.9.4 Synthesis of *N*-allyl-*N*-(4-bromophenyl)acetamide 73a[28,65]

N-(4-Bromophenyl)acetamide **72a** (1.00 g, 4.67 mmol) was dissolved in dry DMF (10 ml), followed by addition of allyBr (0.530 ml, 6.07 mmol) and TBAI (1.73 g, 4.67 mmol). The mixture was stirred at 0 °C for 20 minutes, and then NaH (0.336 g, 0.140 mmol) was added. The yellow heterogeneous mixture was left stirring at room temperature under a N_2 atmosphere for 5 hours. After this time, water was added and the organic material was extracted into diethyl ether. The organic layer was dried with $MgSO_4$, and the solvent was removed under reduced pressure. A clear oil was isolated after purification by silica gel column chromatography (30% EtOAc/Hex) (0.98 g, 83%).

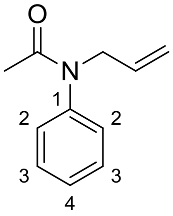


R_f = 0.53 (3:7 EtOAc/Hex); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.53 (2H, d, *J* = 8.5 Hz, H-2), 7.05 (2H, d, *J* = 8.4 Hz, H-3), 5.83 (1H, tt, *J* = 12.7, 5.2 Hz, =CH), 5.17 – 5.01 (2H, m, =CH_2), 4.27 (2H, dt, *J* = 6.3, 1.4 Hz, CH_2), 1.87 (3H, s, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.7 (C=O), 142.0 (C-1), 132.9 (=CH_2), 132.8 (C-3), 129.8 (C-2), 121.7 (C-4), 118.2 (=CH), 51.9 (CH_2), 22.7 (CH_3).

The following compounds were prepared by the method described for *N*-allyl-*N*-(4-bromophenyl)acetamide **73a**.

4.9.5 Synthesis of *N*-allyl-*N*-phenylacetamide 73b[68]

To a solution of *N*-phenylacetamide **72b** (2.00 g, 14.8 mmol) in DMF (30 ml) was added allylBr (2.56 ml, 29.6 mmol), NaH (1.07 g, 44.4 mol) and TBAI (5.47 g, 1.48 mmol)to yield *N*-allyl-*N*-(4-bromophenyl)acetamide **73b** as a clear oil (2.0 g, 76%).

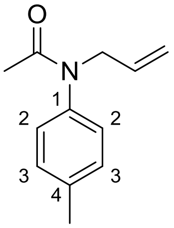


R_f = 0.44 (3:7 EtOAc/Hex); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45 – 7.26 (3H, m, H-2 and H-4), 7.17 (2H, t, *J*=6.7 Hz, H-3), 5.97 – 5.75 (1H, m, =CH), 5.17 – 4.96 (2H, m, =CH_2), 4.37 – 4.20 (2H, m, CH_2), 1.86 (3H, s, CH_3)**^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.0 (C=O), 143.0 (C-1), 133.1 (=CH_2), 129.6

(C-3), 128.1 (C-2), 127.9 (C-4), 117.7 (=CH), 52.0 (CH_2), 22.6 (CH_3).

4.9.6 Synthesis of *N*-allyl-*N*-(*p*-tolyl)acetamide 73c[67]

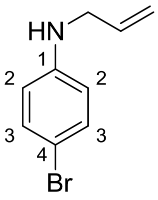
N-(*p*-tolyl)acetamide **72c** (6.54 g, 43.9 mmol), allylBr (7.59 ml, 87.7 mmol), NaH (3.15 g, 0.131 mmol) and TBAI (16.2 g, 43.9 mmol) in DMF (60 ml) afforded *N*-allyl-*N*-(*p*-tolyl)acetamide **73c** as a brown oil (7.0 g, 85%)



R_f = 0.53 (3:7 EtOAc/Hex); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.29 – 7.13 (2H, m, H-2), 7.10 – 6.95 (2H, m, H-3), 5.86 (1H, ddt, *J* = 16.7, 10.3, 6.3 Hz, =CH), 5.18 – 4.94 (2H, m, =CH_2), 4.28 (2H, dt, *J* = 6.3, 1.4 Hz, CH_2), 2.37 (3H, s, CH_3), 1.85 (3H, s, Ac-CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.1 (C=O), 140.4 (C-1), 137.7 (C-4), 133.3 (=CH_2), 130.1 (C-3), 127.8 (C-2), 117.6 (=CH), 52.0 (CH_2), 22.6 (Ac-CH_3), 21.0 (CH_3).

4.9.7 Synthesis of *N*-allyl-4-bromoaniline 74a[69,70]

N-allyl-*N*-(4-bromophenyl)acetamide **73a** (2.70 g, 10.6 mmol) was dissolved in ethanol/water (10:1, 20:2 ml) and then treated with NaOH (4.25 g, 0.106 mol). The mixture was heated at reflux for 2 hours, until the starting material had been consumed. 1M aqueous HCl (15 ml) was added to neutralize the excess base and the organic layer was extracted into diethyl ether. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The resulting oily residue was further purified by silica gel column chromatography (15% EtOAc/Hex). The product **74a** was isolated as a clear brown oil (2.0 g, 88%).

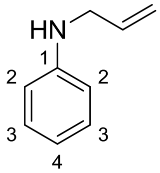


R_f = 0.63 (3:7 EtOAc/Hex); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.27 – 7.14 (2H, m, H-2), 6.49 – 6.39 (2H, m, H-3), 5.88 (1H, ddt, *J* =1 7.2, 10.4, 5.3 Hz, =CH), 5.29 – 5.09 (2H, m, =CH_2), 3.80 (1H, s, NH), 3.69 (2H, dt, *J* = 5.3, 1.7 Hz, CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 146.9 (C-1), 134.8 (=CH), 131.8 (C-3), 116.4 (=CH_2), 114.4 (C-2), 108.9 (C-4), 46.3 (CH_2).

The following compounds were prepared using the above described procedure for *N*-allylaniline **74a**

4.9.8 Synthesis of *N*-allylaniline 74b[71]

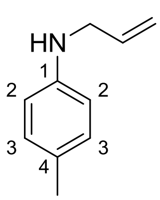
N-allylaniline **74b** was successfully synthesized by reacting *N*-allyl-*N*-phenylacetamide **73b** (10.0 g, 57.07 mmol)in ethanol/water (60:6 ml) with NaOH (22.9 g, 0.571 mol) to yield a yellow oil (6.8 g, 90%).



R_f = 0.86 (3:7 EtOAc/Hex); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.48 – 7.33 (2H, m, H-3), 6.99 – 6.88 (1H, m, H-4), 6.85 – 6.75 (2H, m, H-2), 6.14 (1H, ddt, *J* = 17.4, 10.2, 5.0 Hz, =CH), 5.56 – 5.30 (2H, m, $=CH_2$), 4.10 – 3.78 (3H, m, CH_2 and NH); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 148.0 (C-1), 135.5 (=CH), 129.1 (C-3), 117.4 (C-4), 116.0 ($=CH_2$), 112.9 (C-2), 46.4 (CH_2).

4.9.9 Synthesis of *N*-allyl-4-methylaniline 74c[71]

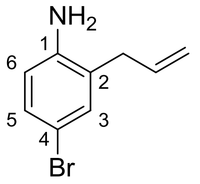
N-allyl-*N*-(*p*-tolyl)acetamide **73c** (6.00 g, 31.7 mmol) was treated with NaOH (12.7 g, 0.317 mol) in a mixture of ethanol/water (30:3 ml). *N*-allyl-4-methylaniline **74c** was isolated as a yellow oil (4.3 g, 92%).



R_f = 0.75 (3:7 EtOAc/Hex); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.06 – 6.88 (2H, m, H-2), 6.60 – 6.44 (2H, m, H-3), 6.04 – 5.81 (1H, m, =CH), 5.32 – 5.04 (2H, m, $=CH_2$), 3.83 – 3.46 (3H, m, CH_2 and NH), 2.22 (3H, s, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 145.8 (C-1), 135.7 (=CH), 129.7 (C-3), 126.6 (C-4), 116.0 ($=CH_2$), 113.2 (C-2), 46.9 (CH_2), 20.4 (CH_3).

4.9.10 Synthesis of 2-allyl-4-bromoaniline 75[49]

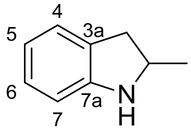
N-allyl-4-bromoaniline **74a** (0.200 g, 0.943 mmol) was dissolved in xylene (5 ml) in a round bottom flask equipped with a stirrer bar and sealed with a Teflon adaptor. $BF_3.OEt_2$ (0.140 ml, 1.13 mmol) was added and the reaction mixture was heated at 140 °C for 19 hours. After cooling to room temperature, brine was added and the organic layer was extracted into diethyl ether. The organic layer was dried over $MgSO_4$, filtered through a pad of Celite and the solvent was removed by rotary evaporation. The resulting dark residue was purified by silica gel column chromatography (15% EtOAc/Hex). 2-allyl-4-bromoaniline **75** was isolated as dark brown oil (19 mg, 10 %).



R$_f$ = 0.86 (3:7 EtOAc/Hex); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.20 – 7.10 (3H, m, H-3 and H-5), 6.55 (1H, d, *J* = 9.0 Hz, H-6), 5.91 (1H, td, *J* = 10.5, 10.5, 5.1 Hz, =CH), 5.23 – 5.01 (2H, m, =CH_2), 3.66 (2H, s, NH_2), 3.25 (2H, d, *J*=5.9 Hz, CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 143.9 (C-4), 135.0 (=CH), 132.6 (C-3), 130.1 (C-5), 126.1 (C-2), 117.2 (C-6), 116.8 (=CH_2), 110.4 (C-4), 36.1 (CH_2).

4.9.11 Synthesis of 2-methylindoline 76[50]

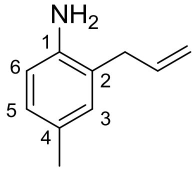
N-allylaniline **74b** (1.50 g, 11.3 mmol) was dissolved in xylene (20 ml) in a round bottom flask fitted with a reflux condensor. $BF_3.OEt_2$ (1.66 ml, 13.5 mmol) was added and the reaction mixture was heated in an oil bath set at 170 °C over 3 days. After cooling to room temperature, brine was added and the organic layer was extracted into diethyl ether. The organic layer was dried over $MgSO_4$ and filtered through a pad of celite. The solvent was removed under reduced pressure. The resulting dark residue was purified by silica gel column chromatography (5% EtOAc/Hex) to afford 2-methylindoline **76** (0.97 g, 65%)



R$_f$ = 0.63 (3:7 EtOAc/Hex); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 – 7.03 (1H, m, H-4), 6.99 (1H, td, *J* = 7.6, 1.3 Hz, H-6), 6.66 (1H, td, *J* = 7.4, 1.1 Hz H-5), 6.57 (1H, d, *J* = 7.8 Hz, H-7), 4.01 – 3.83 (1H, m, CH), 3.69 (1H, s, NH), 3.16 – 2.54 (2H, m, CH_2), 1.23 (3H, d, *J* = 6.3 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 150.9 (C-7a), 128.8 (C-3a), 127.2 (C-6), 124.6 (C-4), 118.4 (C-5), 109.1 (C-7), 55.2 (CH), 37.7 (CH_2), 22.2 (CH_3); **HRMS** (m/z), calculated for $C_9H_{12}N$: 134.0970, found $(M + H)^+$: 134.0987.

4.9.12 Synthesis of 2-allyl-4-methylaniline 77[72]

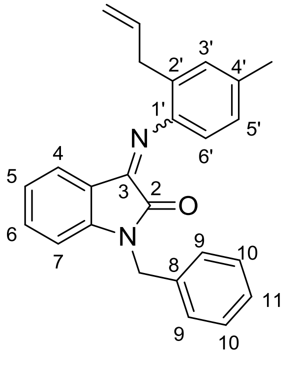
To a solution of *N*-allyl-4-methylaniline **74c** (3.00 g, 20.3 mmol) and chlorobenzene (20 ml) was added $BF_3.OEt_2$ (12.6ml, 0.102 mol) and the reaction mixture was stirred at reflux (120 °C) under a N_2 atmosphere for 3 days. After cooling to room temperature, EtOAc was added and the organic layer partitioned between brine and diethyl ether. The mixture was dried over $MgSO_4$ and filtered through a pad of Celite. The solvent was removed under reduced pressure, resulting in a dark residue purified by silica gel column chromatography (5% EtOAc/Hex). The product **77** was afforded as a dark brown oil (0.55 g, 18%).



R*f* = 0.69 (3:7 EtOAc/Hex); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.91 – 6.80 (2H, m, H-3 and H-5), 6.56 (1H, d, *J* = 7.7 Hz, H-6), 6.08 – 5.79 (1H, m, =CH), 5.27 – 4.94 (2H, m, $=CH_2$), 3.57 (2H, s, NH_2), 3.33 – 3.15 (2H, m, CH_2), 2.22 (3H, s, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 142.2 (C-1), 136.1 (=CH), 130.7 (C-3), 128.0 (C-4), 127.9 (C-5), 124.1 (C-2), 116.0 (C-6), 115.9 ($=CH_2$), 36.4 (CH_2), 20.4 (CH_3).

4.9.13 Synthesis of 3-((2-allyl-4-methylphenyl)imino)-1-benzylindolin-2-one 78[56]

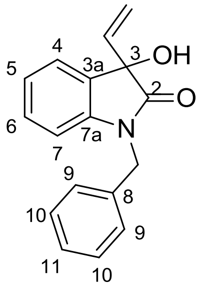
1-Benzylindoline-2,3-dione **62** (0.411 g, 1.73 mmol) and 2-allyl-4-methylaniline **77** (0.255 g, 1.73 mmol) were dissolved in a solution of acetonitrile and acetic acid (2:1, 10:5 ml), followed by the addition of $MgSO_4$ (1.67 g, 13.8 mmol). The mixture was stirred at reflux overnight under a N_2 atmosphere. $MgSO_4$ was filtered off through a pad of celite and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5% EtOAc/Hex) to afford 3-((2-allyl-4-methylphenyl)imino)-1-benzylindolin-2-one **78** as a blood red oil (0.24 g, 38%).



R*f* = 0.69 (3:7 EtOAc/Hex); **IR** ν_{max} (cm^{-1}): 3061 (=C-H), 2963 (C-H), 2897 (C-H), 1725 (C=O), 1654 (C=C), 1638 (C=N); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.42 – 7.27 (5H, m, Bn-H), 7.25 – 7.17 (1H, m, H-6), 7.16 – 7.11 (1H, m, H-3'), 7.09 – 7.02 (1H, m, H-5'), 6.80 – 6.62 (4H, m, H-4, H-5, H-6' and H-7), 5.87 – 5.72 (1H, m, =CH), 5.01 (2H, s, Bn-CH_2), 4.98 – 4.81 (2H, m, $=CH_2$), 3.31 (2H, dd, *J* = 6.7, 1.5 Hz, Allyl CH_2), 2.38 (3H, s, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 163.2 (C=O), 154.2 (C=N), 146.9 (Ar-C), 146.2 (Ar-C), 136.7 (=CH), 135.3 (Ar-C), 135.2 (Ar-C), 133.7 (C-6), 130.7 (C-3'), 129.0 (Ar-C), 128.9 (C-10), 127.9 (C-11), 127.7 (C-5'), 127.4 (C-9), 126.0 (C-4), 122.7 (C-5), 116.8 (C-6'), 116.3 (Ar-C), 115.8 ($=CH_2$), 110.2 (C-7), 44.0 (Bn-CH_2), 36.0 (Allyl CH_2), 21.1 (CH_3); **HRMS** (m/z), calculated for $C_{25}H_{23}N_2O$: 367.1810, found $(M + H)^+$: 367.1805.

4.9.14 Synthesis of 1-benzyl-3-hydroxy-3-vinylindolin-2-one 83[55]

A solution of 1-benzylindoline-2,3-dione **62** (1.00 g, 4.22 mmol) and THF (10 ml) was cooled to -40 °C, then vinylmagnesium bromide (5.06 ml, 5.06 mmol, 1M in THF) was added. After stirring for an hour at -40 °C, the reaction temperature was raised to room temperature overnight under a N_2 atmosphere. Saturated aqueous NH_4Cl was added and the organic layer was extracted into EtOAc. The solvent was removed *in vacuo* and the product was purified by silica gel column chromatography (30% EtOAc/Hex) yielding 1-benzyl-3-hydroxy-3-vinylindolin-2-one **83** as orange crystals (0.92 g, 82%).



R_f = 0.34 (3:7 EtOAc/Hex); **m.p.**: 103-104 °C **IR** ν_{max} (cm^{-1}): 3314 (OH), 3030 (=C-H), 1707 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.33 (1H, dd, *J* = 7.4, 1.3 Hz, H-7), 7.29 – 7.18 (5H, m, Bn-H), 7.14 (1H, td, *J* = 7.7, 1.4 Hz, H-6), 7.01 (1H, td, *J* = 7.5, 1.0 Hz, H-5), 6.67 (1H, d, *J* = 7.8 Hz, H-4), 6.09 (1H, dd, *J* = 17.2, 10.5 Hz, =CH), 5.47 – 5.21 (2H, m, $=CH_2$), 5.02 – 4.63 (3H, m, OH and Bn-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.3 (C=O), 142.2 (C-7a), 136.4 (=CH), 135.3 (C-6), 129.6 (Ar-C), 129.5 (Ar-C), 128.7 (C-10), 127.6 (C-11), 127.1 (C-9), 124.8 (C-4), 123.2 (C-5), 116.8 ($=CH_2$), 109.6 (C-7), 77.2 (C-3), 43.7 (Bn-CH_2); **HRMS** (m/z), calculated for $C_{17}H_{16}NO_2$: 266.1181, found $(M + H)^+$: 266.1180.

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6 APPENDIX