Evaluating the Fungistatic and Fungicidal Activities of Bianthrones as Synthetic Intermediates to Novel Hypericin Analogues.

By

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A thesis submitted in partial fulfilment for the requirements of the degree of MSc (by Research) at the University of Central Lancashire.

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STUDENT DECLARATION

I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution

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Type of Award **MSc (by Research)**

School **The School of Forensic and Investigative Sciences**

ABSTRACT

There is increasing demand for new and improved antifungal agents due to an increase in fungal infections and diseases in patients being resistant to previous antifungals. The focus of this research is on the synthesis of anthraquinone, anthrone, bianthrone and hypericin type derivatives and the effect they have on the growth of the yeast species *Schizosaccharomyces pombe, Lipomyces starkeyi* and *Saccharomyces cerevisiae*. It is hoped that a new antifungal agent could be identified to combat the threat of these resistant fungi in order to treat patients who have compromised immune systems due to HIV/AIDS, cancer treatments or in the case of organ transplant patients due to the presence of immune suppressants. The research approach adopted in this study includes the synthesis of anthraquinone, anthrone and bianthrones as hypericin derivatives and their effect on yeast by a growth inhibition assay. The findings of this research are that these derivatives give interesting results in the yeast growth inhibition assays with a few of the compounds synthesised being fungicidal in their activity and many of the compounds showing low inhibitory concentrations. The main conclusions drawn from this research are that the anthraquinone, anthrone, bianthrone and hypericin type derivative compounds, especially the amine derivatives of anthraquinone, show promise in the area of antifungal agents. The recommendations are that more derivatives are synthesised and the growth inhibition assay is repeated, testing for resorufin fluorescence inhibition, which gives greater sensitivity.

CONTENTS

LIST OF FIGURES, SCHEMES AND TABLES

ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immuno Deficiency Syndrome
SSRI	Selective Serotonin Reuptake Inhibitor
DNA	Deoxyribonucleic Acid
MIC	Minimum Inhibitory Concentration
1,8-DHA	1,8-Dihydroxyanthraquinone
p-TsCl	4-Toluenesulphonyl chloride
t-BuOK	Potassium *tert*-butoxide
S. pombe	Schizosaccharomyces pombe
L. starkeyi	Lipomyces starkeyi
S. cerevisiae	Saccharomyces cerevisiae
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
$CHCl_3$	Chloroform
DMF	Dimethylformamide
TMS	Tetramethylsilane
LCMS – EI	Liquid Chromatography Mass Spectrometry – Electron Ionisation
UV	Ultra Violet
TLC	Thin Layer Chromatography
TEA	Triethylamine
AcOH	Acetic acid
YE	Yeast Extract
YPD	Yeast Peptone Dextrose

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CHAPTER 1. INTRODUCTION

1.1 Literature Review

1.1.1 Fungal Infections

Out of the more than 50,000 species of fungi present in the environment only 200 species are associated with human diseases and of these only 20 – 25 are common causes of infection.[1] Fungal infections are all highly contagious and thrive in warm, moist conditions. There are many different types of fungal infection of which the most common are:

- Athletes foot,[2] or *Tineapedis,* causes scaling, flaking and itching in infected areas,
- Thrush,[3] or *Candida albicans,* causes itching, redness and soreness of infected areas,
- Ringworm,[4] or dermatophytosis, is caused by fungi feeding on keratin in the skin, hair or nails,
- Intertrigo,[5] or Candidalintertrigois, is raw-looking, itchy and sore developing in warm moist areas of the body.

The most common yeast responsible for causing infections is *Candida* and it accounts for the majority of all fungal infections found.[6] For most people a fungal infection is an inconvenient irritation which only causes some soreness or itching and can mostly be treated with creams and ointments applied topically, or with oral antifungal medication. However, for some people a fungal infection can prove dangerous or even fatal.[7] These people have a compromised immune system which has been caused by a variety of different reasons: HIV/AIDS,[8] transplant patients,[9] oncology patients,[9] premature babies and patients who are suffering from long serious illness or injury.[10,11] There have been cases, however, where people have been struck

down with serious fungal infections when they were previously perfectly healthy although these instances are scarce.[12]

Sometimes these infections can lead to more serious diseases like:

- Blastomycosis,[13] which is where the fungus is inhaled into the lungs and can then spread to other areas of the body like the skin, bones and joints. It is extremely rare with most of the cases occurring in India, Israel, Saudi Arabia and Africa.
- Cryptococcosis,[14] is another fungal infection of the pulmonary system, but when it spreads it can cause meningoencephalitis which is a swelling of the brain.[15]
- Mucormycosis,[16] is a fungal infection of the sinuses, brain or lungs and is associated most commonly with diabetes.[17]
- Fungal sinusitis,[18] controversially blamed for causing most of the cases of chronic rhino sinusitis, but are mostly benign or non-invasive and brought on by allergies.[19]

1.1.2 Antifungal Agents

Antifungal medications are used to treat fungal infections and they are usually obtained by prescription from the doctor or over the counter. Both fungi and humans are eukaryotes which mean they are similar at the molecular level, but this means that discovering a drug which will kill the fungus without affecting the mammalian host is much more difficult to find or chemically design. Many antifungal drugs can have side effects and these can prove life threatening, sometimes by causing anaphylaxis, if not used properly.[20]

There are five different classes of antifungals all of which are designed to exploit the differences between mammalian and fungal cells:

- Polyene antifungals, which have multiple conjugated double bonds, usually macrocyclic. They are amphiphilic and bind with the sterol in the fungal cell wall. The shorter the conjugation, the sterol binding is increased, which would make it more likely to bind to cholesterol and therefore toxic to animals. For example Natamycin

(Figure 1) a macrocyclicpolyene which binds to ergosterol in the fungal membrane making it more crystalline, allowing ions and small molecules to leak out causing cell death.[21]

- Azole antifungals, which inhibit the enzyme lanosterol 14 α-demethylase, necessary for converting lanosterol to ergosterol and depletion in ergosterol in the fungal membrane disrupts the structure and ultimately the cell growth. For example the imidazole Daktarin (Figure 1).[22]
- Allylamines, inhibit squalene epoxidase, another enzyme required for ergosterol synthesis. For example Lamisil (Figure 1).[23]
- Echinocandins, can be used for systemic fungal infections in immuno compromised patients, they inhibit the synthesis of glucan in the cell wall via the enzyme 1,3-β glucan synthase. They are absorbed poorly when administered orally, but have a greater effect when given by localised injection. For example Anidulafungin.[24]
- Alternatives, which includes mostly essential oils, for example Citronella Oil.[25]

Due to the similarity between fungi and mammalian cells antifungal medications can have side effects such as liver damage, oestrogen unbalancing and allergic reactions leading to anaphylaxis shock.[26] They can also have interactions with other drugs (cytochrome P450, immunosuppressant's, chemotherapeutics, antidepressants and selective serotonin reuptake inhibitors (SSRIs)) causing serious problems.[27]

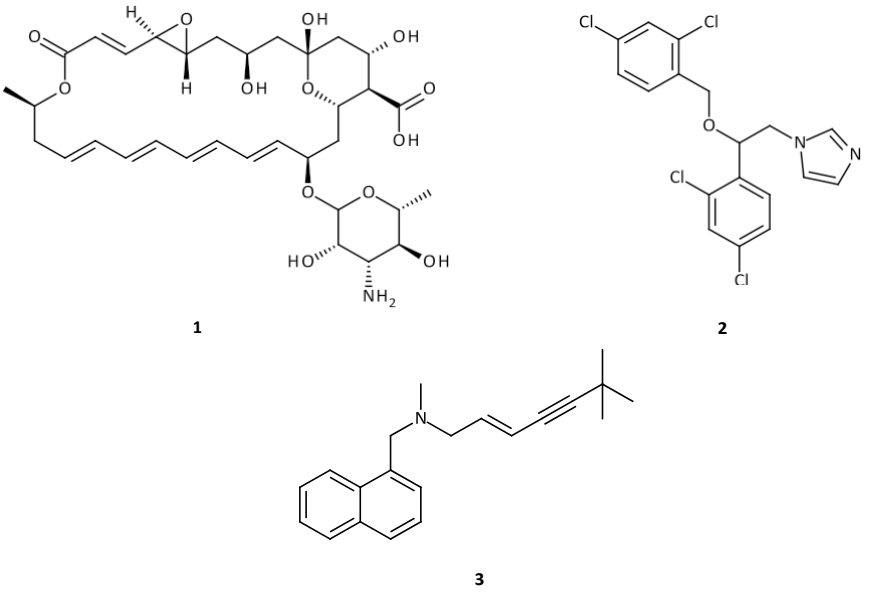


Figure 1The structures of Natamycin (1), Daktarin (2) and Lamisil (3)

One of the major downfalls of today's antifungal agents is that the fungi are becoming resistant to them.[28, 29] This is most probably due to the fact fungi are living organisms and therefore can evolve and develop mutations which will change the way the fungi react to antifungal medications.[30, 31] This leaves the area of developing new antifungal agents of critical importance to the medical world.[32, 33]

1.1.3 Antifungal Agents Derived From Plants

It was not until fairly recently that natural products became of interest to the research of synthetic chemists due to the realisation that simple combinatorial libraries did not have the complexity needed to treat antifungal infections and so natural products became the starting point for many new combinatorial libraries to be developed.[34] Many natural products have seen an emergence of research and one of those is St John's Wort.[35]

St John's Wort (*Hypericum perforatum*) is a perennial herb which grows up to 1m high, with a pungent aromaticity and bright yellow-orange flowers (Figure 2). It has been used throughout history for various medicinal applications with the earliest reference being in *c*1525 when the philosopher Paracelcus recommended it for treating wounds and hallucinations.[36] The plant

gets its name from St John the Baptist whose feast day on the 24th June coincides with when it is in full bloom.[37]

Figure 2 Hypericum Perforatum

The five yellow petals bear a resemblance to the halo of the saint with the red sap symbolising his blood. Hypericum means 'greatest health' in Greek and the term perforatum comes from the tiny oil glands present in the leaves, which give them a perforated look. The most common use for the herbal remedy of St. John's Wort is as a treatment for depression; it is thought that it inhibits serotonin reuptake similar to more conventional SSRIs and has been suggested to be more effective than the common SSRI, paroxetine (Figure 3), in moderate to severe depression.[38] St. John's Wort has medicinally active compounds in the petals, buds, leaves and stem of the plant which could contribute to the pharmacological effects shown, but it is the naphthodianthrones and flavanoids which are the two most active. It is the naphthodianthrones which are thought to be responsible for the anti-depressive nature of St. John's Wort; two of the most studied are the quinones, hypericin **(1)** and pseudohypericin **(2)** (Figure 4).[39]

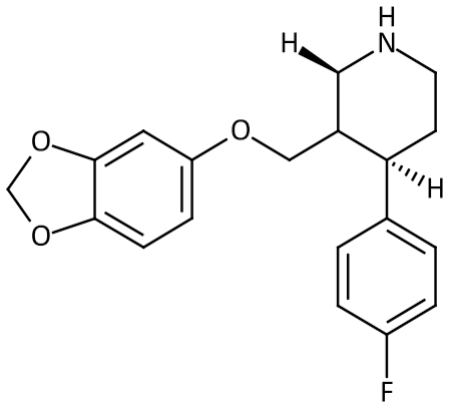
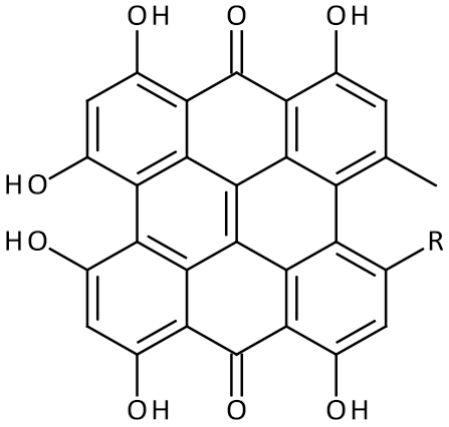


Figure 3 The structure of Paroxetine



(1) Hypericin: R=CH_3
(2) Psuedohypericin: R=CH_2OH

Figure 4 Structures of Hypericin and Pseudohypericin

These quinones are naturally occurring naphthodianthrones, which give a distinctive red, orange or yellow pigmentation in the plants and are found in high concentrations within the flower petals and buds. Pasture fed animals have been known to develop a condition known as hypericism, which is a heightened sun sensitivity brought on by consuming large quantities of St. John's Wort and this can result in severe sunburn and even death in some cases. It is thought that the photoactive action of the quinones is directly responsible.[39]

1.1.4 Hypericin

Hypericin can be isolated from St. John's Wort using an extraction method which yields 0.21% w/w of the product in its crudest form,[40] or it can be synthesised using techniques outlined by

Faulk and Motoyoshiya.[41-43] X-ray diffraction studies have shown hypericin to be non-planar and this is because the side chains repel each other preventing a planar conformation.[44] There are 16 possible hypericin tautomers of which the 7,14-dioxoisomer is the most stable configuration,[45] even when it forms salts with alkali metals in both solution and crystalline states[46]. Hypericin is poorly soluble in water,[47] but if bound to human serum albumin, the dissociation into the monomeric form aids solubility in aqueous physiological solutions.[48]

Hypericin has been studied extensively for its application in photodynamic therapy due to it being one of the most powerful photosensitisers present in nature, possibly due to its high uptake in cells.[49] The reason for this is probably because of its ability to intercalate with or distort DNA. The activity of hypericin is oxygen dependant and this suggests that both the Type I (generation of reactive oxygen species) and Type II (generation of singlet oxygen) mechanisms of photodynamic action are involved.[50] For total necrosis of cells a high light dose needs to be applied which is typically long wavelength red light to aid deeper cell penetration.[50] The high affinity hypericin has for cellular uptake makes it interesting for the treatment of other diseases or infections where cellular penetration of the agent is advantageous, however there has been very little research conducted as to whether hypericin has any antimicrobial function, or in particular antifungal action.

Many synthetic analogues of hypericin have been produced in recent research for a variety of reasons including: improved physiochemical properties,[51] enhanced interaction with DNA,[52] better solubility and accumulation in desired tissues.[53,54] Much of the work has been conducted by Falk and his co-workers who are renowned in this particular field, particularly with their new class of hypericin derivatives containing extra heterocyclic rings,[55-57] but it is their work on the amino functionalised derivatives which gives particular promise due to excellent yields and enhanced solubility.[37] Most of the research to date has focused on the

functionalisation of hydroxyl groups in the *bay* region of hypericin and the biological activity of derivatised *peri* hydroxyl groups has been largely overlooked (Figure 5).

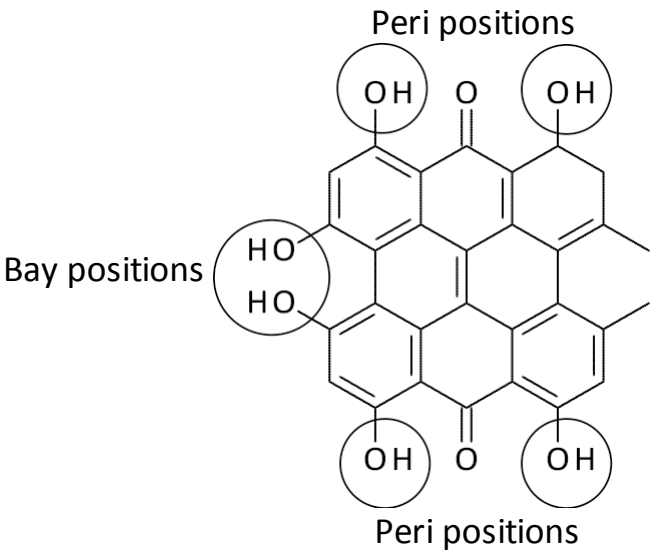


Figure 5 Hypericin showing the bay and peri positions

1.1.5 Anthraquinones

The major building block of Hypericin is anthraquinone. This molecule is a three membered ring with keto groups at the 9th and 10th positions (Figure 6) although there can be many other functional groups attached to the other positions around the rings. As well as hypericin being made up from anthraquinone, many dyes and drugs are based on the anthraquinone structure, for example rubiadin extracted from Morinda officionalis (Figure 7).[56-58] The synthesis of anthraquinone is a simple oxidation of anthracene using chromium(VI),[59] but it can also be produced via a Friedel-Crafts reaction between benzene and phthalic anhydride.[60]

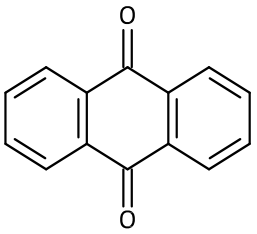


Figure 6 The structure of Anthraquinone

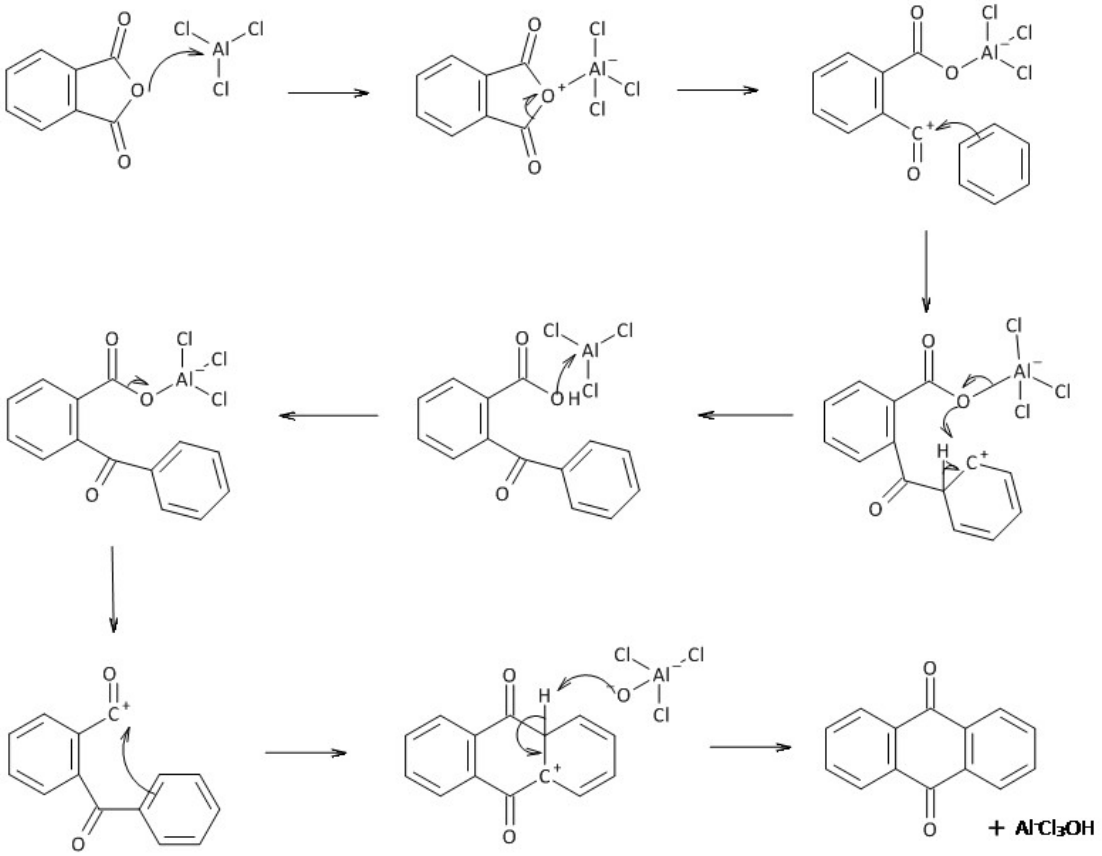


Figure 7 Mechanism showing the synthesis of Anthraquinone

There are many medical uses of anthraquinone based molecules: laxatives (dantron and emodin),[65,61] antimalarials (rufigallol)[62] and as antineoplastics used in the treatment of cancer (mitoxantrone and pixantrone)[63,64] (Figure 8).

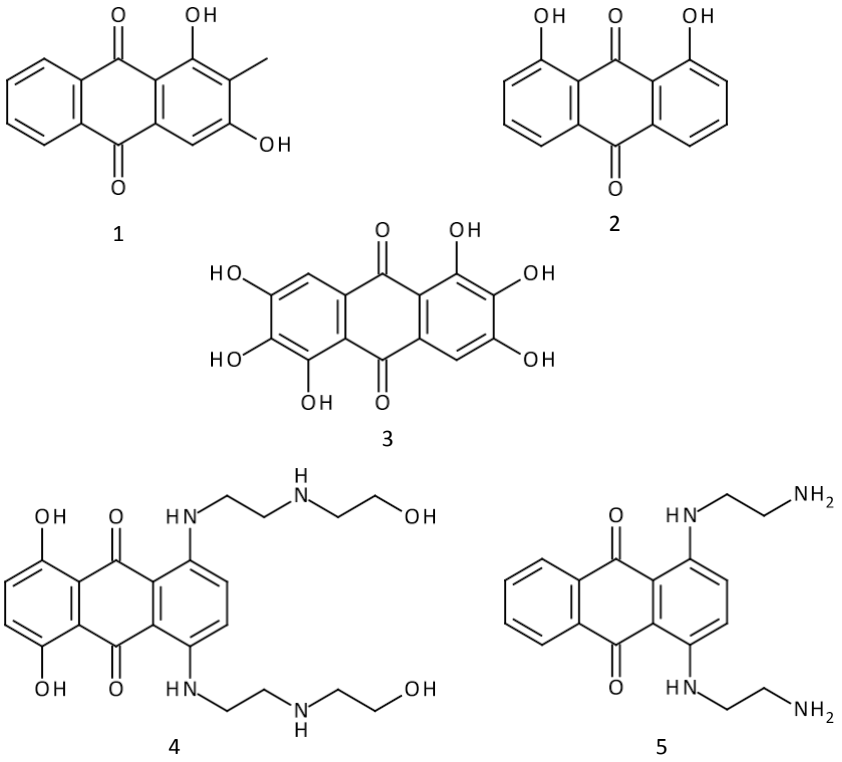


Figure 8 The structures of Rubiadin (1), Dantron (2), Rufigallol (3), Mitoxantrone (4) and Pixantrone (5)

One of the most recent, promising applications of an anthraquinone derivative is as an inhibitor of Tau aggregate formation and as a solvent of paired helical filaments, both of which are considered to be significant to Alzheimer's disease progression, and this has so far been shown in both mouse models and *in vitro* testing.[65] Anthraquinones have also shown antioxidant activity in food,[66] antiviral activity and have also been used in paper pulp manufacture of the raw materials for speciality paper.[67,68]

1.1.6 Anthrones and Bianthrones

Bianthrones are a class of compounds where two anthraquinones are joined via a linker or an immediate bond and they are an intermediate between anthraquinones and hypericin, (Figure 9). They can be found in the natural world in *Alternariaporri,*[69] *Cassia hirsuta* and *Sennamultiglandulosa.*[70,71] Much of the research into bianthrones has focused on the linked

variety with applications ranging from intercalating agents,[72,73] new generation antibiotics and electrochemically switched ion binders.[74,75]

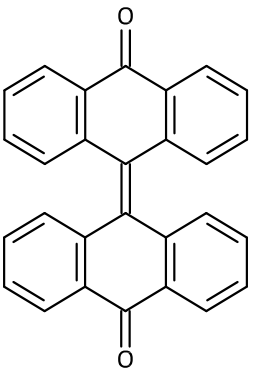


Figure 9 The structure of Bianthrone

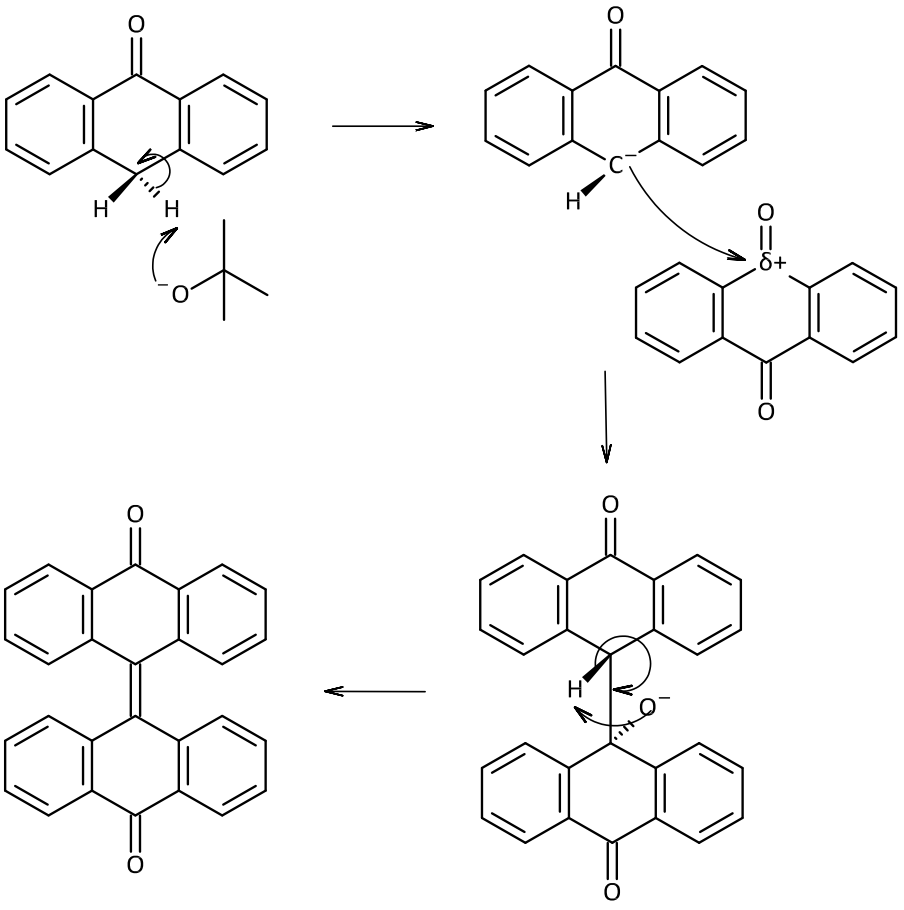


Figure 10 Mechanism showing the synthesis of Bianthrone

1.1.7 Mechanism of Action of Antifungal Agents

Almost all antifungal agents today take on the role of being either fungistatic or fungicidal. If an agent restricts the ability of the fungi to grow then it is termed fungistatic, much like when they are refrigerated, the fungi are preserved keeping them alive for long term storage or further study, or just to inhibit growth. Fungicidal means the killing of all fungi. Other forms of fungicidal activity are the use of heat,[76] electromagnetic or ultraviolet radiation and also exposure to chemicals.[77-78] The term fungicidal generally refers to antifungals which show no further growth at concentrations equal to and greater than the Minimum Inhibitory Concentration (MIC) and all others which will show further growth at the MIC and above are termed fungistatic. However most antifungal agents will have a concentration at which they become fungicidal even if this concentration is much greater than the MIC.[79]

Cell death occurs in one of two ways: apoptosis, the regulated process which is programmed and is led by biochemical events, and necrosis, which is the premature death of cells led by external factors; both are shown in Figure 11 below.[80] It is thought that programmed cell death has evolved to regulate growth and development in unicellular organisms as well as multicellular organisms.[81] Apoptosis is a process for removing unnecessary, damaged or aged cells and can be initiated by a number of intra- or extracellular factors. Dying cells undergo a distinct set of structural changes, which are characteristic to apoptosis and are typically: shrinkage, membrane blebbing, chromatin condensation and DNA fragmentation and finally formation of apoptotic bodies, which are ingested by surrounding cells. Cellular content is never exposed to the immune system minimising inflammatory reactions.[82] Necrosis, by contrast, occurs due to external physical or chemical insult and is characterised by swelling of the entire cytoplasm and organelles which causes the membrane to burst. This results in spillage of the cellular components, ultimately resulting in inflammatory response.[80]

In unicellular organisms, like yeast, cell death always takes place via apoptosis and can occur during development, ageing and reproduction, but it can also be brought on by environmental trauma and contact with toxic agents.[83-87]

Malignant cells are often incapable of triggering apoptosis, so the selective activation of the process is an increasingly attractive therapeutic target. Hypericin has been shown to induce apoptosis in cultured human malignant glioma cells and was followed shortly by other cell lines.[88-97] The conditions used had been poorly defined until the work of Miccoli *et al* in 1998,[89] closely followed by others, which confirmed that under controlled irradiation of different tumour cell lines with hypericin there follows activation of the apoptotic pathway.[90-92]

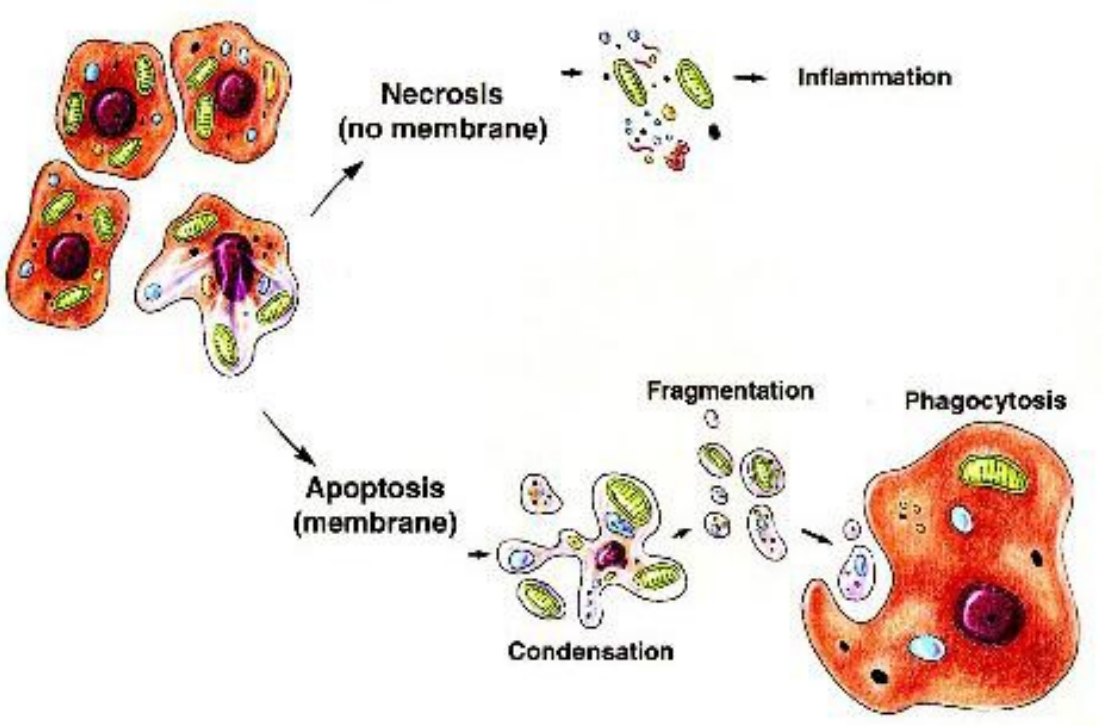


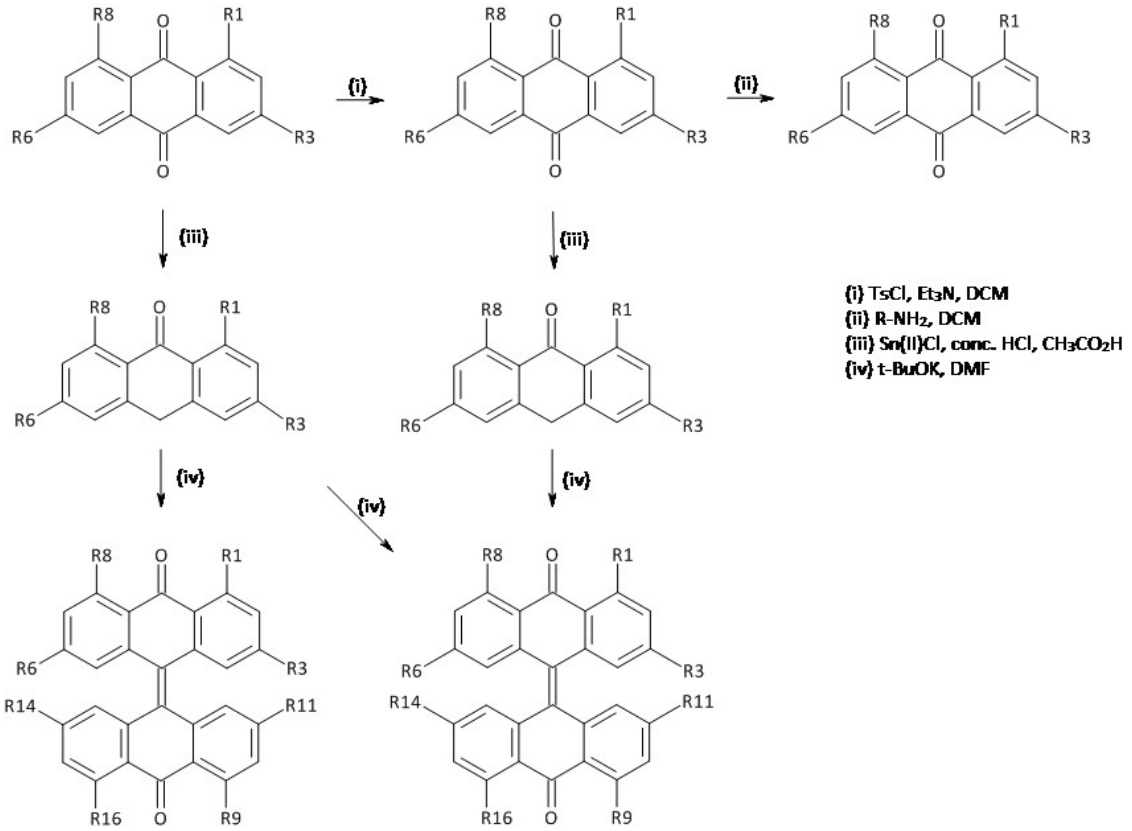
Figure 11 The cell death pathways of necrosis and apoptosis.[93]

1.2 Rationale

Outlined below are the synthetic routes taken for the synthesis of hypericin derivatives, anthraquinone derivatives and bianthrone derivatives. Also detailed is the suggested route to the determination of fungistatic and fungicidal activity of the compounds.

1.2.1 Anthraquinones, Anthrones and Bianthrones

The synthetic route to the anthraquinone, anthrone and bianthrone derivatives (Scheme 1 and Table 1) all start with 1,8-dihydroxyanthraquinone (1,8-DHA). For the various anthraquinones either one or both the hydroxyl groups on the 1,8-DHA is protected with a tosyloxy group using the protecting reagent 4-toluenesulphonyl chloride (p-TsCl) under basic conditions using dichloromethane (DCM) as solvent. The ditosylated anthraquinone is then treated with either ethylenediamine, trimethylenediamine or putrescine to create the aminated anthraquinones. For the synthesis of the anthrones the 1,8-DHA and the tosylated anthraquinones have one of the keto groups reduced using tin(II) chloride ($SnCl_2$) and concentrated hydrochloric acid in acetic acid and these are then irradiated in the microwave to produce the bianthrones using potassium *tert*-butoxide (t-BuOK) to deprotonate the methylene on the central ring.



Scheme 1 The synthetic route

Table 1 Table showing compound functionality

	Compound														
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
R1	OTs	OTs	OH	OH	OTs	OTs	OH	OH	OTs	OTs	OH	$NHC_2H_4NH_2$	$NHC_3H_6NH_2$	$NHC_3H_6NH_2$	$NHC_4H_8NH_2$
R3	H	H	OH	H	H	H	OH	H	H	H	H	H	H	H	H
R6	H	H	CH_3	H	H	H	CH_3	H	H	H	H	H	H	H	H
R8	OH	OTs	OH	OH	OH	OTs	OH	OH	OH	OTs	OH	OH	OH	OTs	OTs
R9							OH	OH	OTs	OTs	OTs				
R11							OH	H	H	H	H				
R14							CH_3	H	H	H	H				
R16							OH	OH	OH	OTs	OTs				

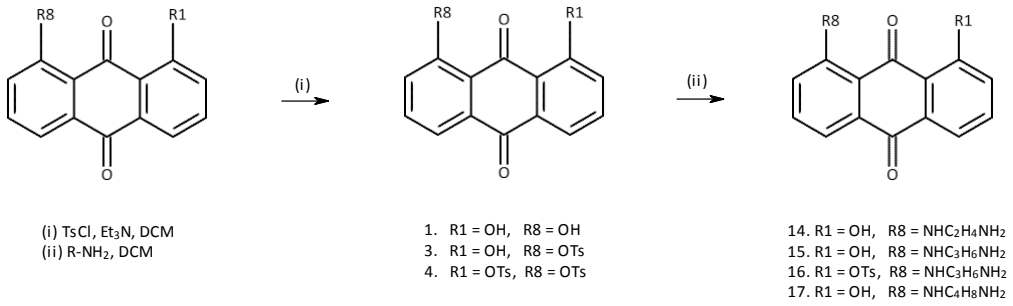
1.2.3 Determination of Antifungal Activity

Three strains of yeast will be used to test, first for the toxicity of the compounds made and then for their fungistatic and fungicidal affects. The three strains of yeast to be used are *Schizosaccharomyces pombe* (*S. pombe*), *Lipomyces starkeyi* (*L. starkeyi*) and *Saccharomyces cerevisiae* (*S. cerevisiae*). These have been chosen because they have been demonstrated to be good representative yeast species and they are non-pathogenic.[1] Therefore, their use in antifungal susceptibility testing and in the screening of chemical libraries for fungicidal effects is advantageous because it reduces exposure to pathogenic fungi.[94] The first step will involve the determination of solubility for each of the compounds being tested: it is hoped that the compounds will be water soluble, but if this is not the case then dimethyl sulfoxide (DMSO) will be used to dissolve the compounds as this is known to be non-toxic up to 10% concentration.[95] For this 0.01g of the compound will be treated with 100µl of media repeatedly until the compound dissolves. If there is no solubility in the media then the process will be repeated in DMSO. Once all the compounds are dissolved they will then be introduced to the yeast strains using serial dilutions and then left to grow overnight. This will be done by transferring 3 x 10^4 yeast cells into each well of a 96-well microtitre plate. The compound will then be transferred into the first column of the plate and then a 1:2 serial dilution will be performed down the row. This will be repeated for each compound, in each yeast, in duplicate. If there is growth in the cell then the compound concentration will be determined to be non-growth inhibitive, but if there is no growth then the compound concentration will be determined as growth inhibitive. The wells with inhibitive concentrations will then be diluted down in 50 ml of media to ensure the compound is strongly diluted, and left to incubate for 16 hrs with shaking at 200rpm at 30°C to see if any growth will occur. If there is growth of yeast present then the compound can be determined as fungistatic; if there is no growth of yeast then this suggests the compound is fungicidal. There were no comparisons made.

CHAPTER 2. RESULTS AND DISCUSSION

2.1 Synthesis of Compounds

Including the two anthraquinones purchased from Sigma Aldrich (Emodin and 1,8-DHA), six pure 1,8-substituted anthraquinone compounds were synthesised. These fitted into two categories, the tosyloxy and amino substituted anthraquinones.



Scheme 2 The anthraquinone synthesis

The synthetic pathway as seen in Scheme 2 shows the route to the tosyloxy protected anthraquinones. This resulted in two pure compounds, the first of which 1-hydroxy-8-tosyloxyanthraquinone (**3**) was produced in reasonable yield (30%) and of excellent purity as judged by the ^{1}H NMR spectrum (Figure 12). This was done using a 4:3 stoichiometric ratio of 1,8-DHA to p-TsCl. After removal of the excess 1,8-DHA using solvent extraction, purification via column chromatography using silica gel, isolated the product as a fine, bright yellow powder.

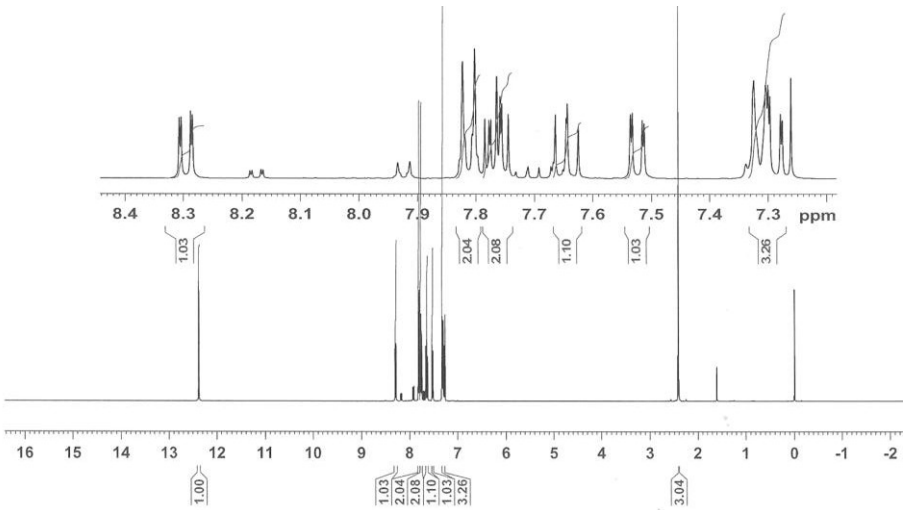


*Figure 12 NMR spectrum of 1-hydroxy-8-tosyloxyanthraquinone (**3**).*

The second compound, 1,8-ditosyloxyanthraquinone (**4**), was produced in good yield (61%) and outstanding purity as judged by the ^{1}H NMR spectrum (Figure 13). This was accomplished using a 10:1 stoichiometric ratio of p-TsCl. The pure compound being isolated after the excess p-TsCl was removed using boiling petroleum ether. This yielded the pure compound as a fine, pale flaxen brown coloured powder.

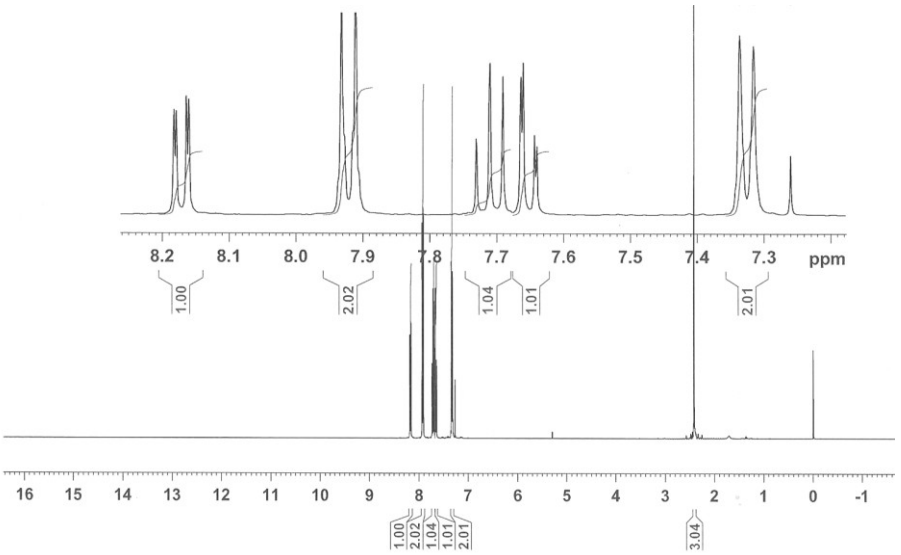


*Figure 13 NMR spectrum of 1,8-ditosyloxyanthraquinone (**4**).*

Four pure compounds were synthesised from the starting materials of 1,8-ditosyloxyanthraquinone (**4**) and the amines ethylenediamine, trimethylenediamine and putrescine. This was possible due to 4-toluenesulphonate being a highly stable leaving group with a distributed negative charge (Figure 14).

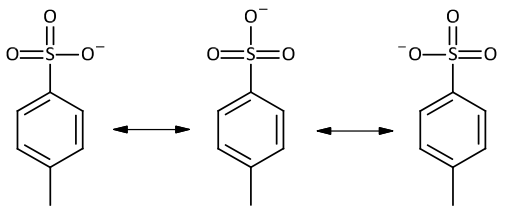


Figure 14 The resonance structures of 4-toluenesulphonate.

1-[(2-Aminoethyl)amino]-8-hydroxyanthraquinone (**14**) was produced in a reasonable yield (34%) with ethylenediamine on a 1:1 ratio with compound 4. The ^{1}H NMR spectrum (Figure 15) shows the methylene chain triplets present implying the synthesis was successful. The product was produced as black crystals and a bright pink solution was observed on addition of chloroform ($CHCl_3$).

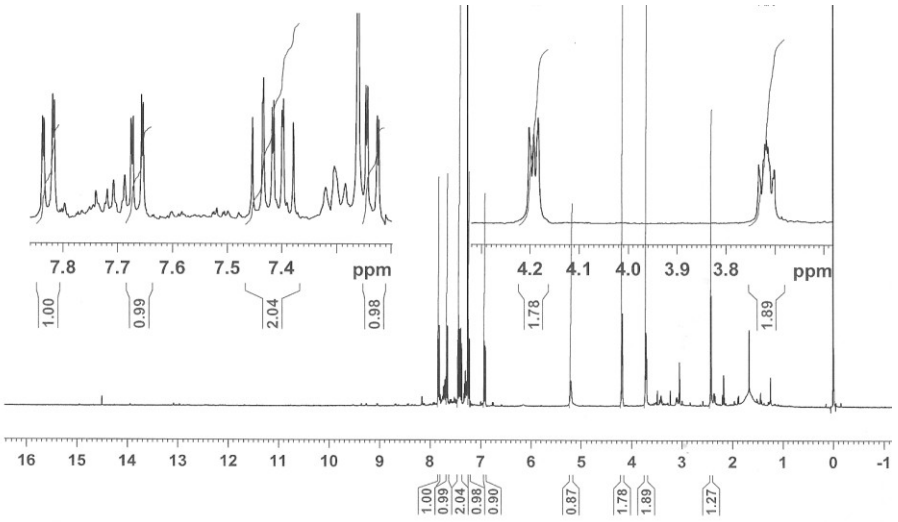


*Figure 15 NMR spectrum of 1-[(2-aminoethyl)amino]-8-hydroxyanthraquinone (**14**).*

The second amine, trimethylenediamine, gave two pure products after purification by column chromatography from one reaction, both of which gave the two triplets and quintet from the methylene chain in their ^{1}H NMR spectra (Figures 16 and 17). The first compound 1-[(3-aminopropyl)amino]-8-hydroxyanthraquinone (**15**), lost the extra tosyloxy group to leave a hydroxyl group and gave a dark purple powder which turned into a bright purple solution on addition of $CHCl_3$. The second compound 1-[(3-aminopropyl)amino]-8-tosyloxyanthraquinone (**16**) retained the tosyloxy group at position 8 and gave a dark red powder which turned into a bright pink solution on addition of $CHCl_3$.

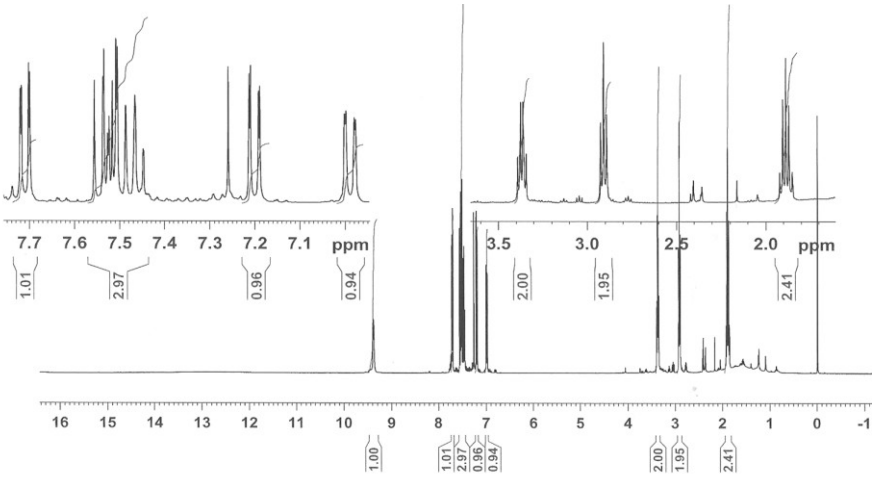


*Figure 16 NMR spectrum of 1-[(3-aminopropyl)amino]-8-hydroxyanthraquinone (**15**).*

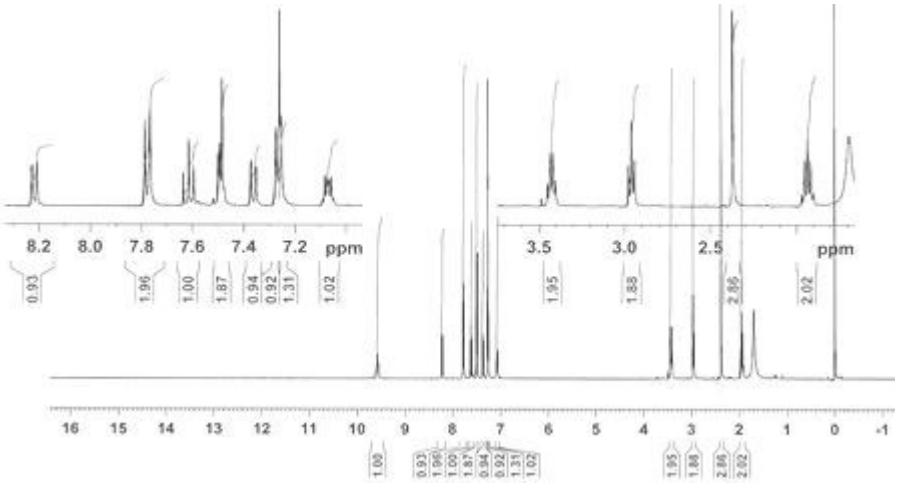


*Figure 17 NMR spectrum of 1-[(3-aminopropyl)amino]-8-tosyloxyanthraquinone (**16**).*

The third amine used, putrescine, gave 1-[(4-aminobutyl)amino]-8-hydroxyanthraquinone (**17**). This product showed two triplets and two quintets for the methylene chain in the ^{1}H NMR spectrum (Figure 18) and gave a dark purple powder which turned into a bright purple solution on addition of $CHCl_3$.

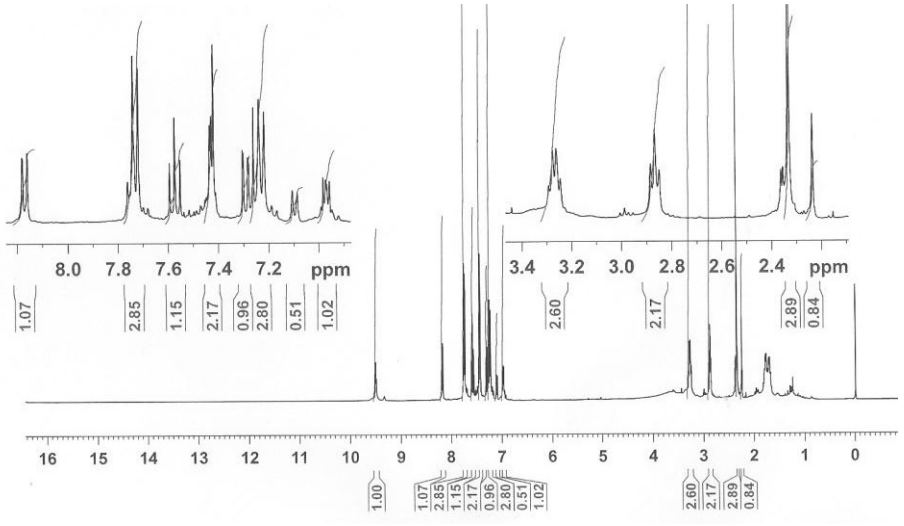
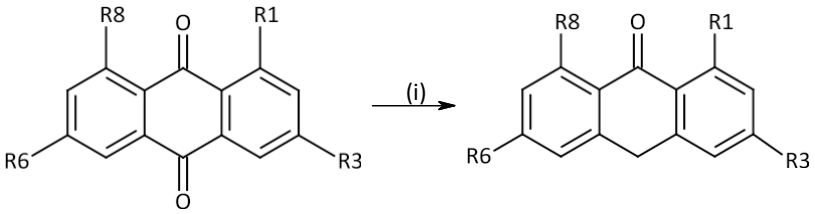


*Figure 18 NMR spectrum of 1-[(4-aminobutyl)amino]-8-hydroxyanthraquinone (**17**).*

The anthraquinone compounds were produced in mostly reasonable yields and of excellent purity. The tosylation reactions worked with fantastic results and were relatively easy to work up, but they were difficult to reproduce on a large scale. Purification of the substituted amine derivatives using column chromatography was tedious requiring on occasion two or more attempts. The reaction itself was straightforward, however, only needing approximately 2 hours to complete.

The next group of compounds in the synthesis of hypericin derivatives, the anthrones, were produced by reducing the carbonyl group chemo-selectively at the 10^{th} position with $SnCl_2$ in the presence of concentrated hydrochloric acid and acetic acid and the reduction was then followed up by a simultaneous acid hydrolysis as shown in Scheme 3. In addition to the two tosyloxy compounds, emodin and 1,8-DHA were also reduced to give four pure compounds.



(i) Sn(II)Cl, Conc. HCl, CH_3CO_2H

5. R1 = OH, R3 = CH3, R6 = OH, R8 = OH
6. R1 = OH, R3 = H, R6 = H, R8 = OH
7. R1 = OTs, R3 = H, R6 = H, R8 = OH
8. R1 = OTs, R3 = H, R6 = H, R8 = OTs

Scheme 3 The anthrone synthesis.

The first: emodin anthrone (**5**) is the reduced form of emodin and this was synthesised as a pale green powder in excellent yield (91%), showing the signature singlet at 4.313ppm on the ^{1}H NMR spectrum (Figure 19).

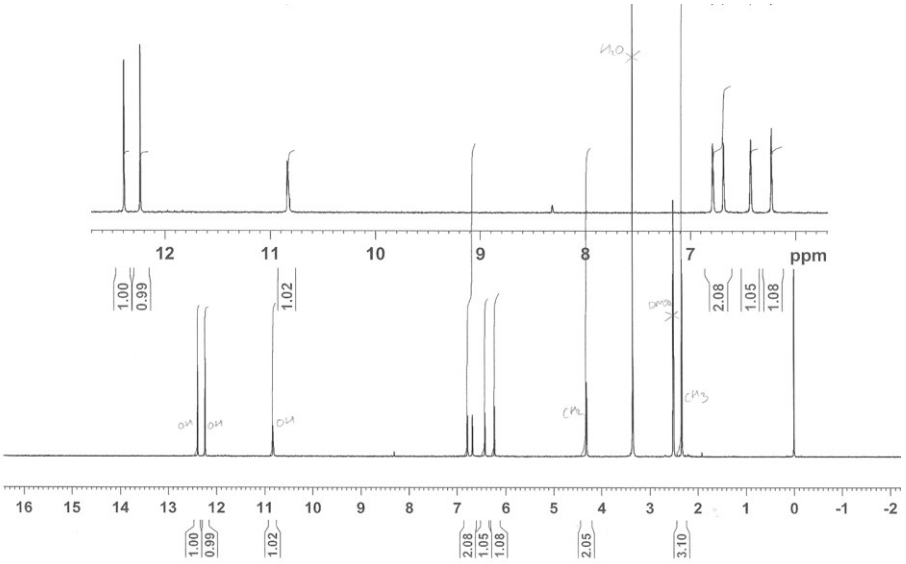


*Figure 19 NMR spectrum of emodin anthrone (**5**).*

The next compound, 1,8-dihydroxyanthrone (**6**), was produced as a buff coloured powder in superb yield (91%) and showed the singlet on the ^{1}H NMR spectrum at 4.472ppm (Figure 20).

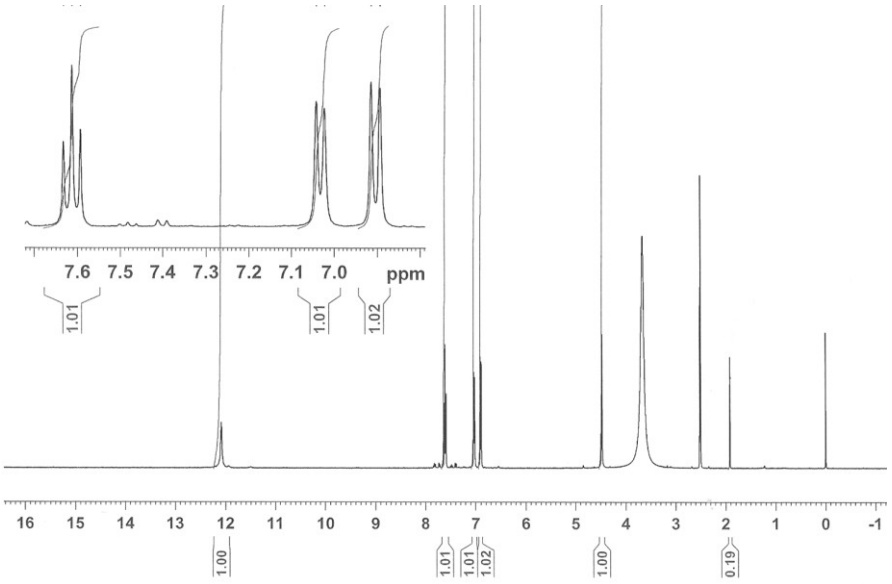
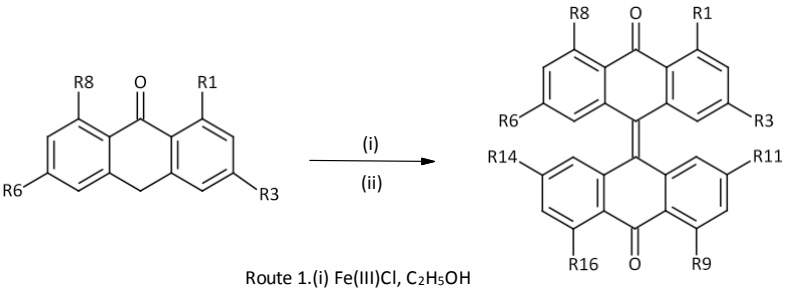


*Figure 20 NMR spectrum of 1,8-dihyroxyanthrone (**6**).*

The next two compounds gave slightly lower yields at 82% for 1-hydroxy-8-tosyloxyanthrone (**7**) and 79% for 1,8-tosyloxyanthrone (**8**). Both were chartreuse coloured powders with singlets present in the ^{1}H NMR spectra at 3.982ppm and 4.313ppm respectively.

The bianthrones followed a different synthetic route to the one shown in Scheme 1 originally, which involved an oxidative coupling using iron(III) chloride hydrate in ethanol as seen in Scheme 4. This method resulted in a very poor yield (<15%), so another method was found using microwave synthesis and t-BuOK in dimethylformamide (DMF). This method greatly improved the process giving five compounds with yields of between 90% at the highest to 32% at the lowest, but was also a much greener method towards the synthesis.



Route 1.(i) Fe(III)Cl, C_2H_5OH
Route 2.(ii) K-t-butoxide, DMF

9. R1 = OH, R3 = CH_3, R6 = OH, R8 = OH, R9 = OH, R11 = CH_3, R14 = OH, R16 = OH
10. R1 = OH, R3 = H, R6 = H, R8 = OH, R9 = OH, R11 = H, R14 = H, R16 = OH
11. R1 = OTs, R3 = H, R6 = H, R8 = OH, R9 = OTs, R11 = H, R14 = H, R16 = OH
12. R1 = OTs, R3 = H, R6 = H, R8 = OTs, R9 = OTs, R11 = H, R14 = H, R16 = OTs
13. R1 = OH, R3 = H, R6 = H, R8 = OH, R9 = OTs, R11 = H, R14 = H, R16 = OTs

Scheme 4 The bianthrone synthesis.

Emodin bianthrone (**9**) gave black crystals at 67% yield and when dissolved in $CHCl_3$ gave a solution with an intense purple colour. The oxidative coupling across the centre went to the double bond, which was seen through the loss of the singlet in the ^{1}H NMR spectrum at 4.313ppm in the starting material. 1,8,9,16-tetrahydroxybianthrone (**10**) had the best yield at 90% of a saffron coloured powder. Only the single bond was achieved with a singlet still present at 4.327ppm in the ^{1}H NMR spectrum (Figure 21), this is distinguishable from the anthrone due to the integration being for just 1 proton.

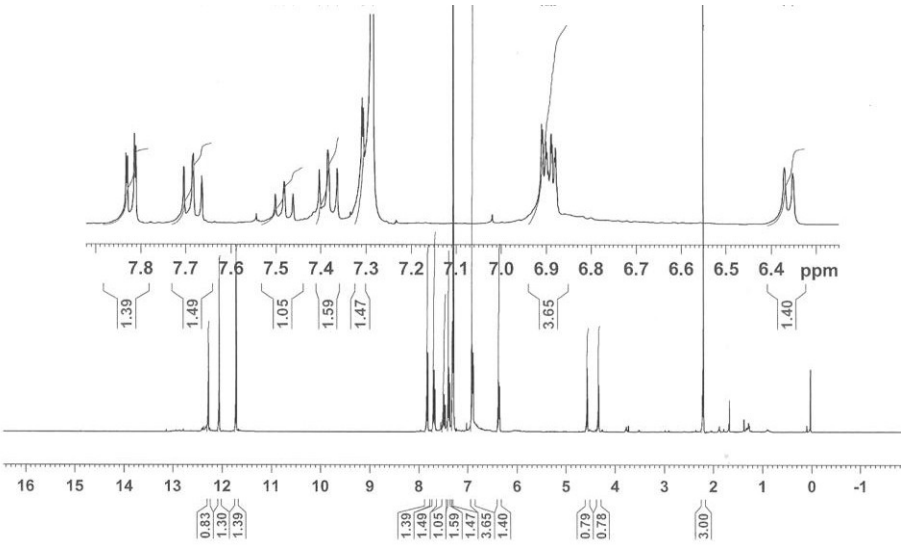


*Figure 21 NMR spectrum of 1,8,9,16-tetrahydroxybianthrone (**10**).*

The next compound 1,9-dihydroxy-8,16-ditosyloxybianthrone (**11**) gave a brown powder with a yield of 80% and again only had the single bond due to the singlet at 4.349ppm present in the ^{1}H NMR spectrum. The last two compounds, 1,8,9,16-tetratosyloxybianthrone (**12**) and 1,8-dihydroxy-9,16-ditosyloxybianthrone (**13**) both gave red coloured powders with compound (**13**) being slightly darker. The yields were 82% and 32% respectively and both have the double bond as shown by the lack of the singlet around 4ppm in their ^{1}H NMR spectra (Figure 22).

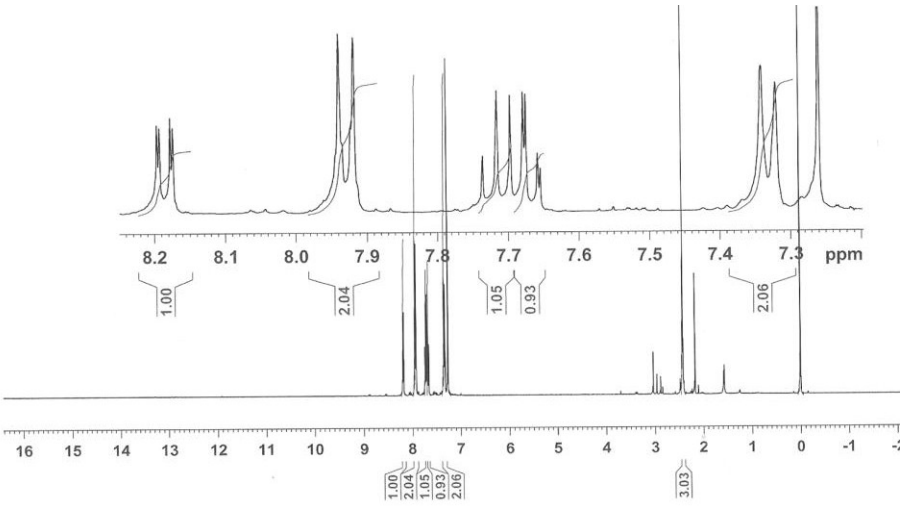


*Figure 22 NMR spectrum of 1,8,9,16-tetratosyloxybianthrone (**12**)*

The procedure for synthesising the anthrones and the bianthrones gave pure products in reasonable to excellent yields. Although the tin(II) chloride reduction for the anthrones was time consuming, needing to run for approximately 12 hours, the reaction worked favourably with good yields and the precipitate which resulted in the work up needed no further purification.

The microwave synthesis improved the reaction time of the bianthrones to just 30 minutes instead of the 5 hours stated in the previous method and the work-up was much simpler, giving a near pure product in the initial precipitate, with only two of the compounds needing further purification by column chromatography. Although two of the compounds (**10** and **11**) gave just the single bond connecting the central rings, when the compounds were put back on to react further, the double bond could not be obtained and they remained as just the single bonded products.

2.2 Antifungal Results

Three types of yeast were used for the determination of growth inhibition: *S. pombe*, a fission yeast used in eukaryotic microbiological research; *S. cerevisiae*, a budding yeast; and *L. starkeyi*, an oleaginous budding yeast from the same genetic tree as *S. cerevisiae*.

All the compounds used for testing were compared against hypericin as a standard. The Table of data and the annotated pictures of the plates can be found in the Appendix.

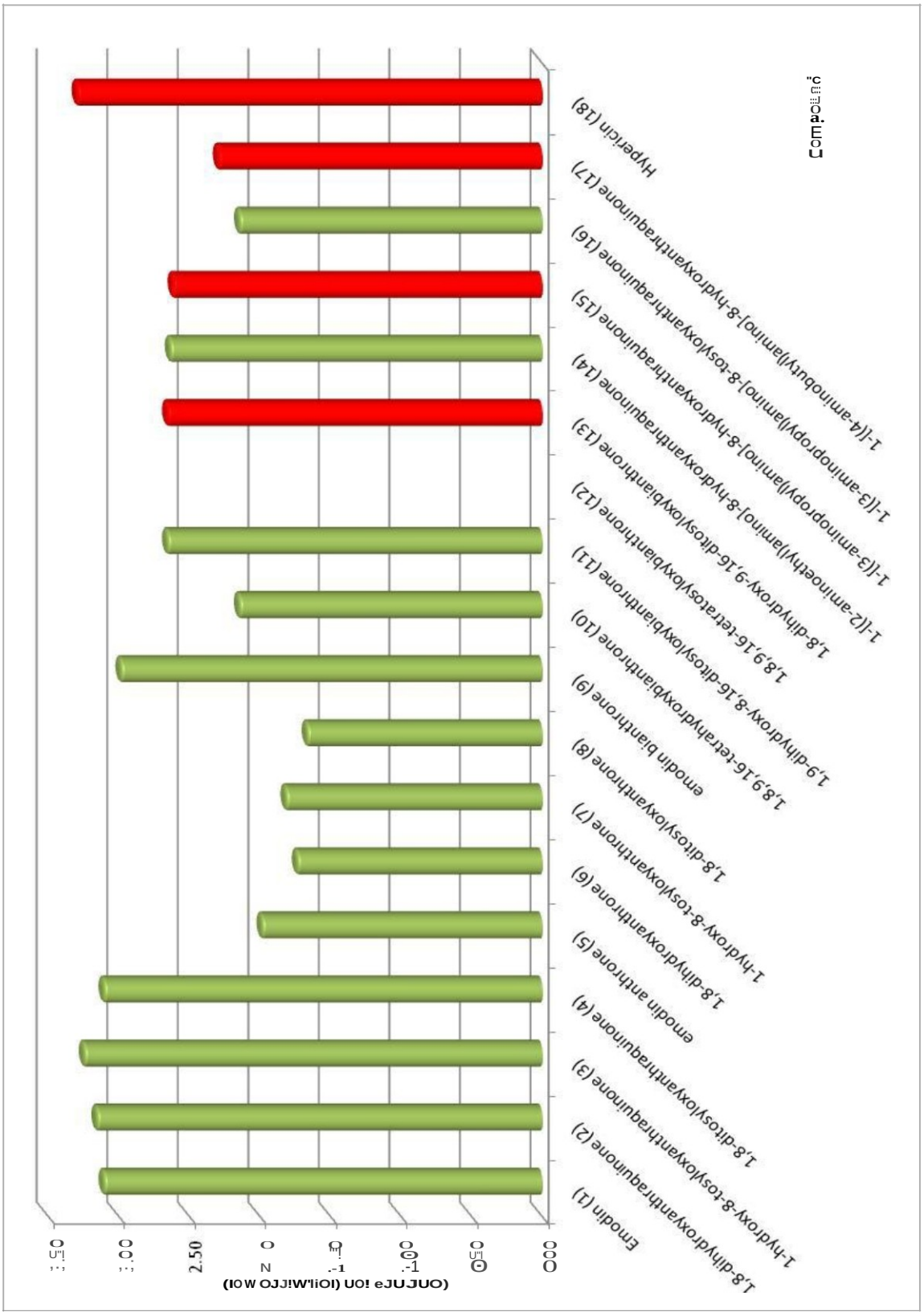


Figure 23 Minimum Inhibitory Growth Concentration (MIC} of synthesised compounds tested in Schizosocchoromyces pombe. Cells were inoculated ot o concentration of 3 x 10 ⁴/ml. Culture media ested were in yeast extract broth (YE).Growth of yeast was determined visually after 24 hours of incubation at 30 C. The MIC of the com pounds was determined to be the well be fore yeast growth was first seen. The experiment was repeated three times to ensure reproducibility of the results. Compound s were determined to be fungicidal (red bars), if no growth was observed and fungistatic (green bars}, if normal growth was seen after inoculation into fresh YE

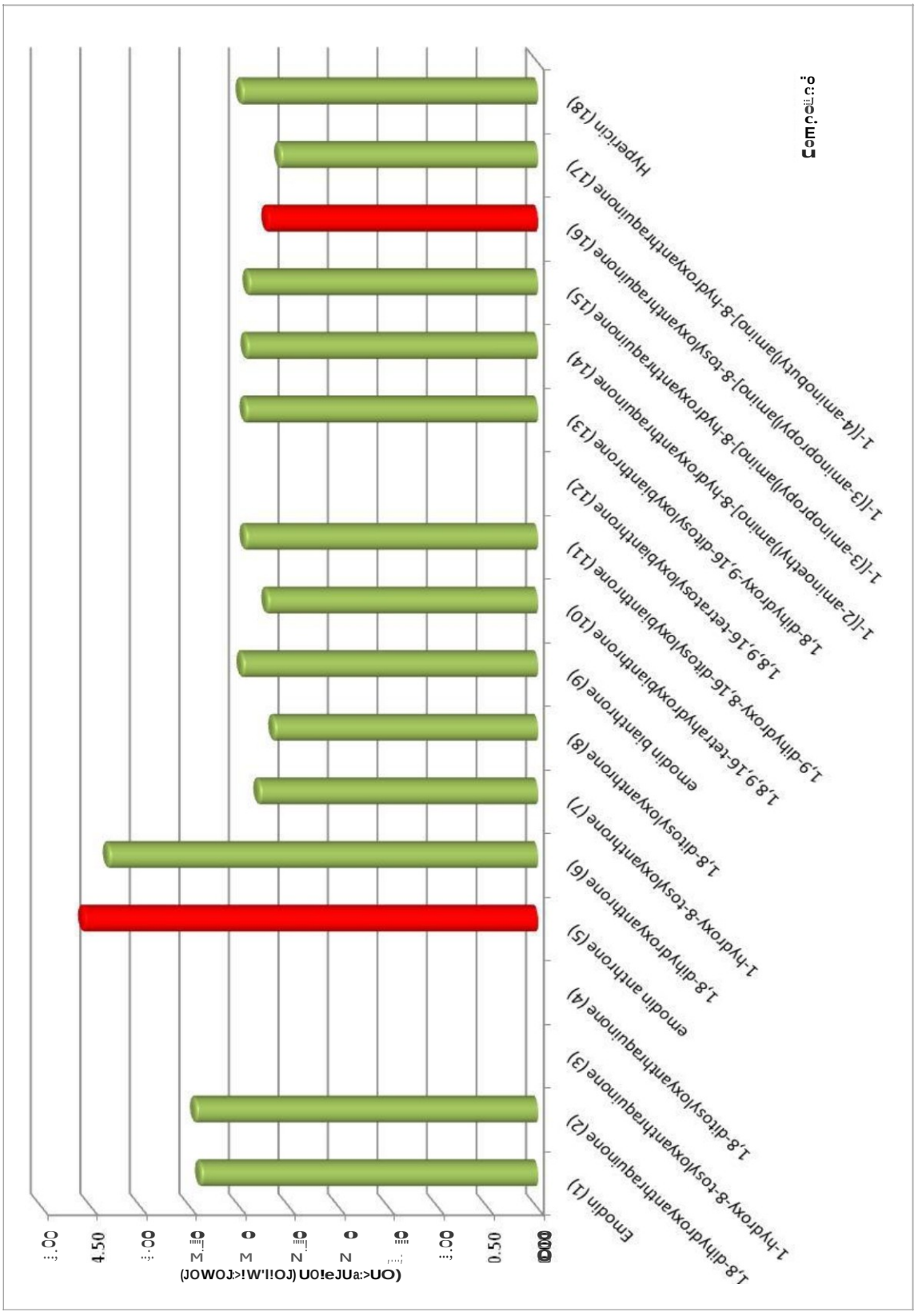


Figure 24 Minimum Inhibitory Growth Concentration {MIC) of synthesised compounds tested in Upomyces starkeyi. Cells were inoculated at a concentration of 3 x 10 /ml. Culture med!a tested were in yeast extract broth (YE). Growth of yeast was determined visually after 24 hours of incubation at 30 C. The MIC of the compounds was determined to be the well before yeast growth was first seen. The experiment was repeated three times to ensure reprod ucibilit y of the results. Compound s were determined to be f un gicidal (red bars), if no growth was observed and fungistatic (green bars), if normal growth was seen after inoculation into fresh YE media.

Figure 25 Minimum Inhibitory Growth Concentration (MJC) of synthesised compounds tested in Saccharomyces cerevisiae. Cells were inoculated at a concentration of 3 x 10 4/ml. Culture media tested were in yeast extract broth (YE). Growth of yeast was determined visually after 24 hours of incubation at 3a·c. The MIC of the compounds was determined to be the well before yeast growth was first seen. The experiment was repeated three times to ensure reproducibility of the results. Compounds were determined to be fungicidal (red bars), if no growth was observed and fungistatic (green bars), if normal growth was seen after inoculation into fresh YE media.

The anthraquinones showed varied results between the yeast strains. In the *S. pombe* they were consistently inhibitive at a concentration greater than 1mM. In the *L. starkeyi,* the emodin and 1,8-DHA were inhibitive at concentrations greater than 2.5 and 2.9 mM, respectively, but 1-hydroxy-8-tosyloxyanthraquinone (**3**) and 1,8-ditosyloxyanthraquinone (**4**) suggested that these compounds protected the yeast cells against the DMSO in which they were dissolved, as growth could be seen in the 1st lane which contained DMSO to a concentration of 10%, known to be inhibitive to growth. In the *S. cerevisiae* the results were varied, with emodin and compound (**3**) having similar results to that of the *S. pombe*, 1,8-DHA showed the same result as for *L. starkeyi* and compound (**4**) had a reduced inhibitive concentration of 0.638nM.

All the compounds, except compounds (**3**) and (**4**) in *L. starkeyi* which were not tested due to being non-growth inhibitive, this can be seen in the growth present in the well containing a toxic concentration of DMSO, showed growth after removal of the product suggesting that they are fungistatic.

The amine anthraquinone compounds all had growth inhibition concentrations at greater than 0.1nM but less than 1nM. The concentrations for *L. starkeyi* and *S. cerevisiae* were higher than those for *S. pombe*, except for 1-[(4-aminobutyl)amino]-8-hydroxyanthraquinone (**17**) in *S. cerevisiae* which was equal. In the antifungal tests for *S. pombe*, 1-[(3-aminopropyl)amino]-8-hydroxyanthraquinone (**15**) and compound (**17**) were fungicidal and 1-[(2-aminoethyl)amino]-8-hydroxyanthraquinone (**14**) and 1-[(3-aminopropyl)amino]-8-tosyloxyanthraquinone (**16**) were fungistatic. In both the *L. starkeyi* and *S. cerevisiae* all the compounds except (**16**) were fungistatic.

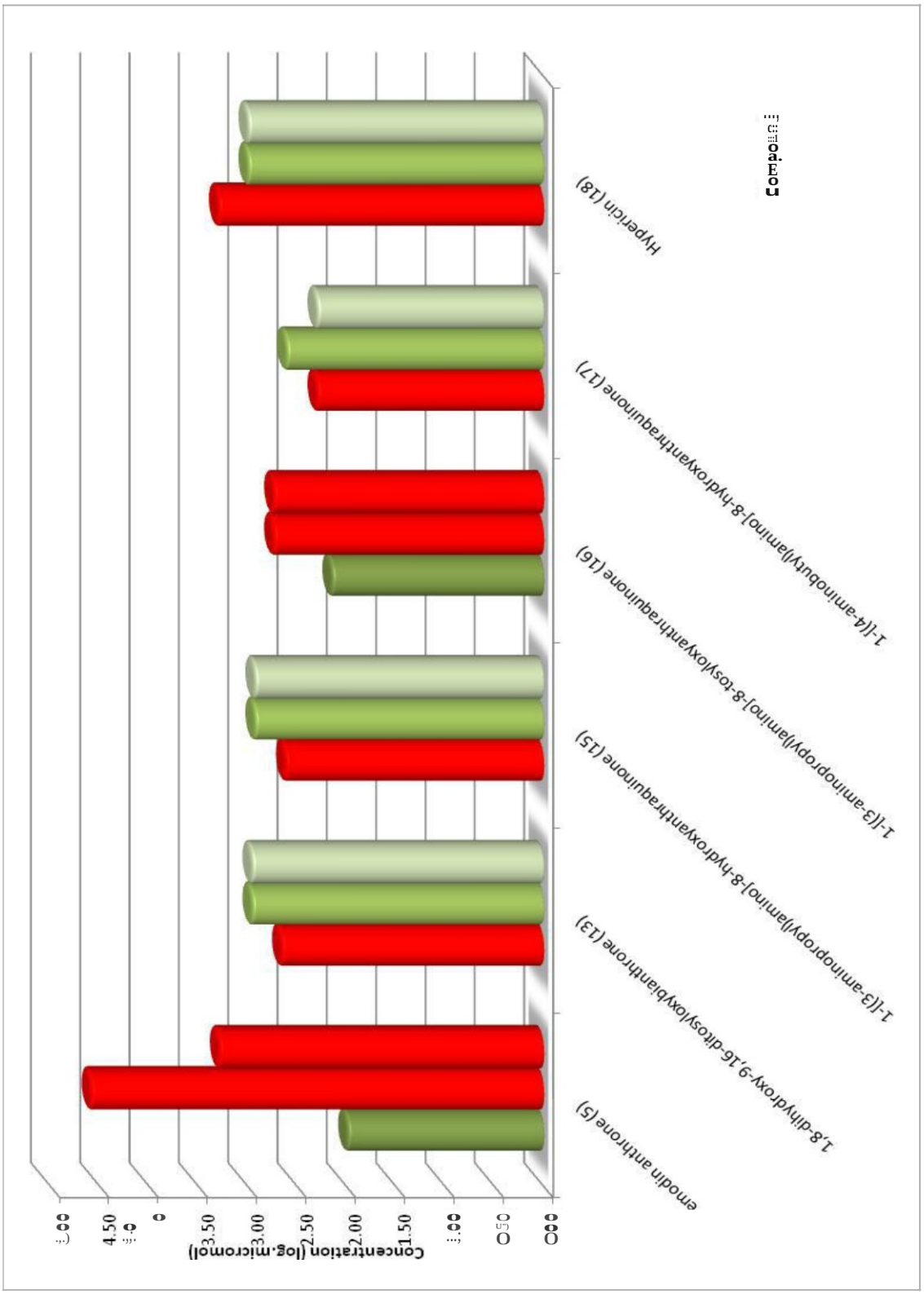


Figure 26 The results for the compounds which showed different results for the different yeast strains. For each of the compounds the 1st column shows the MIC for 5. pombe, the 2nd column for L. starkeyi and the 3rd for 5. cerevisiae. The fungistatic compounds are denoted by a green column and the fungicidal compounds by a red column.

The growth inhibition concentrations for the anthrones in *S. pombe* were all consistently under 0.1mM, which is similar to those for *S. cerevisiae*, except for emodin anthrone (**5**), which is at 1.95mM. In the *L. starkeyi* compound **5** and 1,8-dihydroxyanthrone (**6**) were both significantly less growth inhibitive with concentrations of 39mM and 22.1mM respectively, but 1-hydroxy-8-tosyloxyanthrone (**7**) and 1,8-ditosyloxyanthrone (**8**) gave similar results to these of *S. pombe* and *S. cerevisiae*. With *S. pombe* all the compounds were seen to be fungistatic, but in both *L. starkeyi* and *S. cerevisiae* all but compound (**5**) were fungistatic, which itself was fungicidal.

One of the bianthrones stood out from all the others, 1,8,9,16-tetratosyloxybianthrone (**12**) did not show any growth inhibition concentration and, like compounds (**3**) and (**4**) with *L. starkeyi*, seemed to protect the cells against the growth inhibitive effect of the DMSO. The case could be however that the DMSO aided the membrane transport of the compound inside the yeast cells and it was the presence of the DMSO which altered the inhibitive effects of the compound.[95] For all the other compounds the growth inhibition concentrations were similar. Emodin bianthrone (**9**) had the same growth inhibition concentration for all of the yeasts, 1,8,9,16-tetrahydroxybianthrone (**10**) and 1,8-dihydroxy-9,16-ditosyloxybianthrone (**13**) were less growth inhibitive for both *L. starkeyi* and *S. cerevisiae.* 1,9-Dihydroxy-8,16-ditosyloxybianthrone (**11**) was less growth inhibitive for just the *L. starkeyi*. All the compounds: except compound (**12**) which was not tested (again this can be seen by the growth present in the well containing a toxic concentration of DMSO similar to compounds (**3**) and (**4**) in *L. starkeyi*) and compound (**13)** which was fungicidal for *S. pombe*, were found to be fungistatic for all of the yeasts.

Of all the compounds tested, hypericin was the only one to show it had less growth inhibitive effect for *S. pombe* than the *L. starkeyi* and *S. cerevisiae*. Only two of the compounds synthesised came close with compound (**9**) having equal growth inhibitive concentrations for all of the yeasts and compound (**12**) which was non-growth inhibitive in all the yeast strains.

This is most probably due to the increased lipophilic nature of the compounds; increased lipophilicty will aid the membrane crossing of the compound and if the compound is present within the cell rather than just in the locality it is more likely to have an effect on the yeast cell. It is therefore beneficial to concentrate on the most lipophilic compounds due to the possibility of membrane crossing and develop them to be more responsive to fungi rather than human cells.

Initially, it was expected that the tosyloxy substituted compounds would show more growth inhibition (because of the toxic nature of the starting material)[96,97] than the hydroxyl and amine substituted compounds, because tosyloxy compounds with more tosyloxy groups present increase the growth inhibition. This was shown to be the case in the anthraquinone and anthrone compounds (**3**), (**4**), (**7**) and 9**8**) which all showed a more inhibitive effect on the growth of yeast than the hydroxy and amino substituted compounds. With the bianthrone compounds (**11**), (**12**) and (**13**), however, it was discovered that the more tosyloxy groups that were substituted on the molecule, the less inhibited was the growth of the yeast.

This was the complete opposite of the anthraquinones where, aside from in *L. starkeyi*, more tosyloxy groups increased the growth inhibition of the yeast. In the anthrone group the compounds with increasing number of tosyloxy groups increased the growth inhibition in both the *L. starkeyi* and *S. cerevisiae*, but in the *S. pombe*, the difference between the growth inhibition concentrations was insignificant.

In the amino anthraquinones it was found that the shorter the methylene chain between the amino groups, the less growth inhibition was shown, and this is true for all the yeast strains. It was also observed that the presence of the tosyloxy group in compound (**16**) reduces the growth inhibition in comparison to compound (**15**), where the tosyloxy group at position **8** on the molecule is the only difference between the two compounds.

Due to the close genetic relationship between *L. starkeyi* and *S. cerevisiae* the antifungal results were expected to show similarity for these two yeast strains and for *S. pombe* to show the opposite result was not totally unexpected, as although it is from the same family of yeast it does not share as close a genetic relationship as the other two yeasts.[98,99] One possible explanation for this could be that the majority of the compounds show fungistatic properties, as they are more organic in nature with tosyloxy groups and methylene chains. This would be preferable for the *L. starkeyi* due to its ability to accumulate lipids and therefore a predisposition towards more organic substances.[100] As *S. cerevisiae* is similar genetically to *L. starkeyi,*[98] this explains the parallel results.

CHAPTER 3. CONCLUSIONS AND FUTURE WORK

3.1 Conclusions

The anthraquinone derivatives were developed in a straightforward method according to the method of Schio, Chatreaux and Kilch.[101] The tosylation reactions had been done many times previously, so this was a case of following the protocol and purifying the products formed, although an improvement was made in the work-up which involved getting a much cleaner product by heating it in hexane to remove most of the p-TsCl starting material due to it being less polar and therefore more soluble in the warm hexane than the product. The amine anthraquinones caused a few problems with an initial reaction plan involving long reaction times and triethylamine as strong base, as stated in the protocol according to Zielske,[102] possibly there for the purpose of activating the carbon with the tosyloxy group attached. As the tosyloxy group acts as a strong activating group in its own right,[103] the use of triethylamine was abandoned. It was also discovered that the 250x excess of amine was extreme and so was scaled down to 4x excess and after this the further reactions proceeded in an improved behaviour and in much less time.

The protocol leading to the synthesis of the anthrone derivatives as outlined by Motoyoshiya, Masue, Nishi and Aoyama in their paper on the synthesis of hypericin was relatively straightforward, so this was the method followed for the synthesis of the anthrone derivatives.[43] These progressed with no problems and so no improvement could be made on their synthesis. As this method proceeded with pure products as the outcome from a very simple work-up, there was no need to tamper with the route.

The initial protocol for the synthesis of the bianthrone compounds involving iron(III) chloride, also from the paper by Motoyoshiya, Masue, Nishi and Aoyama, was complicated and did not

yield much product; it also involved using a large amount of solvent for a small scale reaction, so it was not preferable.[43] An improved method involving the microwave synthesis from the research by Aigner and Falk was done with minimal solvent and gave mostly pure product with little work-up.[104] As developing newer, green methods are of major interest due to the importance of modern synthetic chemistry being more environmentally friendly; this enhanced synthetic route is a most important improvement.[105]

The results from the growth inhibition testing did not give the results expected. It was hoped that some of the compounds may be less inhibitive in *S. pombe* than *L. starkeyi*, but this was not the case for any of the compounds synthesised. The increased growth inhibition of the anthrone derivatives in *S. pombe* was unexpected with a possible reason being the increased organic nature of the molecules.

Another reason could be the metabolism of the yeast; mostly this is done by aerobic respiration.[106] However, some yeasts are able to ferment sugars such as glucose, producing ethanol and carbon dioxide even when there is oxygen available and this is known as the Crabtree effect.[107,108] The balance between respiration and fermentation may increase the sensitivity of the yeast to the compounds therefore accounting for the increased growth inhibition in the anthrone derivatives.[94]

It has been suggested that the mechanism by which antifungals affect yeast is by changing the physical properties of the yeast cell membrane, particularly the permeability, which could account for the differences in the growth inhibition for the compounds.[109, 110] There is also evidence to suggest that quinone containing species can create reactive oxygen species and therefore induce lipid peroxidation of the yeast.[111]

For the majority of the compounds to be fungistatic was unexpected, especially after the low growth inhibition concentrations of some of them, these would be expected to be fungicidal

due to the low concentrations of the compound needed to affect the yeast. However, for the few compounds that were fungicidal in one of the yeasts, but not the others, this could also be due to the metabolism of the yeast tested.

Looking at the functionality of the compounds there seems to be no consistency in whether they will be either fungistatic or fungicidal.

3.2 Future Work

There is much scope to continue work on this project in the future. The derivatives could be developed to hypericin-like compounds with all the bonds connected between the rings. There is also the possibility of creating more derivatives with enhanced features such as better water solubility, increased absorption into the near infrared and increased cellular uptake.

The results of the growth inhibition assay show that the best amine to use to increase the water solubility is ethylenediamine as it did not become inhibitive towards growth until much greater concentrations than both the trimethylenediamine and putrescine. It would not be wise to rule out these amines completely; however, as when the molecules are taken to the anthrone and bianthrone this could influence the inhibitive effects.

There are many different anthraquinones that can be studied, with a variety of different functional groups and the next step in this project would have been to synthesise some derivatives from 1,4-diyhdroxyanthraquinone, which is readily available. Another route would be to reduce 1,8-dinitroanthraquinone, which will cut out the protection / activation step with p-TsCl and possibly reduce the growth inhibitive effect again. This could be to a distinct advantage if it is to be believed that the shorter the methylene chain between the amines the lower effect on growth inhibition of the yeast, then no chain present should be a distinct advantage.

Another possibility for future research would be using the resorufin fluorescence inhibition technique outlined by Fai et al,[94] this would provide a greater sensitivity to the result of the inhibition assay and also an insight to the general metabolism of the yeast.

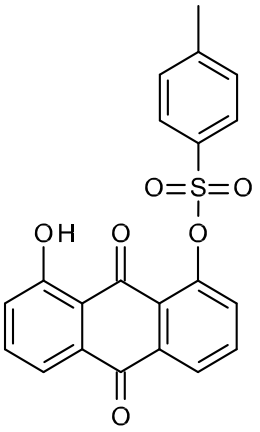
CHAPTER 4. SYNTHESIS AND EXPERIMENTAL

4.1 Chemical Synthesis

^{1}H NMR spectra were measured on a Bruker Avance 400MHz spectrometer, δ values are quoted relative to tetramethylsilane (TMS, δ_H 0.00ppm). Mass spectra were recorded on a Thermo Scientific Trace LC Ultra DSQ II using Electron Impact Ionisation (LCMS-EI). The Ultra Violet absorptions were recorded on a WPA Lightwave II UV/Visible spectrophotometer. Infrared spectra were recorded on a Specac ATR with a He/Ne 633nm laser. Thin Layer Chromatography (TLC) was carried out on Machery-Nagel polygramSil/G/UV_{254} pre-coated plates. All chemicals, solvents and silica gel were obtained commercially from either Sigma Aldrich or Alfa Aesar and used without further purification.

Emodin (**1**), 1,8-DHA (**2**) and Hypericin (**18**) were obtained from Sigma Aldrich to be used for testing alongside the synthesised molecules below.

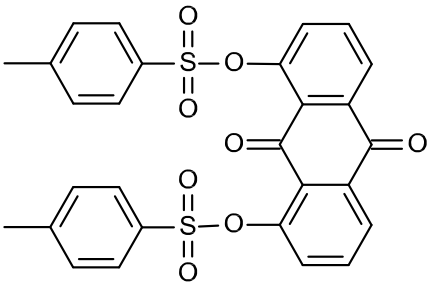
1. **Synthesis of 1-hydroxy-8-tosyloxyanthraquinone (3)**



1,8-DHA (9.61g, 0.04mols) in DCM (100cm^3) was stirred in an ice bath for 10 mins. Triethylamine (TEA) (4.05g, 0.04mols) and p-TsCl (5.72g, 0.03mols) were added slowly to the

reaction flask to ensure the reaction did not exceed 40°C for 6 hours. The reaction mixture was extracted with DCM (50cm^3) and the organic layer was washed with 2M hydrochloric acid (HCl) (6 x 50cm^3). The organic layer was isolated, dried with anhydrous sodium sulphate and the solvent removed under pressure. The resulting solid was heated in hexane (50cm^3) and filtered under pressure. The crude product was then purified by silica column chromatography eluting with $CHCl_3$ to give the pure product as the second band (R_f = 0.68). The 1-hydroxy-8-tosyloxyanthraquinone was obtained as a yellow powder, yield 4.63g, 29.3%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.42 (s, CH_3, 3H), 7.29 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.31 (d, J = 8Hz, Ar-H, 2H), 7.55 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.64 (t, J = 8Hz, Ar-H, 1H), 7.76 (t, J = 8Hz, Ar-H, 1H), 7.77 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.81 (d, J = 8Hz, Ar-H, 2H), 8.29 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 12.38 (s, OH, 1H). IR (ATR) 2000, 1699, 1380, 1180, 980, 870, 750, 650, 550 cm-1. MS-EI: 393.03 [M+]. UV abs λ_{max} = 247nm.

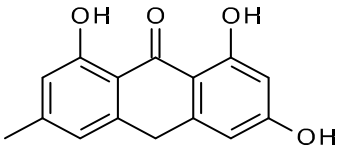
2. Synthesis of 1,8-ditosyloxyanthraquinone (4)



1,8-DHA (5.00g, 0.02mols) in DCM (120cm^3) was stirred in an ice bath for 10mins. TEA (25.30g, 0.25mols) and p-TsCl (43.80g, 0.23mols) were added slowly to the reaction flask to ensure the reaction did not exceed 40°C for 6 hours. The reaction mixture was extracted with DCM (50cm^3) and the organic layer was washed with 2M HCl (6 x 50cm^3). The organic layer was isolated, dried with anhydrous sodium sulphate and the solvent removed under pressure. The resulting solid was heated in hexane (50cm^3) and filtered under reduced? pressure. The crude product was then purified by silica column chromatography eluted with $CHCl_3$ to give the pure

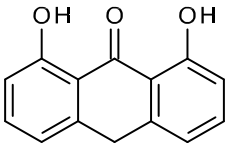
product as the second band (R_f = 0.62). This yielded 1,8-ditosyloxyanthraquinone as a pale flaxen powder, 13.32g, 60.7%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.41 (s, CH_3, 3H), 7.33 (d, J =8Hz, Ar-H, 2H), 7.65 (dd, J =8Hz, J = 1Hz, Ar-H, 1H), 7.71(t, J = 8Hz, Ar-H, 1H), 7.92 (d, J = 8Hz, Ar-H, 2H), 8.17 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H). IR (ATR) 1380, 1180, 980, 860, 750, 680, 570, 560 cm-1. MS-EI: 548.94 *M++. UV abs λ_{max} = 250nm.

3. Synthesis of emodin anthrone (5)



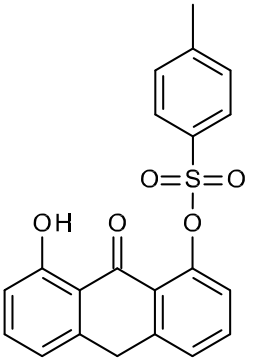
A warm solution of $SnCl_2$ (0.35g, 1.83mmols) in conc. HCl (3cm^3) was added to a suspension of emodin (0.05g, 0.183mmols) in acetic acid (AcOH) (5cm^3) and the reaction mixture was left to stir under reflux for 15 hours. The reaction mixture was allowed to cool and poured slowly into ice water, which resulted in the isolation of emodin anthrone as a pale green powder, yield 0.043g, 91.5%,^{1}H NMR (DMSO, 400MHz) δppm 2.33 (s, CH_3, 3H), 4.31 (s, CH_2, 2H), 6.23 (s, Ar-H, 1H), 6.43 (s, Ar-H, 1H), 6.68 (s, Ar-H, 1H), 6.79 (s, Ar-H, 1H), 10.84 (s, OH, 1H), 12.24 (s, OH, 1H), 12.39 (s, OH, 1H). IR (ATR) 3300, 1600, 1490, 1290, 1240, 1150, 1070, 800, 770, 690, 550 cm^{-1}. MS-EI: 257.76 [M^++. UV abs λ_{max} = 224nm.

4. Synthesis of 1,8-dihydroxyanthrone (6)



A warm solution of $SnCl_2$ (1.07g, 4.78mmols) in conc. HCl (5.5cm^3) was added to a suspension of 1,8-DHA (0.12g, 0.478mmols) in AcOH (10cm^3) and the reaction mixture was left to stir under reflux for 24 hours. The reaction mixture was allowed to cool and poured slowly into ice water, which resulted in the isolation of 1,8-dihydroxyanthrone as a buff powder, yield 0.10g, 90.9%, ^{1}H NMR (DMSO, 400MHz) δppm 4.31 (s, CH_2, 2H), 6.90 (d, J = 8Hz, Ar-H, 2H), 7.03 (d, J = 8Hz, Ar-H, 2H), 7.61 (t, J = 8Hz, Ar-H, 2H), 12.09 (s, OH, 2H). IR (ATR) 3000, 2150, 1600, 1450, 1280, 1200, 780, 700, 620 cm-1. MS-EI: 226.18 *M++. UV abs λ_{max} = 226nm.

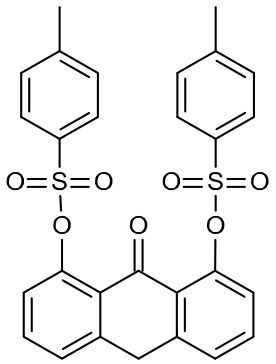
5. Synthesis of 1-hydroxy-8-tosyloxyanthrone (7) (NOVEL)



A warm solution of $SnCl_2$ (5.69g, 0.03mols) in conc. HCl (30cm^3) was added to a suspension of compound (**3**) (1.18g, 3.0mmols) in AcOH (60cm^3) and the reaction mixture was left to stir under reflux for 48 hours. The reaction mixture was allowed to cool and poured slowly into ice water, which resulted in the isolation of 1-hydroxy-8-tosyloxyanthrone as a chartreuse powder, yield 0.93g, 81.6%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.33 (s, CH_3, 3H), 3.98 (s, CH_2, 1H),

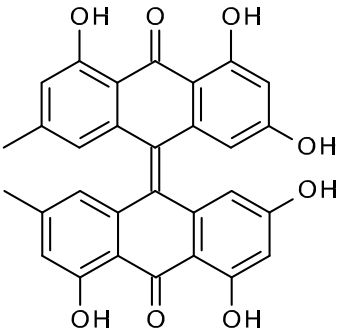
7.16 (d, J = 8Hz, Ar-H, 2H), 7.26 (d, J = 8Hz, Ar-H, 1H), 7.39 (dd, J = 8Hz, J = 2Hz, Ar-H, 1H), 7.41 (t, J = 8Hz, Ar-H, 1H), 7.55 (t, J = 8Hz, Ar-H, 1H), 7.78 (d, J = 8Hz, Ar-H, 1H), 7.88 (d, J = 8Hz, Ar-H, 1H), 8.24 (dd, J = 8Hz, J = 2Hz, Ar-H, 1H), 12.52 (s, OH, 1H). IR (ATR) 3000, 1600, 1490, 1460, 1370, 1280, 1250, 1170, 730, 620, 550 cm^{-1}. MS-EI: 380.76 [M^{+}+. UV abs λ_{max} = 224nm.

6. Synthesis of 1,8-ditosyloxyanthrone (8) (NOVEL)



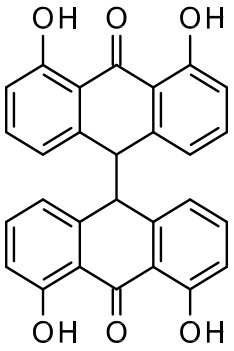
A warm solution of $SnCl_2$ (5.69g, 0.03mols) in conc. HCl (30cm^3) was added to a suspension of compound (**4**) (1.64g, 3.0mols) in AcOH (60cm^3) and the reaction mixture was left to stir under reflux for 48 hours. The reaction mixture was allowed to cool and poured slowly into ice water, which resulted in the isolation of 1,8-ditosyloxyanthrone as a chartreuse powder, yield 1.26g, 78.8%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.37 (s, CH_3, 6H), 4.31 (s, CH_2, 1H), 7.22 (d, J = 8Hz, Ar-H, 2H), 7.26 (d, J = 8Hz, Ar-H, 4H), 7.55 (t, J = 8Hz, Ar-H, 2H), 7.79 (d, J = 8Hz, Ar-H, 4H), 7.88 (d, J = 8Hz, Ar-H, 2H). IR (ATR) 2170, 2040, 2000, 1990 cm^{-1}. MS-EI: 534.12 [M^{+}+. UV abs λ_{max} = 221nm.

7. Synthesis of emodin bianthrone (9)



A mixture of compound (**5**) (0.03g, 0.117mmols), t-BuOK (0.0022g, 0.0198mmols) and DMF (0.02cm^3) were irradiated in the microwave with stirring at 150W (t=130°C) for 30 minutes under sealed conditions. After cooling the mixture was then quenched with water, acidified to pH6 with 1M HCl and the resulting precipitate filtered using suction to give a pure product of emodin bianthrone as black crystals, yield 0.04g, 66.7%, ^{1}H NMR (DMSO, 400MHz) δppm 2.73 (s, CH_3, 6H), 6.76 (s, Ar-H, 2H), 7.22 (s, Ar-H, 2H), 7.95 (s, Ar-H, 2H), 8.01 (s, Ar-H, 2H). IR (ATR) 2170, 2050 cm^{-1}. MS-EI: 509.46 [M^++. UV abs λ_{max} = 588nm.

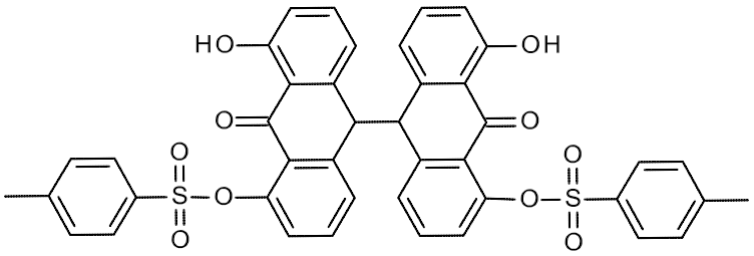
8. Synthesis of 1,8,9,16-tetrahydroxybianthrone (10)



A mixture of compound (**6**) (0.45g, 2mmols), t-BuOK(0.038g, 0.34mmols) and DMF (1cm^3) were irradiated in the microwave with stirring at 150W (t=130°C) for 30 minutes under sealed conditions. After cooling the mixture was then quenched with water, acidified to pH 6 with 1M

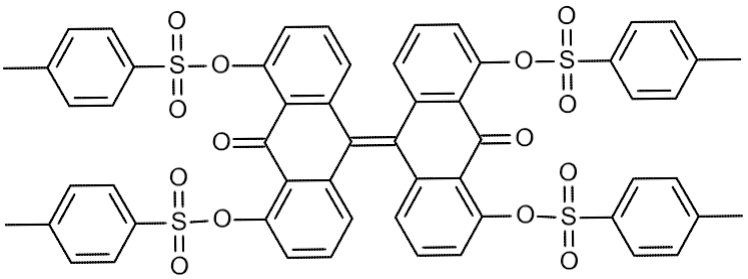
HCl and the resulting precipitate filtered using suction to give a pure product of 1,8,9,16-tetrahydroxybianthrone as a saffron powder, yield 0.81g, 90.0%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 4.33 (s, CH_2, 1H), 6.36 (d, J = 8Hz, Ar-H, 2H), 6.89 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 6.89 (dd, J = 8Hz, J = 1Hz, Ar-H, 2H), 7.38 (t, J = 8Hz, Ar-H, 2H), 7.68 (t, J = 8Hz, Ar-H, 2H), 7.82 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 11.71 (s, OH, 2H), 12.06 (s, OH, 2H); there is a possibility of diasteriomeric mix present due to the H's on the linking bond being either cis or trans in configuration hence there being more signals. IR (ATR) 2180, 2020, 2000, 1020, 1000 cm^{-1}. MS-EI: 541.51 [M^{+}+. UV abs λ_{max} = 260nm.

9. Synthesis of 1,9-dihydroxy-8,16-ditosyloxybianthrone (11) (NOVEL)



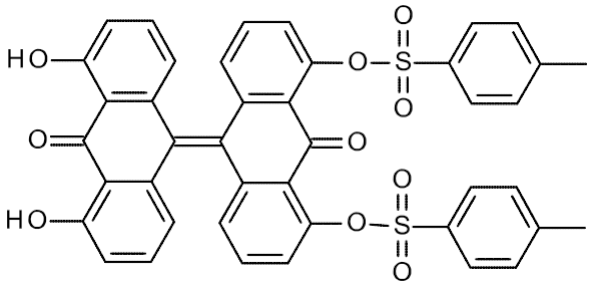
A mixture of compound (**7**) (0.45g, 1.17mmols), t-BuOK (0.02g, 0.117mmols) and DMF (1cm^3) were irradiated in the microwave with stirring at 150W (t=130°C) for 30 minutes under sealed conditions. After cooling the mixture was then quenched with water, acidified to pH 6 with 1M HCl and the resulting precipitate filtered using suction to give a pure product of 1,9-dihydroxy-8,16-ditosyloxybianthrone as a brown powder, yield 0.71g, 79.8%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.46 (s, CH_3, 6H), 4.35 (s, CH, 1H), 7.16 (d, J = 8Hz, Ar-H, 4H), 7.55 (t, J = 8Hz, Ar-H, 4H), 7.78 (d, J = 8Hz, Ar-H, 4H), 7.88 (d, J = 8Hz, Ar-H, 4H), 8.24 (dd, J = 8Hz, J = 1Hz, Ar-H, 4H), 12.40 (s, OH, 2H). IR (ATR) 3120, 2180, 2040, 1990 cm^{-1}. MS-EI: 758.41 [M^{+}+. UV abs λ_{max} = 258nm.

10. Synthesis of 1,8,9,16-tetratosyloxybianthrone (12) (NOVEL)



A mixture of compound (**8**) (0.79g, 2.0mmols), t-BuOK (0.038g, 0.34mmols) and DMF (1cm^3) were irradiated in the microwave with stirring at 150W (t=130°C) for 30 minutes under sealed conditions. After cooling the mixture was then quenched with water, acidified to pH 6 with 1M HCl and the resulting precipitate filtered using suction to give a pure product of 1,8,9,16-tetratosyloxybianthrone as a red powder, yield 1.74g, 81.7%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.42 (s, CH_3, 9H), 7.33 (d, J = 8Hz, Ar-H, 8H), 7.67 (dd, J = 8Hz, J = 2Hz, Ar-H, 4H), 7.72 (t, J = 8Hz, Ar-H, 4H), 7.93 (d, J = 8Hz, Ar-H, 8H), 8.18 (dd, J = 8Hz, J = 2Hz, Ar-H, 4H) there are two lines of symmetry through the molecule hence there are only 6 signals. IR (ATR) 2160, 2050, 1990, 1620, 1480, 1320 cm^{-1}. MS-EI: 1075.61 [M$^+$+. UV abs λ_{max} = 249nm.

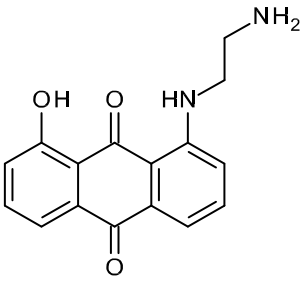
11. Synthesis of 1,8-dihydroxy-9,16-ditosyloxybianthrone (13) (NOVEL)



A mixture of compound (**6)** (0.226g, 1.0mmols), compound (**8)** (0.549g, 1mmols), t-BuOK (0.038g, 0.34mmols) and DMF (1cm^3) were irradiated in the microwave with stirring at 150W

(t=130°C) for 30 mins under sealed conditions. After cooling the mixture was then quenched with water, acidified to pH 6 with 1M HCl and the resulting precipitate filtered using suction to give the crude product. This was then purified by silica column chromatography, elution with $CHCl_3$ gave the pure product 1,8-dihydroxy-9,16-ditosyloxybianthrone, the third band (R_f = 0.48) as a dark red powder, yield 0.24g, 31.6%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.40 (s, CH_3, 6H), 7.27 (dd, J = 8Hz, J = 2Hz, Ar-H, 2H), 7.31 (d, J = 8Hz, Ar-H, 4H), 7.51 (dd, J = 8Hz, J = 1Hz, Ar-H, 2H), 7.63 (t, J = 8Hz, Ar-H, 2H), 7.75 (dd, J = 8Hz, J = 1Hz, Ar-H, 2H), 7.76 (t, J = 8Hz, Ar-H, 2H), 7.80 (d, J = 8Hz, Ar-H, 4H), 8.28 (dd, J = 8Hz, J = 1Hz, Ar-H, 2H), 12.37 (s, OH, 2H). IR (ATR) 3070, 1650, 1600, 1400, 1290, 1170, 980, 880, 750, 720, 680, 550 cm^{-1}. MS-EI: 758.79 [M^+]. UV abs λ_{max} = 406nm.

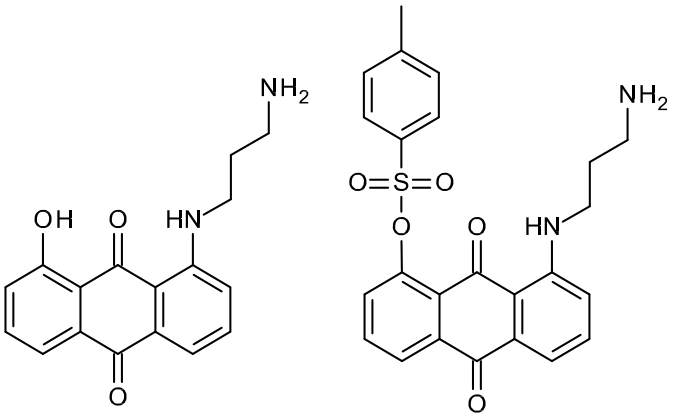
12. Synthesis of 1-[(2-aminoethyl)amino]-8-hydroxy anthraquinone (14)



A mixture of compound (**4**) (5.48g, 0.01mols), ethylenediamine (2.4g, 0.04mols) in DCM (30cm^3) was stirred under reflux for 2 hours. The solvent was removed under vacuum and the resulting crude product was purified twice using silica column chromatography, first as the second band (R_f = 0.61) eluted with DCM and second with $CHCl_3$: methanol (MeOH) (5:1) giving a pure product of 1-[(2-aminoethyl)amino]-8-hydroxy anthraquinone as the second band (R_f = 0.59) yielding black crystals, 0.97g, 34.4%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 2.42 (s, NH_2, 2H), 3.72 (t, J = 5Hz, CH_2, 2H), 4.19 (t, J = 5Hz, CH_2, 2H), 5.20 (s, NH, 1H), 6.92 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.23 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.40 (t, J = 8Hz, Ar-H, 1H), 7.43 (t, J = 8Hz, Ar-H,

1H), 7.66 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.83 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H). IR (ATR) 3290, 3040, 2850, 2170, 1610, 1300, 1200, 1180, 740 cm^{-1}. MS-EI: 282 [M^{+}+. UV abs λ_{max} = 525nm.

13. Synthesis of 1-[(3-aminopropyl)amino]-8-hydroxyanthraquinone (15) and 1-[(3-aminopropyl)amino]-8-tosyloxyanthraquinone (16)

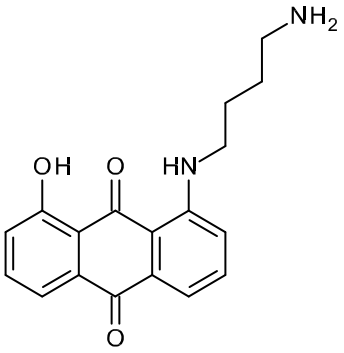


15 **16**

A mixture of compound (**4**) (5.48g, 0.01mols) and 1,3-diaminopropane (2.97g, 0.04mols) in DCM (30cm^3) were stirred under reflux for 2 hours. The solvent was removed under vacuum and the resulting crude product was purified twice using silica column chromatography, first as the second band (R_f = 0.78) eluted with DCM and second with $CHCl_3$: MeOH (5:1) to give two products: 1-[(3-aminopropyl)amino]-8-hydroxyanthraquinone the first band (R_f = 0.67) as a dark purple powder, yield 1.18g, 9.96%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 1.88 (quin, J = 6Hz, CH_2, 2H), 2.37 (s, CH_3, 3H), 2.91 (t, J = 6Hz, CH_2, 2H), 3.37 (quart, J = 6Hz, CH_2, 2H), 7.00 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.20 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.47 (t, J = 8Hz, Ar-H, 1H), 7.52 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.54 (t, J = 8Hz, Ar-H, 1H), 9.38 (s, OH, 1H). IR (ATR) 3300, 2960, 2870, 1630, 1590, 1520, 1480, 1280, 1210, 1170, 1070, 820, 730 cm^{-1}. MS-EI: 296 [M^{+}]. UV abs λ_{max} = 512nm and 1-[(3-aminopropyl)amino]-8-tosyloxyanthraquinone the second band (R_f =

0.54) as a dark red powder, yield 1.35g, 7.49%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 1.94 (quin, J = 7Hz, CH_2, 2H), 2.96 (t, J = 7Hz, CH_2, 2H), 3.43 (quar, J = 7Hz, CH_2, 2H), 7.07 (dd, J = 6Hz, J = 3Hz, Ar-H, 1H), 7.26 (d, J = 8Hz, Ar-H, 2H), 7.36 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 7.49 (t, J = 8Hz, Ar-H, 1H), 7.49 (d, J = 8Hz, Ar-H, 1H), 7.61 (t, J = 8Hz, Ar-H, 1H), 7.77 (d, J = 8Hz, Ar-H, 2H), 8.22 (dd, J = 8Hz, J = 1Hz, Ar-H, 1H), 9.57 (s, NH, 1H). IR (ATR) 2980, 2910, 1670, 1600, 1510, 1310, 1220, 1180, 850, 740, 670, 550 cm^{-1}. MS-EI: 450.97 [M^+]. UV abs λ_{max} = 521nm.

14. Synthesis of 1-[(4-aminobutyl)amino]-8-hydroxyanthraquinone (17)



A mixture of compound (**4**) (5.48g, 0.01mols) and putrescine (3.53g, 0.04mols) in DCM (30cm^3) were stirred under reflux for 2 hours. The solvent was removed under vacuum and the resulting crude product was purified twice using silica column chromatography, first as the second band (R_f = 0.71) eluted with DCM and second as the second band (R_f = 0.63) with $CHCl_3$: MeOH (5:1) to give 1-[(4-aminobutyl)amino]-8-hydroxyanthraquinone as a dark purple powder, yield 1.29g, 10.39%, ^{1}H NMR ($CDCl_3$, 400MHz) δppm 1.69 (quin, J = 7Hz, CH_2, 2H), 1.77 (quin, J = 7Hz, CH_2, 2H), 2.24 (s, NH, 1H), 2.86 (t, J = 7Hz, CH_2, 2H), 3.27 (quar, J = 7Hz, CH_2, 2H), 6.96 (d, J = 6Hz, Ar-H, 1H), 6.97 (d, J = 6Hz, Ar-H, 1H), 7.24 (t, J = 8Hz, Ar-H, 1H), 7.29 (d, J = 8Hz, Ar-H, 1H), 7.57 (t, J = 8Hz, Ar-H, 1H), 8.17 (d, J = 8Hz, Ar-H, 1H), 9.50 (s, OH, 1H). IR (ATR) 3050, 2970, 2850, 2160, 1590, 1300, 1180, 870, 750, 550 cm^{-1}. MS-EI: 310.95 [M^++. UV abs λ_{max} = 527nm.

4.2 Yeast Growth Inhibition Assay

The growth inhibitory activity of the compounds was determined by screening *S. pombe*, *L. starkeyi* and *S. cerevisiae* using the following method.

Yeast species were inoculated into relevant media; *S. pombe* (NJ2 *h⁻ ura4-D18 leu1-32 ade6-M210 his7-366*)[112] into yeast extract broth (YE)[113], *L. starkeyi* (strain NCYC2710)[1] and *S. cerevisiae* (strain BY4741a, a derivative of S288C Genotype is *MAT***a***his3* Δ1 *leu2* Δ0 *met15* Δ 0*ura3* Δ0)[114] into complex media (YPD).[115] The culture was then incubated for 12 hours at 30°C with shaking at 200 rpm. Stock solutions of the compounds were prepared in 20% DMSO and culture media. DMSO and culture media were all used as controls for the experiment. 3 x 10^4 yeast cells were transferred into the wells of a 96- well plate. A 1 in 2 serial dilution of the compounds was then performed starting at the stock concentration indicated in the Table. The well plates were finally inspected visually for growth of yeast after 24 hours of incubation at 30°C. Growth was indicated by full or partial white appearance of yeast on the bottom of the wells. The MIC of the compounds was determined to be the well before yeast growth was first seen. The experiment was repeated three times to ensure reproducibility of the results.

To determine whether compounds had antifungal activity, cells were taken from the well on the micro-titre plate at the concentration where lack of yeast growth was first observed. These cells were inoculated into fresh culture media, to remove presence of compound and grown for 24hours at 30°C with shaking at 200rpm. Compounds were determined to be fungicidal, if no growth was observed and fungistatic, if normal growth was seen.

REFERENCES

1. Suzuki, M.; Prasad, G. S.; Kurtzman, C. P., *The Yeasts*. North Holland Publishers: Amsterdam, 1952.
2. William C.R, A., Athlete's foot: When all else fails! *Foot and Ankle Surgery* **2006,** *12*, 209-210.
3. Philip, B., CHAPTER 53 - Oral Candidiasis: (Thrush or Yeast Infection). In *Minor Emergencies (Second Edition)*, Mosby: Philadelphia, 2007; pp 210-211.
4. J.L, B., SABOURAUD'S METHOD OF RINGWORM TREATMENT. *The Lancet* **1905,** *165*, 414-416.
5. Within the fold: Treatments of intertrigo. *Journal of the American Academy of Dermatology* **2007,** *56*, AB126.
6. Slavin, M.; Fastenau, J.; Sukarom, I.; Mavros, P.; Crowley, S.; Gerth, W. C., Burden of hospitalization of patients with Candida and Aspergillus infections in Australia. *International Journal of Infectious Diseases* **2004,** *8*, 111-120.
7. Rassl, D. M.; Suvarna, S. K.; Cooper, G. J., Fatal fungal infection complicating aortic dissection following coronary artery bypass grafting. *Cardiovascular Surgery* **2000,** *8*, 79-81.
8. Durden, F. M.; Elewski, B., Fungal infections in HIV-infected patients. *Seminars in Cutaneous Medicine and Surgery* **1997,** *16*, 200-212.
9. Person, A. K.; Kontoyiannis, D. P.; Alexander, B. D., Fungal Infections in Transplant and Oncology Patients. *Hematology/Oncology Clinics of North America* **2011,** *25*, 193-213.
10. Garland, J. S.; Uhing, M. R., Strategies to Prevent Bacterial and Fungal Infection in the Neonatal Intensive Care Unit. *Clinics in Perinatology* **2009,** *36*, 1-13.
11. Zilberberg, M. D.; Shorr, A. F., Fungal Infections in the ICU. *Infectious Disease Clinics of North America* **2009,** *23*, 625-642.
12. Fan, Y.-M.; Huang, W.-M.; Yang, Y.-P.; Li, W.; Li, S.-F., Primary cutaneous trichosporonosis caused by Trichosporon dermatis in an immunocompetent man. *Journal of the American Academy of Dermatology* **2011,** *65*, 434-436.
13. McKinnell, J. A.; Pappas, P. G., Blastomycosis: New Insights into Diagnosis, Prevention, and Treatment. *Clinics in Chest Medicine* **2009,** *30*, 227-239.
14. McDonnell, J. M.; Hutchins, G. M., Pulmonary cryptococcosis. *Human Pathology* **1985,** *16*, 121-128.
15. Lee, S. C.; Dickson, D. W.; Casadevall, A., Pathology of cryptococcal meningoencephalitis: Analysis of 27 patients with pathogenetic implications. *Human Pathology* **1996,** *27*, 839-847.
16. Arnáiz-García, M. E.; Alonso-Peña, D.; del Carmen González-Vela, M.; García-Palomo, J. D.; Sanz-Giménez-Rico, J. R.; Arnáiz-García, A. M., Cutaneous mucormycosis: report of five cases and review of the literature. *Journal of Plastic, Reconstructive & Aesthetic Surgery* **2009,** *62*, e434-e441.
17. Turunc, T.; Demiroglu, Y. Z.; Aliskan, H.; Colakoglu, S.; Arslan, H., Eleven cases of mucormycosis with atypical clinical manifestations in diabetic patients. *Diabetes Research and Clinical Practice* **2008,** *82*, 203-208.
18. Torres, C.; Ro, J. Y.; El-Naggar, A. K.; Sim, S. J.; Weber, R. S.; Ayala, A. G., Allergic fungal sinusitis: A clinicopathologic study of 16 cases. *Human Pathology* **1996,** *27*, 793-799.
19. Bozeman, S.; deShazo, R.; Stringer, S.; Wright, L., Complications of Allergic Fungal Sinusitis. *The American Journal of Medicine* **2011,** *124*, 359-368.
20. Kauffman, C. A.; Carver, P. L., Use of Azoles for Systemic Antifungal Therapy. In *Advances in Pharmacology*, J.Thomas August, M. W. A. F. M.; Joseph, C., Eds. Academic Press: 1997; Vol. Volume 39, pp 143-189.

21. Baginski, M.; Czub, J., Amphotericin B and its new derivatives - mode of action. *Current Drug Metabolism* **2009,** *10*, 459-469.
22. Sheehan, D. J.; Hitchcock, C. A.; Sibley, C. M., Current and Emerging Azole Antifungal Agents. *Clin. Microbiol. Rev.* **1999,** *12*, 40-79.
23. Ryder, N. S.; Dupont, M. C., Inhibition of squalene epoxidase by allylamine antimycotic compounds. A comparative study of the fungal and mammalian enzymes. *Biochemical Journal* **1985,** *230*, 765-770.
24. Denning, D. W., Echinocandins: a new class of antifungal. *Journal of Antimicrobial Chemotherapy* **2002,** *49*, 889-891.
25. S., P.; R., S. V.; C., K., Antibacterial and antifungal activity of ten essential oils in vitro. *Microbiology* **1996,** *86*, 237-246.
26. Galindo, P. A.; Borja, J.; Feo, F.; Gómez, E.; Garcia, R.; Cabrera, M.; Martinez, C., Anaphylaxis to omeprazole. *Annals of Allergy, Asthma & Immunology* **1999,** *82*, 52-54.
27. Lewis, R. E., Managing drug interactions in the patient with aspergillosis. *Medical Mycology* **2006,** *44*, 349-356.
28. Thomas R, R., Antifungal drug resistance: limited data, dramatic impact? *International Journal of Antimicrobial Agents* **2006,** *27, Supplement 1*, 7-11.
29. Rodriguez-Tudela, J. L.; Alcazar-Fuoli, L.; Cuesta, I.; Alastruey-Izquierdo, A.; Monzon, A.; Mellado, E.; Cuenca-Estrella, M., Clinical relevance of resistance to antifungals. *International Journal of Antimicrobial Agents* **2008,** *32, Supplement 2*, S111-S113.
30. Leah E, C., Predicting the emergence of resistance to antifungal drugs. *FEMS Microbiology Letters* **2001,** *204*, 1-7.
31. Dominique, S., Resistance of human fungal pathogens to antifungal drugs. *Current Opinion in Microbiology* **2002,** *5*, 379-385.
32. Wingard, J. R.; Leather, H., A new era of antifungal therapy. *Biology of Blood and Marrow Transplantation* **2004,** *10*, 73-90.
33. Vincent T, A., Current and future antifungal therapy: new targets for antifungal therapy. *International Journal of Antimicrobial Agents* **2000,** *16*, 317-321.
34. Di Santo, R., ChemInform Abstract: Natural Products as Antifungal Agents Against Clinically Relevant Pathogens. *ChemInform* **2010,** *41*, no-no.
35. Walter E, M., Current St. John's wort research from mode of action to clinical efficacy. *Pharmacological Research* **2003,** *47*, 101-109.
36. Muller, W. E., *St. John's Wort and its Active Principles in Depression and Anxiety*. Birkhauser: Frankfurt, 2005.
37. Lackner, B.; Bretterbauer, K.; Schwarzinger, C.; Falk, H., A Route to Amino Functionalized Hypericin Derivatives and their Chemical and Photochemical Properties Pertaining to Photodynamic Therapy. *Monatshefte für Chemie / Chemical Monthly* **2005,** *136*, 2067-2082.
38. Szegedi, A.; Kohnen, R.; Dienel, A.; Kieser, M., Acute treatment of moderate to severe depression with hypericum extract WS 5570 (St John's wort): randomised controlled double blind non-inferiority trial versus paroxetine. *BMJ* **2005,** *330*, 503.
39. Nelson, R. St John's Wort. http://www.herballegacy.com/Nelson_History.html (8/10/2010),
40. Pace, N.; Mackinney, G., Hypericin, the Photodynamic Pigment from St. John'swort. *Journal of the American Chemical Society* **1941,** *63*, 2570-2574.
41. Falk, H.; Schoppel, G., A synthesis of emodin anthrone. *Monatshefte für Chemie / Chemical Monthly* **1991,** *122*, 739-744.
42. Falk, H.; Meyer, J.; Oberreiter, M., A convenient semisynthetic route to hypericin. *Monatshefte für Chemie / Chemical Monthly* **1993,** *124*, 339-341.

43. Motoyoshiya, J.; Masue, Y.; Nishi, Y.; Aoyama, H., Synthesis of Hypericin via Emodin Anthrone Derived from aTwo-fold Diels-Alder Reaction of 1,4-Benzoquinone. *Natural Product Communications* **2006,** *0*, 1-5.
44. Etzlstorfer, C.; Falk, H.; Müller, N.; Schmitzberger, W.; Wagner, U. G., Tautomerism and stereochemistry of hypericin: Force field, NMR, and X-ray crystallographic investigations. *Monatshefte für Chemie / Chemical Monthly* **1993,** *124*, 751-761.
45. Gutman, I.; Markovid, Z.; Solujid, S.; Sukdolak, S., On the Tautomers of Hypericin. *Monatshefte für Chemie / Chemical Monthly* **1998,** *129*, 481-486.
46. Kapinus, E. I.; Falk, H.; Tran, H. T. N., Spectroscopic Investigation of the Molecular Structure of Hypericin and its Salts. *Monatshefte für Chemie / Chemical Monthly* **1999,** *130*, 623-635.
47. Wirz, A.; Meier, B.; Sticher, O., Solubility of hypericin in methanol and methanol-pyridine. *Pharmazie* **2002,** *57*, 543-545.
48. Miskovsky, P., Hypericin - A New Antiviral and Antitumor Photosensitizer: Mechanism of Action and Interaction with Biological Macromolecules *Current Drug Targets* **2002,** *3*, 55-84.
49. Karioti, A.; Bilia, A. R., Hypericins as Potential Leads for New Therapeutics. *International Journal of Molecular Science* **2010,** *11*, 562-594.
50. Mark, W., Photoantimicrobials—So what's stopping us? *Photodiagnosis and Photodynamic Therapy* **2009,** *6*, 167-169.
51. Saddiqe, Z.; Naeem, I.; Maimoona, A., A review of the antibacterial activity of Hypericum perforatum L. *Journal of Ethnopharmacology* **2010,** *131*, 511-521.
52. Agostinis, P.; Vantieghem, A.; Merlevede, W.; de Witte, P. A. M., Hypericin in cancer treatment: more light on the way. *The International Journal of Biochemistry & Cell Biology* **2002,** *34*, 221-241.
53. Uzdensky, A. B.; Bragin, D. E.; Kolosov, M. S.; Kubin, A.; Loew, H. G.; Moan, J., Photodynamic effect of hypericin and a water-soluble derivative on isolated crayfish neuron and surrounding glial cells. *Journal of Photochemistry and Photobiology B: Biology* **2003,** *72*, 27-33.
54. Huygens, A.; Crnolatac, I.; Develter, J.; Van Cleynenbreugel, B.; Van der Kwast, T.; de Witte, P. A. M., Differential Accumulation of Hypericin in Spheroids Composed of T-24 Transitional Cell Carcinoma Cells Expressing Different Levels of E-Cadherin. *The Journal of Urology* **2008,** *179*, 2014-2019.
55. Lackner, B.; Popova, Y.; Etzlstorfer, C.; Smelcerovic, A. A.; Klampfl, C. W.; Falk, H., Syntheses and Properties of Two Heterocyclically Substituted Hypericin Derivatives: 10,11-Dibenzothiazolyl-10,11-didemethylhypericin and 10,11-Dibenzoxazolyl-10,11-didemethylhypericin. *Monatshefte für Chemie / Chemical Monthly* **2005,** *136*, 777-793.
56. Waser, M.; Popova, Y.; Klampfl, C. W.; Falk, H., 9,12-Dibenzothiazolylhypericin and 10,11-Dibenzothiazolyl-10,11-didemethylhypericin: Photochemical Properties of Hypericin Derivatives Depending on the Substitution Site. *Monatshefte für Chemie / Chemical Monthly* **2005,** *136*, 1791-1797.
57. Waser, M.; Lackner, B.; Zuschrader, J.; Müller, N.; Falk, H., An efficient regioselective synthesis of endocrocin and structural related natural anthraquinones starting from emodin. *Tetrahedron Letters* **2005,** *46*, 2377-2380.
58. Bao, L.; Qin, L.; Liu, L.; Wu, Y.; Han, T.; Xue, L.; Zhang, Q., Anthraquinone compounds from Morinda officinalis inhibit osteoclastic bone resorption in vitro. *Chemico-Biological Interactions*.
59. Firouzabadi, H.; Iranpoor, N.; Kiaeezadeh, F.; Toofan, J., Chromium(VI) based oxidants-1 : Chromium peroxide complexes as versatile, mild, and efficient oxidants in organic synthesis. *Tetrahedron* **1986,** *42*, 719-725.
60. Weissermel, K.; Arpe, H. J., *Industrial Organic Chemistry*. 4th ed.; Wiley VCH GmbH and Co: 2003.

61. Müller, S. O.; Eckert, I.; Lutz, W. K.; Stopper, H., Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: Topoisomerase II mediated? *Mutation Research/Genetic Toxicology* **1996,** *371*, 165-173.
62. Winter, R. W.; Cornell, K. A.; Johnson, L. L.; Isabelle, L. M.; Hinrichs, D. J.; Riscoe, M. K., Hydroxy-anthraquinones as antimalarial agents. *Bioorganic & Medicinal Chemistry Letters* **1995,** *5*, 1927-1932.
63. Keswani, N.; Kishore, N., Calorimetric and spectroscopic studies on the interaction of anticancer drug mitoxantrone with human serum albumin. *The Journal of Chemical Thermodynamics* **2011,** *43*, 1406-1413.
64. Evison, B. J.; Pastuovic, M.; Bilardi, R. A.; Forrest, R. A.; Pumuye, P. P.; Sleebs, B. E.; Watson, K. G.; Phillips, D. R.; Cutts, S. M., M2, a novel anthracenedione, elicits a potent DNA damage response that can be subverted through checkpoint kinase inhibition to generate mitotic catastrophe. *Biochemical Pharmacology*.
65. Pickhardt, M.; Gazova, Z.; von Bergen, M.; Khlistunova, I.; Wang, Y.; Hascher, A.; Mandelkow, E.-M.; Biernat, J.; Mandelkow, E., Anthraquinones Inhibit Tau Aggregation and Dissolve Alzheimer's Paired Helical Filaments in Vitro and in Cells. *J. Biol. Chem.* **2005,** *280*, 3628-3635.
66. Yen, G.-C.; Duh, P.-D.; Chuang, D.-Y., Antioxidant activity of anthraquinones and anthrone. *Food Chemistry* **2000,** *70*, 437-441.
67. Barnard, D. L.; Huffman, J. H.; Morris, J. L. B.; Wood, S. G.; Hughes, B. G.; Sidwell, R. W., Evaluation of the antiviral activity of anthraquinones, anthrones and anthraquinone derivatives against human cytomegalovirus. *Antiviral Research* **1992,** *17*, 63-77.
68. González-García, S.; Teresa Moreira, M.; Artal, G.; Maldonado, L.; Feijoo, G., Environmental impact assessment of non-wood based pulp production by soda-anthraquinone pulping process. *Journal of Cleaner Production* **2010,** *18*, 137-145.
69. Suemitsu, R.; Yamamoto, T.; Miyai, T.; Ueshima, T., Alterporriol A: a modified bianthraquinone from Alternaria porri. *Phytochemistry* **1987,** *26*, 3221-3224.
70. Singh, J.; Singh, J., A bianthraquinone and a triterpenoid from the seeds of cassia hirsuta. *Phytochemistry* **1986,** *25*, 1985-1987.
71. Alemayehu, G.; Abegaz, B. M., Bianthraquinones from the seeds of Senna multiglandulosa. *Phytochemistry* **1996,** *41*, 919-921.
72. Abdou, W. M.; Elkhoshnieh, Y. O.; Sidky, M. M., Photochemical reactions of bianthrone and related substances. *Tetrahedron* **1994,** *50*, 3595-3602.
73. RAGAZZON, P. A.; ILEY, J.; MISSAILIDIS, S., Structure-activity Studies of the Binding of the Flavonoid Scaffold to DNA. *Anticancer Research* **2009,** *29*, 2285-2293.
74. Tsaffack, M.; Nguemeving, J. R.; Kuete, V.; Tchize, B. L. S. N.; Mkounga, P.; Beng, V. P.; Hultin, P. G.; Tsamo, E.; Nkengfack, A. E., Two new antimicrobial dimeric compounds: febrifuquinone, a vismione-anthraquinone coupled pigment and adamabianthrone, from two Psorospermum species. *Chemical Pharmaceutical Bulletin* **2009,** *57*, 1113-1118.
75. Lara-Avila, S.; Danilov, A.; Geskin, V.; Bouzakraoui, S. d.; Kubatkin, S.; Cornil, J. r. m.; Bjørnholm, T., Bianthrone in a Single-Molecule Junction: Conductance Switching with a Bistable Molecule Facilitated by Image Charge Effects†. *The Journal of Physical Chemistry C* **2010,** *114*, 20686-20695.
76. Horta, J. A.; Faganello, J.; Silva, L. K. R. e.; Oliveira, L. T.; Santurio, J. M.; Vaistein, M. H.; Alves, S. H., Susceptibility to heat and antifungal agents of Cryptococcus neoformans var. neoformans (serotype D) isolated from Eucalyptus spp in Rio Grande do Sul, Brazil. *Brazilian Journal of Microbiology* **2005,** *36*.
77. Kurnatowski, P.; Klimiuk, C.; Głowacka, A., *Effect of electromagnetic waves on sensitivity of fungi of the genus Candida to miconazole*. 2003; Vol. 49, p 61-71.

78. Palou, L.; Crisosto, C. H.; Garner, D., Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve storability of 'Wonderful' pomegranates. *Postharvest Biology and Technology* **2007,** *43*, 133-142.
79. Hawser, S.; Islam, K., Comparisons of the effects of fungicidal and fungistatic antifungal agents on the morphogenetic transformation of Candida albicans. *Journal of Antimicrobial Chemotherapy* **1999,** *43*, 411-413.
80. Fiers, W.; Beyaert, R.; Declercq, W.; Vandenabeele, P., More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene* **1999,** *18*, 7719-7730.
81. Debrabant, A.; Lee, N.; Bertholet, S.; Duncan, R.; Nakhasi, H. L., Programmed cell death in trypanosomatids and other unicellular organisms. *International Journal for Parasitology* **2003,** *33*, 257-267.
82. Kerr, J. F.; Wyllie, A. H.; Currie, A. R., Apoptosis: A Basic Biological Phenomenon with Wideranging Implications in Tissue Kinetics. *Br J Cancer* **1972,** *26*, 239-257.
83. Madeo, F.; Engelhardt, S.; Herker, E.; Lehman, N.; Maldener, C.; Proksch, A.; Wissing, S.; Fröhlich, K. U., Apoptosis in yeast: A new model system with applications in cell biology and medicine. *Current Genetics* **2002,** *41*, 208-216.
84. Madeo, F.; Fröhlich, E.; Fröhlich, K. U., A yeast mutant showing diagnostic markers of early and late apoptosis. *Journal of Cell Biology* **1997,** *139*, 729-734.
85. Madeo, F.; Fröhlich, E.; Ligr, M.; Grey, M.; Sigrist, S. J.; Wolf, D. H.; Fröhlich, K. U., Oxygen stress: A regulator of apoptosis in yeast. *Journal of Cell Biology* **1999,** *145*, 757-767.
86. Ludovico, P.; Rodrigues, F.; Almeida, A.; Silva, M. T.; Barrientos, A.; Côrte-Real, M., Cytochrome c release and mitochondria involvement in programmed cell death induced by acetic acid in Saccharomyces cerevisiae. *Molecular Biology of the Cell* **2002,** *13*, 2598-2606.
87. Ludovico, P.; Sousa, M. J.; Silva, M. T.; Leão, C.; Côrte-Real, M., Saccharomyces cerevisiae commits to a programmed cell death process in response to acetic acid. *Microbiology* **2001,** *147*, 2409-2415.
88. Couldwell, W. T.; Gopalakrishna, R.; Hinton, D. R.; He, S.; Weiss, M. H.; Apuzzo, M. L. J., Hypericin: A Potential Antiglioma Therapy. *Neurosurgery* **1994,** *35*, 705-710.
89. Miccoli, L.; Beurdeley-Thomas, A.; De Pinieux, G.; Sureau, F.; Oudard, S.; Dutrillaux, B.; Poupon, M.-F., Light-induced Photoactivation of Hypericin Affects the Energy Metabolism of Human Glioma Cells by Inhibiting Hexokinase Bound to Mitochondria. *Cancer Research* **1998,** *58*, 5777-5786.
90. Agostinis, P.; Assefa, Z.; Vantieghem, A.; Vandenheede, J. R.; Merlevede, W.; De Witte, P., Apoptotic and anti-apoptotic signaling pathways induced by photodynamic therapy with hypericin. *Advances in Enzyme Regulation* **2000,** *40*, 157-182.
91. Colasanti, A.; Kisslinger, A.; Liuzzi, R.; Quarto, M.; Riccio, P.; Roberti, G.; Tramontano, D.; Villani, F., Hypericin photosensitization of tumor and metastatic cell lines of human prostate. *Journal of Photochemistry and Photobiology B: Biology* **2000,** *54*, 103-107.
92. Marekova, M.; Vavrova, J.; Vokurkova, D.; Cerman, J., LIGHT-INDUCED PHOTOACTIVATION OF HYPERICIN INHIBITS CELLULAR GROWTH IN PITUITARY ADENOMA CELL LINE AtT20/D16v-F2 (HYPERICIN INHIBITS CELLULAR GROWTH OF AtT20/D16v-F2). *ACTA MEDICA* **2001,** *44*, 7-13.
93. Dyer, C. Immunology of Cell Death. http://www.sci-mate.org/wiki/index.php/Immunology_of_Cell_Death (20/05/2011),
94. Fai, P. B.; Grant, A., A comparative study of Saccharomyces cerevisiae sensitivity against eight yeast species sensitivities to a range of toxicants. *Chemosphere* **2009,** *75*, 289-296.
95. Jacob, S. W.; Herschler, R., Pharmacology of DMSO. *Cryobiology* **1986,** *23*, 14-27.
96. Jonák, J.; Rychlík, I., Study of conditions for the inhibitory effect of N-tosyl-l-phenylalanylchloromethane on protein synthesis and the possibility of existence of different

forms of the elongation factor S3 in Bacillus stearothermophilus. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* **1973,** *324*, 554-562.
97. Safety data for p-toluenesulfonyl chloride. http://msds.chem.ox.ac.uk/TO/p-toluenesulfonyl_chloride.html (20/09/2011),
98. Liu, H.; Zhao, X.; Wang, F.; Jiang, X.; Zhang, S.; Ye, M.; Zhao, Z. K.; Zou, H., The proteome analysis of oleaginous yeast Lipomyces starkeyi. *FEMS Yeast Research* **2011,** *11*, 42-51.
99. Wilm, K. H. Our Food: Database of Food and Related Sciences. (14/09/2011),
100. Ageitos, J.; Vallejo, J.; Veiga-Crespo, P.; Villa, T., Oily yeasts as oleaginous cell factories. *Applied Microbiology and Biotechnology* **2011,** *90*, 1219-1227.
101. Schio, L.; Chatreaux, F.; Klich, M., Tosylates in palladium-catalysed coupling reactions. Application to the synthesis of arylcoumarin inhibitors of gyrase B. *Tetrahedron Letters* **2000,** *41*, 1543-1547.
102. Zielske, A. G., (Tosyloxy)anthraquinones. Versatile synthons for the preparation of various aminoanthraquinones. *The Journal of Organic Chemistry* **1987,** *52*, 1305-1309.
103. Nilsson, K.; Mosbach, K., p-Toluenesulfonyl Chloride as an Activating Agent of Agarose for the Preparation of Immobilized Affinity Ligands and Proteins. *European Journal of Biochemistry* **1980,** *112*, 397-402.
104. Aigner, S.; Falk, H., A microwave-assisted synthesis of phenanthroperylene quinones as exemplified with hypericin. *Monatshefte für Chemie / Chemical Monthly* **2008,** *139*, 991-993.
105. Agency, U. S. E. P. Green Chemistry. http://www.epa.gov/greenchemistry/ (20/09/2011),
106. Alexander, M. A.; Jeffries, T. W., Respiratory efficiency and metabolite partitioning as regulatory phenomena in yeasts. *Enzyme and Microbial Technology* **1990,** *12*, 2-19.
107. Sussman, I.; Erecioska, M.; Wilson, D. F., Regulation of cellular energy metabolism. The Crabtree effect. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1980,** *591*, 209-223.
108. Charles E, W., [31] Pasteur and crabtree effects—Assay in cells. In *Methods in Enzymology*, Sidney Fleischer, L. P., Ed. Academic Press: 1979; Vol. Volume 55, pp 289-297.
109. Alves, D. S.; Pérez-Fons, L.; Estepa, A.; Micol, V., Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. *Biochemical Pharmacology* **2004,** *68*, 549-561.
110. Haraguchi, H.; Kataoka, S.; Okamoto, S.; Hanafi, M.; Shibata, K., Antimicrobial triterpenes from Ilex integra and the mechanism of antifungal action. *Phytother Res* **1999,** *13*, 151-156.
111. Mendoza, L.; Araya-Maturana, R.; Cardona, W.; Delgado-Castro, T.; Garcia, C.; Lagos, C.; Cotoras, M., In Vitro Sensitivity of Botrytis cinerea to Anthraquinone and Anthrahydroquinone Derivatives. *Journal of Agricultural and Food Chemistry* **2005,** *53*, 10080-10084.
112. Lawrence, C. L.; Maekawa, H.; Worthington, J. L.; Reiter, W.; Wilkinson, C. R. M.; Jones, N., Regulation of Schizosaccharomyces pombe Atf1 Protein Levels by Sty1-mediated Phosphorylation and Heterodimerization with Pcr1. *J. Biol. Chem.* **2007,** *282*, 5160-5170.
113. Moreno, S.; Klar, A.; Nurse, P., Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. *Methods Enzymol* **1991,** *194*, 795-823.
114. Brachmann, C. B.; Davis, A.; Cost, G. J.; Caputo, E.; Li, J.; Hieter, P.; Boeke, J. D., Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **1998,** *14*, 115-132.
115. Treco, D. A.; Lundblad, V., Preparation of Yeast Media. In *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc.: 2001.

APPENDIX

Table showing the raw data from the growth inhibition assay.

a : growth seen in all wells up to 0.0007 g/ml concentration

s : Compound is fungistatic

c : Compound is fungicidal

		S. pombe				*L. starkeyi*				*S. cerevisiae*			
Compound	MW	mmol	µmol	log(µmol)	S or C	mmol	µmol	log(µmol)	S or C	mmol	µmol	log(µmol)	S or C
Emodin (1)	270.24	1.2950	1295.0	3.1123	s	2.590	2590	3.413	s	1.300	1300	3.114	s
1,8-dihydroxyanthraquinone (2)	239.22	1.4600	1460.0	3.1644	s	2.910	2910	3.464	s	2.910	2910	3.464	s
1-hydroxy-8-tosyloxyanthraquinone (3)	394.40	1.7700	1770.0	3.2480	s	*a*	*a*	*a*	*a*	1.770	1770	3.248	s
1,8-ditosyloxyanthraquinone (4)	548.58	1.2800	1280.0	3.1072	s	*a*	*a*	*a*	*a*	0.638	638	2.805	s
emodin anthrone (5)	256.25	0.0976	97.6	1.9894	s	39.000	39000	4.591	c	1.950	1950	3.290	c
1,8-dihydroxyanthrone (6)	226.23	0.0553	55.3	1.7427	s	22.100	22100	4.344	s	0.553	553	2.743	s
1-hydroxy-8-tosyloxyanthrone (7)	380.41	0.0657	65.7	1.8176	s	0.657	657	2.818	s	0.329	329	2.517	s
1,8-ditosyloxyanthrone (8)	534.60	0.0468	46.8	1.6702	s	0.468	468	2.670	s	0.234	234	2.369	s
emodin bianthrone (9)	510.49	0.9800	980.0	2.9912	s	0.979	979	2.991	s	0.979	979	2.991	s
1,8,9,16-tetrahydroxybianthrone (10)	450.44	0.1400	140.0	2.1461	s	0.555	555	2.744	s	0.555	555	2.744	s
1,9-dihydroxy-8,16-ditosyloxybianthrone (11)	758.81	0.4600	460.0	2.6628	s	0.922	922	2.965	s	0.461	461	2.664	s
1,8,9,16-tetratosyloxybianthrone (12)	1065.17	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
1,8-dihydroxy-9,16-ditosyloxybianthrone (13)	758.81	0.4600	460.0	2.6628	c	0.922	922	2.965	s	0.922	922	2.965	s
1-[(2-aminoethyl)amino]-8-hydroxyanthraquinone (14)	282.29	0.4400	440.0	2.6435	s	0.886	886	2.947	s	0.886	886	2.947	s
1-[(3-aminopropyl)amino]-8-hydroxyanthraquinone (15)	296.32	0.4200	420.0	2.6232	c	0.844	844	2.926	s	0.844	844	2.926	s
1-[(3-aminopropyl)amino]-8-tosyloxyanthraquinone (16)	450.51	0.1400	140.0	2.1461	s	0.555	555	2.744	c	0.555	555	2.744	c
1-[(4-aminobutyl)amino]-8-hydroxyanthraquinone (17)	310.35	0.2000	200.0	2.3010	c	0.403	403	2.605	s	0.201	201	2.303	s
Hypericin (18)	504.44	1.9800	1980.0	3.2967	c	0.991	991	2.996	s	0.991	991	2.996	s

Plate pictures for the growth inhibition of *S. **pombe.***

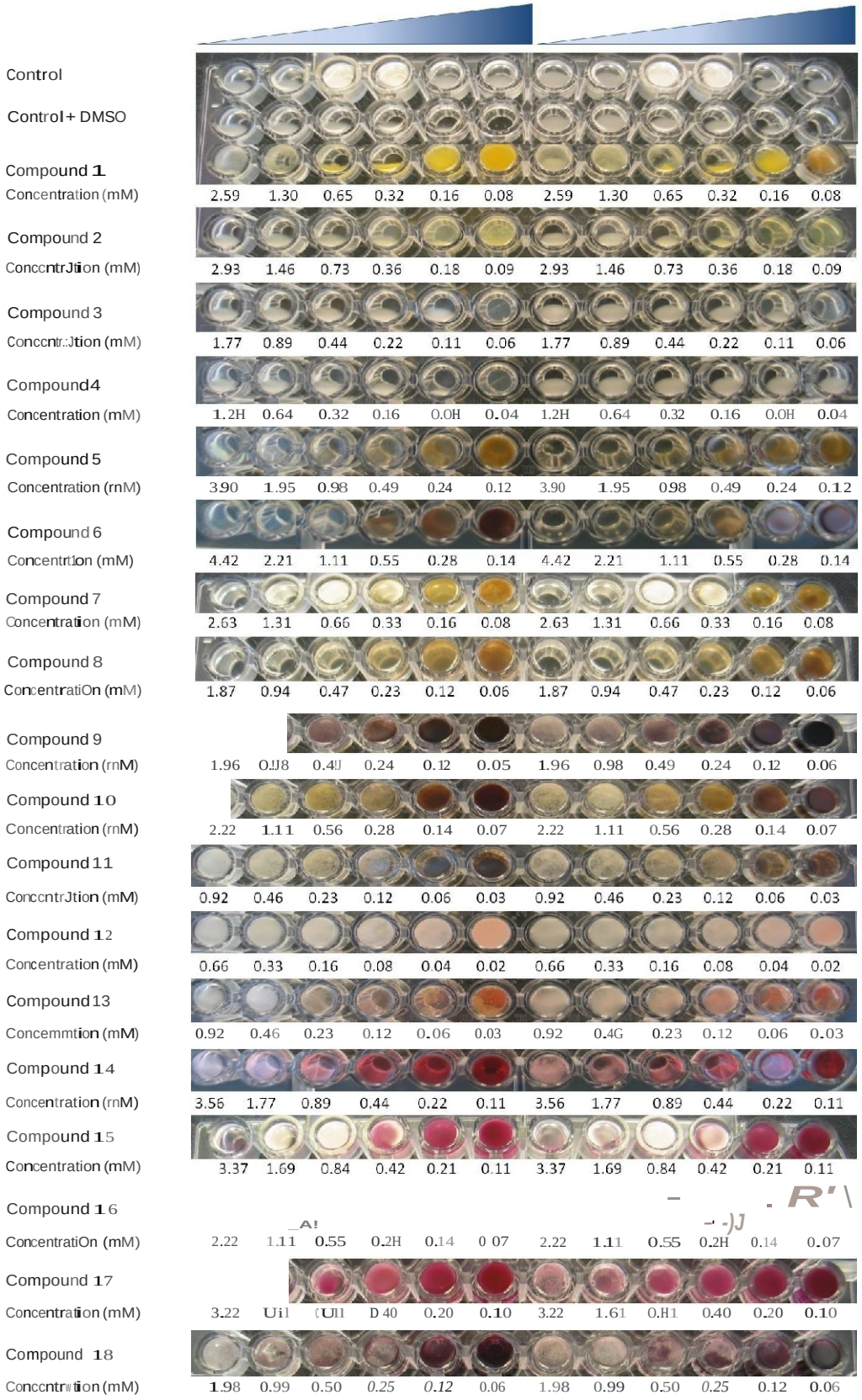


Plate pictures for the growth inhibition of *L starkeyi*

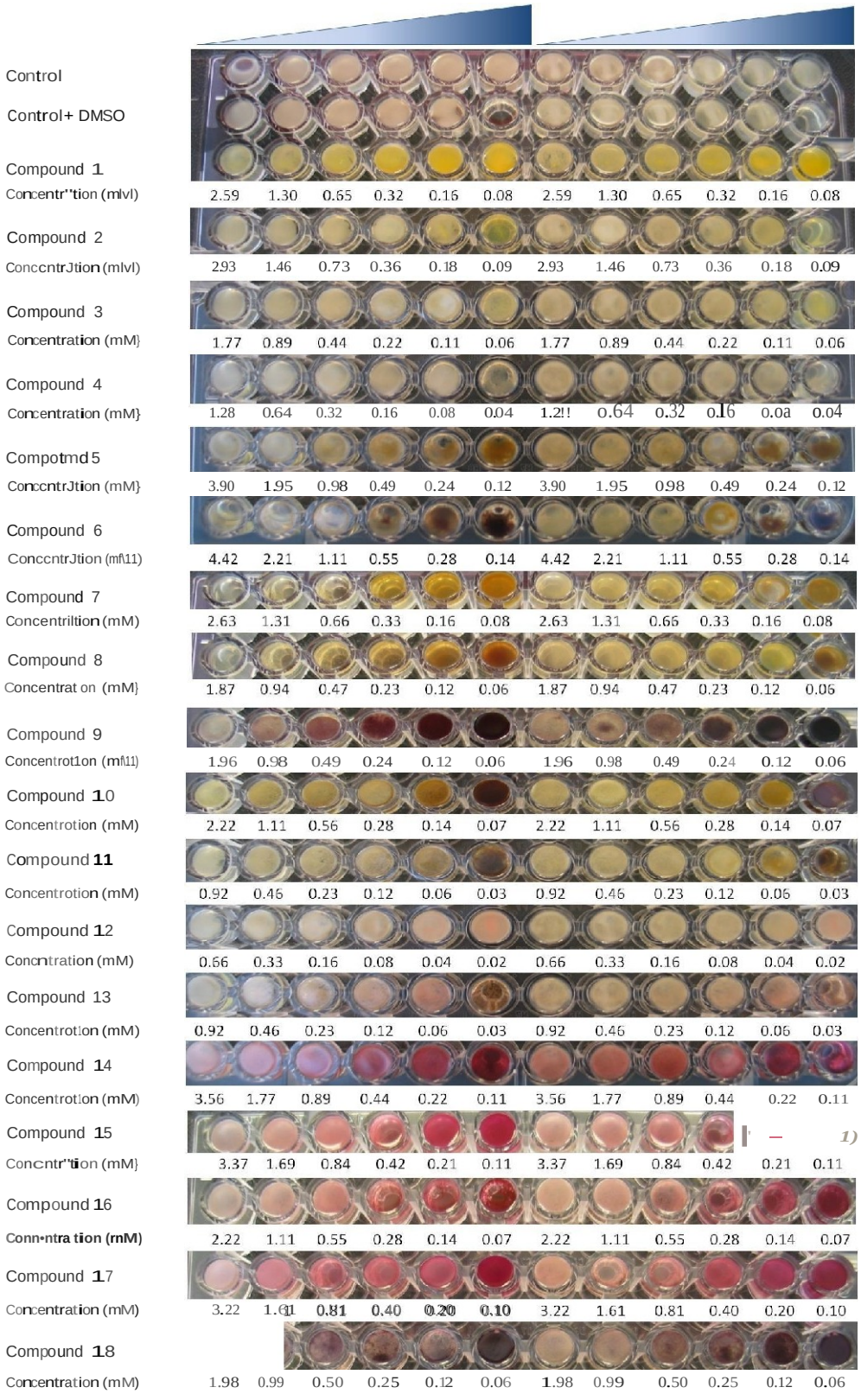
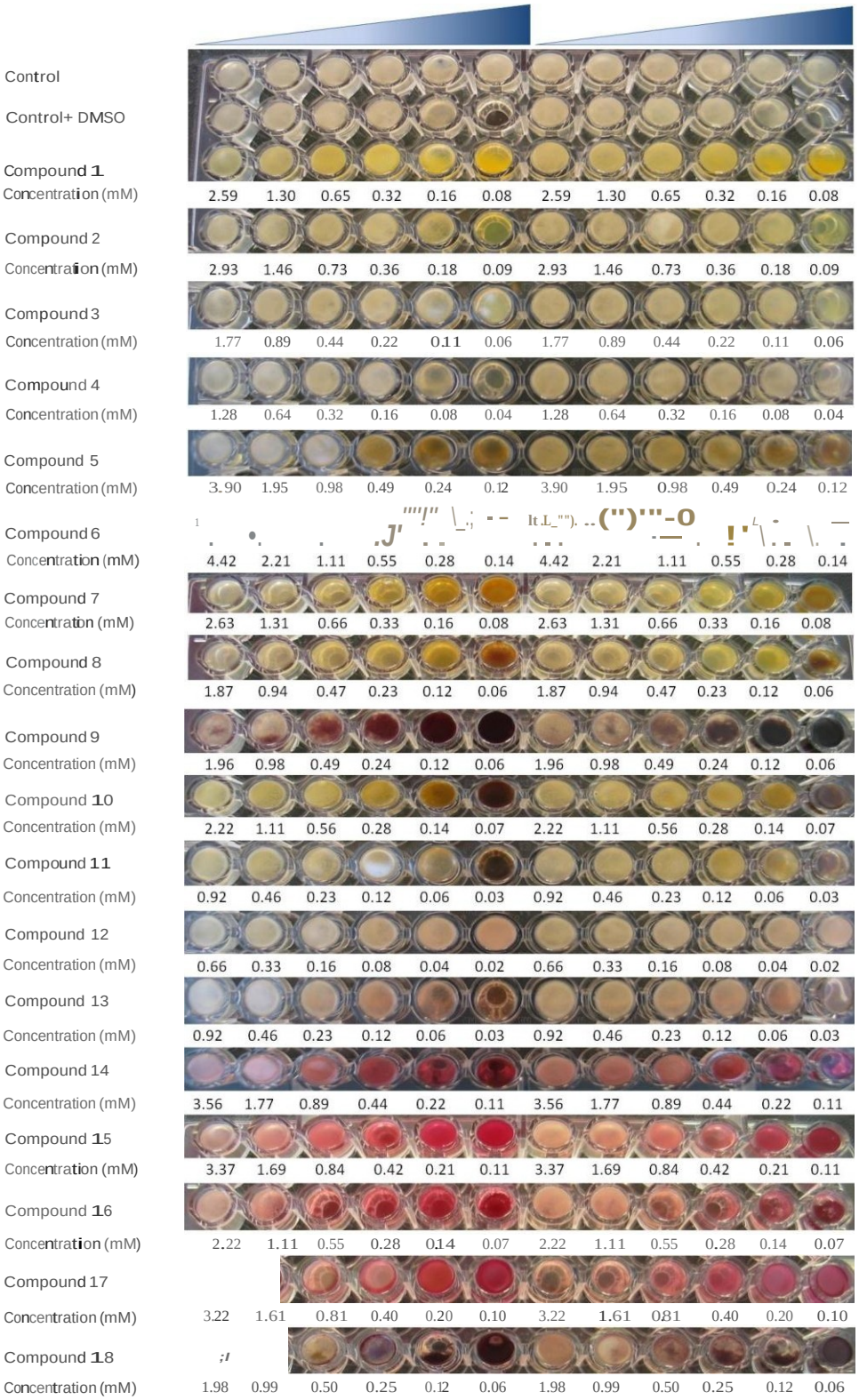


Plate pictures for the growth inhibition of *S. cerevisiae*



Repeat of compounds (5), (6), (7) and (8) at lower concentrations in 5. *pombe.*