THE DESIGN AND SYNTHESIS OF NOVEL TACRINE ANALOGUES

Thesis submitted to the University of Strathclyde
in fulfilment of the requirements for the
Degree of Doctor of Philosophy.

by

MAUREEN T. McKENNA

Department of Pure and Applied Chemistry,
Thomas Graham Building,
295 Cathedral Street,
Glasgow. G1 1XL

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For my parents, Peter and Elizabeth McKenna.

"... (al)chemists, their abominable hodgepodge of ideas and language, their confessed interest in gold, their Levantine swindles typical of charlatans or magicians."

"The Periodic Table." Primo Levi

CONTENTS

RESULTS AND DISCUSSION

ABSTRACT

From the basic structure of 9-amino-1,2,3,4-tetrahydroacridine (36), was designed a range of analogues. These compounds were synthesised using a Lewis acid-mediated cyclodehydration reaction involving anthranilonitrile (150) and various 1-tetralones, 1-benzosuberones and 2-benzosuberone. Heterocyclic ketones such as 7-chloro-1,2,3,4-tetrahydroquinolines, 1,2,3,4-tetrahydrobenzazepinones and 1,2,3,4,5,6-hexahydrobenzazocinone were also substituted into the reaction to produce novel heterocycles. Their protected nitrogen moieties were detosylated with sodium naphthalenide. Bridged tacrine analogues were investigated using norcamphor (210) and R(+)-camphor (212). All the resultant products were subjected to pharmacological assay and from inspection of the results further molecular design was possible. Synthetic routes were extended to include imine and enamine intermediates. In particular, *N*-(4-methyl-5-oxo-cyclopenten-1-yl)-2-aminobenzonitrile (263a) was an important enamine intermediate which cyclised to give 9-amino-2,3-dihydro-2,3-disubstituted-3-hydroxy-[*1H*]-cyclopenta-[*b*]-quinolines with use of organolithium reagents. These were dehydrated to their respective alkenes. This cyclisation was further investigated with the use of vinyl anions produced by the Shapiro reaction.

Compounds were produced which showed activity as anticholinesterase agents and 5-HT uptake inhibitors.

ACKNOWLEDGEMENTS

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The last words go to Craig Johnstone, my bestest friend and staunchest ally, whose boundless patience and support during my Ph.D. I can never repay. Hey, let's do it again some time.

ABBREVIATIONS

ACh - acetylcholine
AD - Alzheimer's disease
Acetyl CoA - acetyl coenzyme A
AChEase - acetylcholinesterase
n-BuLi - *n*-butyllithium
BuChEase - butyrylcholinesterase
ChAT - choline acetyltransferase
cAMP - cyclic adenosine monophosphate
CNS - central nervous system
conc. - concentrated
DMAD - dimethyl acetylene dicarboxylate
DMF - *N,N*- dimethylformamide
DME - dimethoxyethane
glac. - glacial
GABA - *gamma* -aminobutyric acid
5-HT - 5-hydroxytryptamine
HMPT - hexamethylphosphoric triamide
LDA - lithium diisopropylamide
mcpba - *meta*-chloro-perbenzoic acid
NA - noradrenaline
NBS - *N*-bromosuccinimide
NEM - *N*- ethyl-morpholine
ny - no yield reported
p-TSA - *para*-toluenesulphonic acid
PPA - polyphosphoric acid
PCP - phencyclidine
py - pyridine
tlc - thin layer chromatography
THF - tetrahydrofuran
Ts - tosyl, toluenesulphonyl

INTRODUCTION

Chapter 1

ALZHEIMER'S DISEASE

Dementia is the most serious psychiatric disorder of old age and Alzheimer's disease[1] (AD) is its most common cause. First described by Alois Alzheimer in 1907 as a pathological curiosity[2], it has been suggested that the disease will become a major medical problem for the Western World by the year 2000 when the proportion of those people over the age of 65 will have risen to more than 20 percent of the population[3]. A fifth of these people over the age of 65 will have the disease, rising to a quarter over the age of 80, and to more than half over the age of 90. These statistics account for the 0.5 million people affected or killed by AD in the United Kingdom today, becoming the fourth major cause of death after heart disease, cancer and stroke[4]. In the United States, as many as 4 million people are affected and scientists there predict that, in the absence of any advances in treatment, the number of people with AD will grow to 6.2 million, including 2.2 million severe cases, by 2015[5]. AD has therefore become a major target for all types of research investigating the causes, symptoms and treatment of this mysterious condition.

The first problem that is faced when an individual begins to show the symptoms of dementia is one of diagnosis by their doctor. Dementia is a term encompassing mental disturbances which may have any number of causes. Dementias of acute onset, that is, those that occur as a result of major trauma are

non-progressive unless further trauma occurs. These follow such events as severe hypoxic states caused by, for example, carbon monoxide poisoning, strangulation or hanging, severe hypoglycæmic states, meningitic or encephalitic infections, clots or deposits after hæmorrhaging and most commonly, after severe head injury[6]. These dementias do not relate to AD in that they are not progressive like the other dementias of middle and later life that occur for reasons other than physical injury.

Progressive dementias, of which AD is one, have many causes and are often treatable.

(i) Vitamin deficiencies such as niacin or B_{12} **may** present purely as a psychiatric condition of depression or dementia.

(ii) Endocrine disorders such as hypothyroidism give early mental symptoms. For example, cognitive loss and depression frequently develop before the characteristic physical symptoms. This condition is treatable but at the risk of permanent mental impairment if the treatment has not been given within about 2 years of the start of the intellectual decline.

(iii) Vascular conditions – strokes, arrhythmias, heart block – may provoke or aggravate dementing symptoms in elderly patients.

(iv) Head injuries may also result in progressive dementia if a subdural hæmatoma (an accumulation of blood in the subdural space) has occurred causing pressure and further accumulation of fluid. This gives rise to headaches, drowsiness and dementia and requires surgical treatment. A well-known cause of this type of dementia is chronic traumatic encephalopathy (dementia pugilistica) - the "punch drunk" syndrome acquired by boxers. This may present as Parkinson-like tremors, even spasticity, but there is progressive intellectual impairment accompanying emotional and personality changes.

(v) Chronic infections may give rise to dementia, although these have become rarer since the introduction of antibiotics in the treatment of such diseases as syphilis.

(vi) Viral infections such as measles and rubella may give progressive dementia with death occurring within a few months.

It can clearly be seen that there are many deficiencies, disorders and lesions which give rise to mental disturbances of which the above are examples. The appropriate investigations must therefore be carried out in all cases of dementia when first seen so as to distinguish the curable from the incurable – one should not assume the patient is a victim of AD merely because they are elderly.

AD itself has characteristic symptoms and although it is possible to confuse dementias, there is a pattern of changes which may emerge in an Alzheimer patient[3]. In most people AD is first recognised by the striking loss of short term memory in an otherwise healthy individual, and perhaps disorientation, with the person found wandering in unlikely places at unusual hours. At this stage, language and senses remain intact, as do older memories. However, as the disease progresses all aspects of memory fail, with simple tests becoming hazardous when the person cannot recall the order in which to perform the required actions or indeed the names or uses of common objects. The patient loses the ability for judgement, recognition (of family and friends) and the senses, such as the feeling of heat or coldness. The personality deteriorates, bringing with it mood disturbances, anxiety and possibly psychotic episodes. In the final stages of the disease there is total memory loss, loss of language and the patient becomes incontinent, vegetative and hence bedridden. Death is the inevitable conclusion. The time scale of this disease can be anything between 5 and 15 years from the onset of AD until death.

It seems to be the case that the earlier the onset of the disease, the more rapid its progression. As yet, there is no known cause for AD and no known cure.

Although AD is quite difficult to diagnose while the person is alive, definitive diagnosis of the disease is possible *post mortem* as the pathological changes that occur in the brain have been well documented due to extensive research[1,7,8,9]. The characteristic histological findings are located in an area of the brain called the cerebral cortex. The cortex (*figure 1*) is a thin layer of grey matter covering both hemispheres of the cerebrum which is the largest part of the brain in mammals. The cortex consists of interconnecting layers of neurones which communicate with each other and also project to other areas of the brain. It is considered to be the highest region of the central nervous system as it is the end point for information from the periphery, co-ordinating the incoming information with memories previously gained and initiating appropriate motor activity. It is also that part of the brain responsible for higher intellectual functioning; memories, recognition, mathematical skills, emotions and the senses (touch, taste, smell, sight, hearing). The convoluted surface of the cortex is a continuous mass of nervous tissue, divided into four main areas according to the bones of the skull that cover them:

(i) the frontal lobe, containing the motor cortex, premotor cortex, motor speech area and centres associated with co-ordination of autonomic and somatic activity.

(ii) the parietal lobe, containing the sensory cortex.

(iii) the occipital lobe, containing the visual cortex.

(iv) the temporal lobe, containing the auditory cortex.

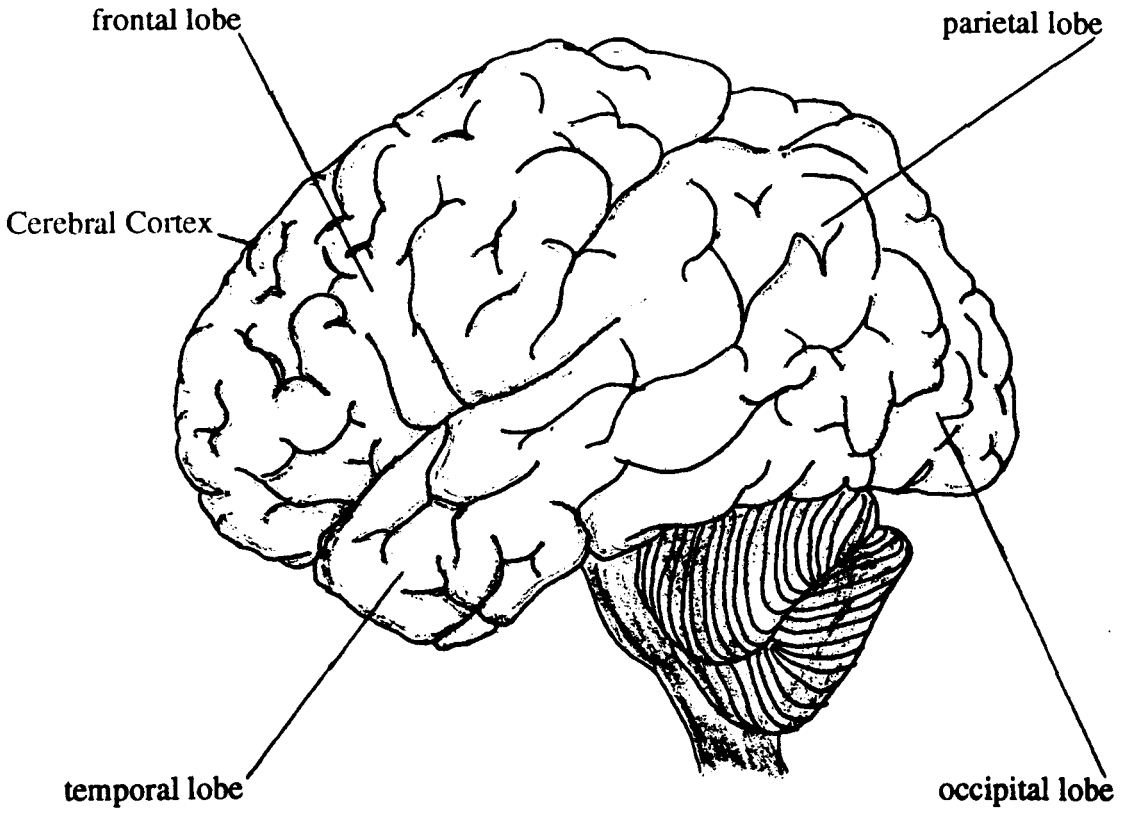


Figure 1: Diagram of the brain, indicating areas of the cerebral cortex.

Although these areas can be mapped in the brain, there are large parts surrounding them which seem to have no specific function, previously thought of as "silent areas". Now it is considered that they work together as a whole, giving the faculties of recognition, memory, imagination, creative thought, consciousness and personality.

The changes in the brain caused by AD, both in its structure and chemistry, occur mainly in the cortex, and give rise to the characteristic symptoms *i.e.* loss of memory, language, senses *etc.* The lesions that occur in the brain tissue are described as senile plaques and neurofibrillary tangles and were first noticed by Alzheimer who wrote, "Scattered through the entire cortex, especially in the upper layers, one found miliary foci that were caused by the deposition of a peculiar substance on the cerebral cortex"[7].

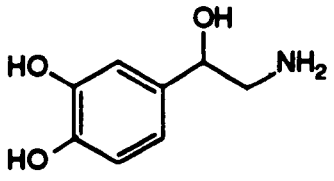
The senile plaque[7] is a slowly evolving and complex structure which has a central core of protein fragment known as β-amyloid protein and a surround of altered axons and dendrites (the long, tapered processes from the bodies of nerve cells). In addition to this, the mature plaque may also contain two types of glial cells (cells not of nervous origin *i.e.* not neurones) - microglial cells and astrocytes. Microglial cells are scavengers capable of responding to inflammation or the destruction of nervous tissue in brain disorders, and astrocytes are often found in injured areas of the brain.

Neurofibrillary tangles[7] are dense bundles of abnormal fibres which occur in the cytoplasm of certain neurones. These fibres consist of paired helical filaments of a modified form of a naturally occurring protein called *tau*, and it is not known how, or even if, these are connected to the β-amyloid deposition.

However, aged healthy people do develop these senile plaques and tangles, particularly in an area of the brain called the hippocampus and other areas associated with memory. This may be responsible for age-associated memory impairment, a normal consequence of ageing. The distinction, then, between a normal ageing brain and the brain of an Alzheimer patient is that of the **quantity** of these lesions. Perhaps a simple description of an "Alzheimer brain" would be that of a brain which has prematurely, rapidly and massively aged beyond the physical years of the person.

In addition to the damage caused by the presence of plaques and tangles, there is also a dramatic loss of neurones, altering the levels of neurotransmitters – those small molecules involved in the communication between neurones. A decrease in the levels of substances such as noradrenaline (1), somatostatin (growth hormone release-inhibiting hormone) (2) and 5-hydroxytryptamine (5-HT,

serotonin) (3) are documented[3,10] and indeed all these changes must contribute to the overall profile of AD.



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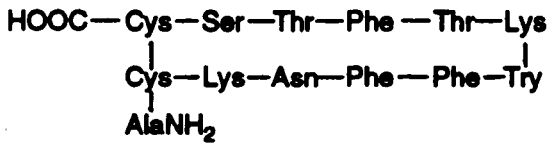

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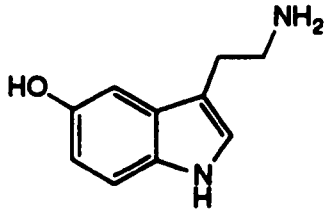


(3)

However, the most consistent deficits are those seen in the cholinergic system of the brain, that is, in those neurones that transmit by releasing acetylcholine. This has been elucidated by histological and biochemical investigation of *post mortem* brain tissue of Alzheimer patients[11,12,13] revealing a degeneration of cholinergic neurones and loss of cortical cholinergic axons, these losses also extending into other brain areas. The marker of defective cholinergic transmission is the enzyme choline acetyltransferase which catalyses the synthesis of acetylcholine in the neurone, and this provides the information on the brain areas involved – loss of this enzyme correlating with loss of cholinergic function[14].

However, the physiology of the cerebral cholinergic system is still not fully understood, and anatomically, the association between activation of individual cholinergic pathways and specific forms of behaviour is not understood at all.

The general opinion of AD is that the cholinergic deficits in the brain are, at least, related to the memory losses characteristic of the disease, and that the other symptoms that are manifest are too subtle and difficult to quantify as resulting from these deficits *e.g.* perception and other cognitive faculties.

There is of course a great deal of research into the cause of AD and it has stirred up much controversy over the years. One of the first things that is generally associated with AD is aluminium[15] which was first implicated when it was found that some dialysis patients were suffering from dementia (the dialysis fluid contained aluminium). Aluminium is a very potent neurotoxin which can interfere with many reactions in the brain, some of which are associated with AD. However, aluminium is also strongly connected with epilepsy and microcyctic anæmia, both of which are not generally found in patients with AD. It has been suggested that the staining techniques used to prepare samples of brain tissue had accumulated aluminium compounds as a contaminant from the dye, which was then detected on subsequent examination of the senile plaques[16]. The role of aluminium is therefore still unclear.

As well as with toxins, viruses and other infective agents, there has been much research into a possible genetic component of AD. The formation of β-amyloid, the constituent of senile plaques, is a protein fragment about 40 amino acids long which arises from the cleavage of a much larger precursor protein encoded by a gene on chromosome 21[17]. This itself has raised the interesting question of a connection between AD and Down's syndrome[18], since people suffering from Down's syndrome are born with three copies of chromosome 21 instead of the normal two. Also, Down's syndrome people develop lesions typical of AD in their forties and fifties, by which time their behaviour and mental abilities are beginning to decline. Research is therefore being carried out on the

assumption that build-up of β-amyloid protein in the brain has a common cause in AD and Down's syndrome in a mutation on chromosome 21[19]. Again, opinions are divided. Chromosome 21 is being closely studied for this mutation but it has been argued[20] that this gene is known to be unaltered in AD and instead other changes must be responsible for accelerated β-amyloid deposition, such as abnormalities in the control of protein synthesis in the brain (*e.g.* transcription or post-translation of the gene). Studies to support these hypotheses were carried out in 1987 when AD was being described as an autosomal dominant disease, with the genetic defect occurring on chromosome 21, and this was therefore the "familial" Alzheimer gene[21]. This was subsequently shown not to be the case - in familial AD the disorder does not segregate exclusively with the amyloid protein gene and this gene is not present in excess in either sporadic AD or Down's syndrome[22,23], and it is now clear that the gene for the amyloid protein is not the gene for familial AD and is therefore not the cause of AD[24], although it is possibly contributory.

At present there is work being carried out to show that AD is a direct consequence of ageing[25] which then manifests itself in (perhaps) genetically susceptible people. These workers have discovered that there is a burst of growth in the brain around middle age when brain connections begin to resprout. This is presumably an attempt by the brain to protect itself from the effects of ageing, but what these researchers have shown is that the subsequent deposition of β-amyloid seems to start in the same area of the brain each time - the medial temporal lobe (see page 5, *figure 1*), which is responsible for memory. The conclusion of these researchers is that the regrowth of the brain, which happens to everyone, results in a vast over-production of β-amyloid in some vulnerable people as an unfortunate by-product of the ageing process.

Another avenue of research has produced yet a different theory – that AD is the result of an inflammatory response to an immunological fault in the brain[26]. Aggregation of β-amyloid can activate a component of the immune system called the complement cascade. This is a chain of events normally only triggered by antibodies when there is a need to destroy damaged or infected cells. This, of course, does not explain the presence of β-amyloid to begin with, but it is proposed that microglial cells, the scavenger cells of the brain, may be involved in producing the protein. All of this evidence and supposition has led to the proposal that AD is an autoimmune disease similar to rheumatoid arthritis. The researchers decided to test this theory of the role of inflammation in AD by comparing the records of Alzheimer's patients with those suffering from arthritis (who would be taking courses of anti-inflammatory drugs). The data showed that there was an unusually small number of people with both diseases – something that would be expected if anti-inflammatories prevented, or slowed, the processes of AD. The researchers then discovered that a similar observation had already been published postulating a genetic link between susceptibility to arthritis and resistance to AD. This additional piece of evidence came from a leper colony, where the leprosy drug being used was not only an antibiotic but an effective anti-inflammatory as well. *Post-mortem* analysis of the brains of lepers showed an absence of senile plaques, a remarkable finding considering that even in normal aged brains plaques are expected to occur.

It can be seen there is a vast and confusing amount of research being carried out today on the current hypotheses. Is it genetic and encoded on chromosome 21; is it a different type of abnormality to do with the synthesis of β-amyloid protein; or is it the consequence of an as yet unknown trigger? With the millions of people affected by AD and the millions spent by drug companies into research on

AD there is much at stake, but as each theory is investigated we are surely coming closer to elucidating its causes and perhaps formulating a cure.

As we wait for the answers, though, we need to provide therapy for the sufferers of AD today. Discovery of a drug for this purpose is therefore the aim of this project.

Chapter 2

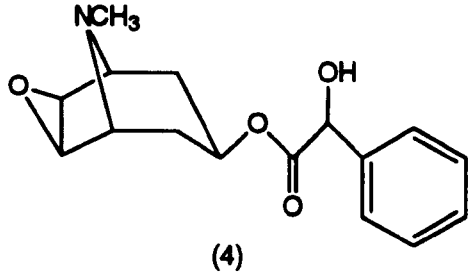
THERAPEUTIC STRATEGIES FOR ALZHEIMER'S DISEASE

(a) The Cholinergic Hypothesis

Prior to the mid-seventies, AD was thought of as a non-specific degenerative disease where a drug acting on a particular neurochemical system was unlikely to have any positive effect. But in 1976, Davis and Maloney carried out a study on the selective loss of central cholinergic neurones in *post mortem* examination of brain tissue from sufferers of AD[12], a finding replicated by Bowen *et al* [13]. They measured the quantities of the enzymes responsible for the synthesis and degradation of acetylcholine and had found remarkable reductions in their levels, particularly around the areas of greatest density of neurofibrillary tangles. It was subsequently found the extent of these changes in the cholinergic system also correlated with the severity of the dementia[14]. The discovery that cholinergic function was severely compromised in AD was an important landmark in the search for a specific pharmacologic agent for symptomatic treatment.

Research into the cholinergic hypothesis began to show direct comparisons to the pharmacological actions of known drugs. For example, as far back as 1906, it had been discovered that a combination of sedatives and centrally active anticholinergic agents produced a state of altered conciousness called "twilight

sleep"[27]. A combination of such drugs was occasionally used in the fifties and sixties during childbirth. Then, in 1974, it was found that the anticholinergic scopolamine (4) produced memory disorders in normal volunteers where learning abilities were severely affected[28]. Significantly, it was discovered that the ability to recall previously learned material was not affected, similarly with AD in the first stages (long-term memories do not deteriorate until the more advanced stages).



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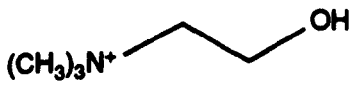
Since then, extensive research has focussed on the central cholinergic system[3,11] as a target for research into the discovery of a method to relieve the more distressing symptoms of AD (such as loss of memory and cognitive impairment). To have an understanding of how this can be achieved requires an outline of the cholinergic system itself, the biochemistry within it and the mechanisms by which it can be affected.

(b) Cholinergic Transmission

Acetylcholine (ACh) (7) is synthesised in cholinergic nerve terminals from two precursors: choline (5) and acetyl coenzyme A (6) in a reaction catalysed by choline acetyltransferase (ChAT, choline acetylase E.C. 2.3.1.6)[8].

The diagram below (*figure 2*) gives a summary of events at a cholinergic synapse.



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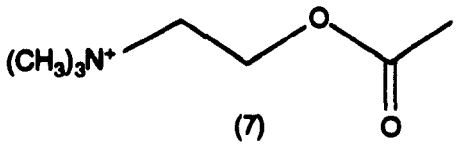

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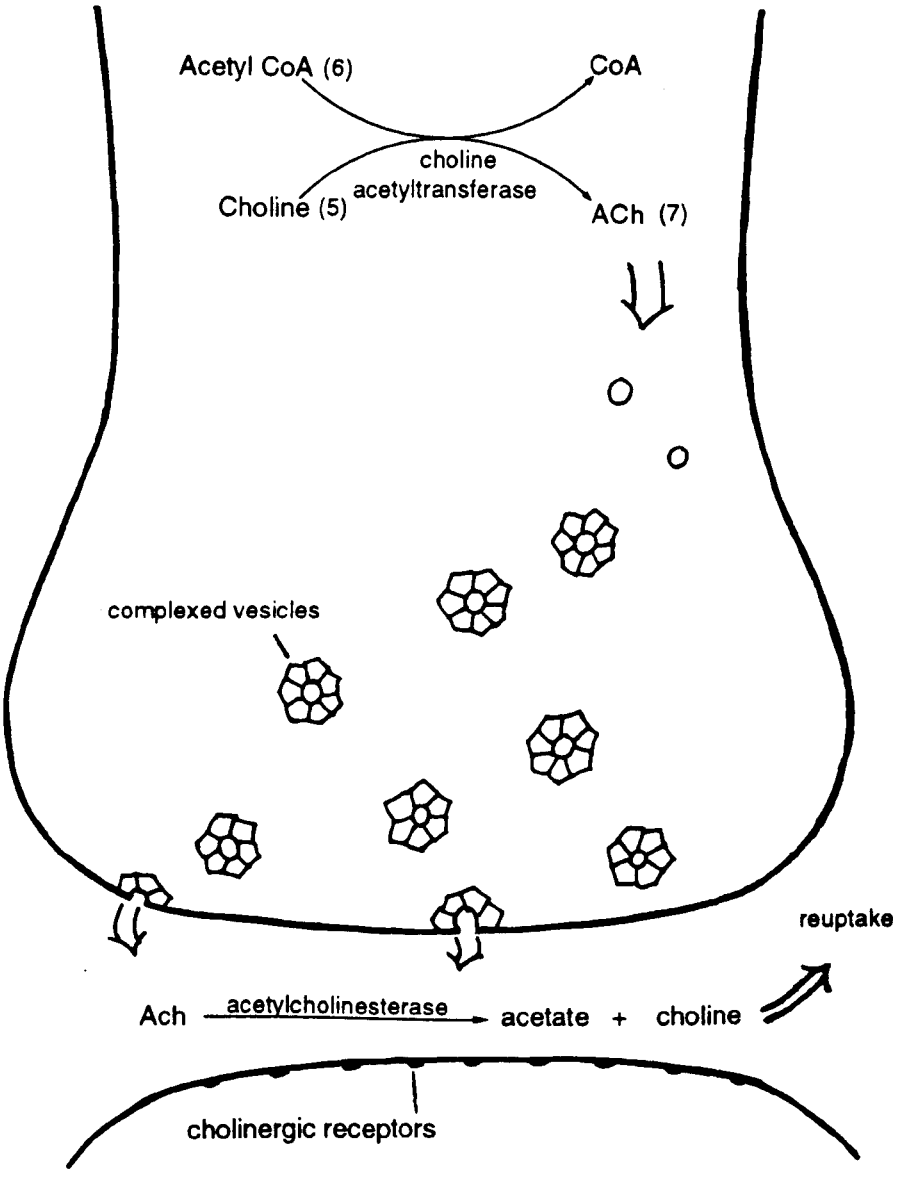


Figure 2: Representation of the synthesis, storage and release of acetylcholine at a cholinergic nerve terminal.

Acetyl coenzyme A (6) synthesis takes place in mitochondria which are present in large numbers in the nerve terminal. Choline, however, is provided by transport into the nerve ending from the extracellular fluid by two distinct mechanisms: (i) a saturable, sodium dependent, high affinity process and (ii) a high-capacity, low

affinity process[30]. The high-affinity transport system is believed to be crucial for the delivery of choline into the nerve terminals.

The ACh is stored prior to use in synaptic vesicles which are activated by the arrival of an action potential which passes along the nerve, or axon, and arrives at the terminal causing depolarisation of the axonal membrane. The term depolarisation describes the reversal of the existing "resting" electrical potential across the membrane, triggering an influx of Ca^{2+} ions in response. The presence of Ca^{2+} ions couples the action potential to the release of ACh[31] which is believed to occur by fusion of the vesicles with the axonal membrane which releases the ACh into the synaptic cleft in a process of exocytosis. The molecules of ACh then diffuse across the synaptic gap and combine with the receptors on the post-synaptic membrane, stimulating secondary processes to take place (*e.g.* cAMP formation or phosphoinositol hydrolysis) hence propagating or inhibiting the functions carried out by that nerve or tissue as appropriate.

To inactivate ACh, there exists a family of enzymes which hydrolyse ACh to its components of choline and acetate, the choline molecules then being taken up by the nerve for its re-use in ACh synthesis. These enzymes are known as cholinesterases and there are two distinct types. Acetylcholinesterase (true, or specific cholinesterase, acetylcholine acetylhydrolase E.C. 3.1.1.7) is found in the synaptic cleft at cholinergic synapses where its function is the hydrolysis of ACh, for which it is a relatively specific enzyme. It exists in several molecular forms[32] and, with the capacity to hydrolyse (3×10^5) ACh molecules per enzyme molecule per minute, is one of the most efficient enzymes known[33].

Butyrylcholinesterase (serum, pseudo- or non-specific cholinesterase) is a group of enzymes that has a wider distribution and is found in such tissues as liver, skin, gastrointestinal smooth muscle and plasma, with no clearly defined functional

rôle. These enzymes are not particularly associated with cholinergic synapses and have broader actions than acetylcholinesterase, hydrolysing butyrylcholine and other esters more rapidly than ACh.

ACh itself is very widespread throughout the body, both in the peripheral and central nervous systems. Peripherally, ACh is responsible for transmission at the neuromuscular junction; all motor nerves leaving the central nervous system (CNS), all post-ganglionic* parasympathetic† fibres and all pre-ganglionic* fibres of peripheral autonomic ganglia[34]. The autonomic nervous system conveys all of the outputs from the CNS to the rest of the body except for motor innervation of skeletal muscle and is largely outside the influence of voluntary control. The main processes that the autonomic nervous system regulates are (i) the contraction and relaxation of smooth muscle, (ii) all exocrine and certain endocrine secretions, (iii) the heartbeat and (iv) certain metabolic steps.

Centrally, ACh is active as a neurotransmitter and, as mentioned before, its actions are not fully understood. It is very widely distributed in the brain but the functional characteristics of cholinergic pathways have been deduced in the main from studies of the actions of drugs which affect cholinergic function in some way. The main functions associated with ACh transmission are related to arousal, learning, short-term memory and motor control.

An understanding of the events occurring at a cholinergic synapse and knowledge of where these synapses operate leads us naturally to investigate the methods by which

* Tissues under the control of the autonomic nervous system are innervated via two neurones connected in series, one originating from the CNS and the other ending at a tissue. The two neurones are known respectively as pre-ganglionic and post-ganglionic and their junction known as the autonomic ganglion synapse, which itself lies outside the CNS[35].

† The autonomic nervous system is divided **anatomically** into two groups, namely sympathetic and parasympathetic.

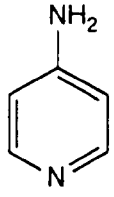
these processes could be modified. In the case of AD, enhancement of the remaining cholinergic function in the patient's brain is the requirement of any therapeutic treatment. But how exactly could this be achieved? There exist several strategies towards solving the problem. Firstly, by attempting to introduce amounts of ACh precursor *i.e.* choline. Secondly, by the stimulation of ACh synthesis or release. Thirdly, by the administration of cholinergic agonists and fourthly, by the inhibition of the enzyme that hydrolyses ACh to its component parts (see *figure 2*).

(c) Acetylcholine Precursors

To administer one of the precursors to ACh for the treatment of AD has a direct analogy in Parkinson's disease which, leaving the patient with a deficit of the neurotransmitter dopamine, is treated by the introduction of the immediate precursor to dopamine, L-Dopa. However, clinical trials[36] with choline (5) and lecithin (phosphatidylcholine, the main dietary source of ACh and a constituent of all cell membranes) have produced disappointing results[37]. Evidence[38,39] to suggest that choline affects neurotransmission at all is inconclusive, indeed some of the results reported are contradictory[40]. Although there may be a place for AD treatment with an ACh precursor, or at least ensuring a lecithin-rich diet, choline has not been shown unequivocally to give an immediate enhancement of cognition in AD.

(d) Acetylcholine Releasing Agents

4-Aminopyridine (8) and 3,4-diaminopyridine (9) are used in facilitating acetylcholine release at the neuromuscular junction[41].



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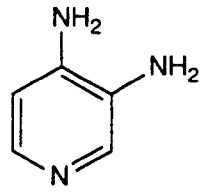

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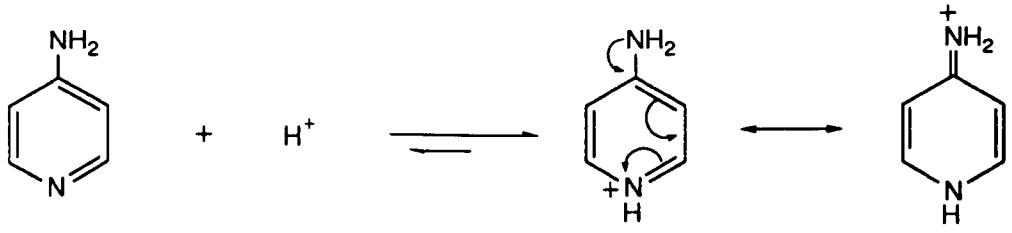
These compounds exert an anti-curare action *i.e.* they reverse the blockade of cholinergic receptors by the drug curare. They also act directly on muscle fibres by augmenting contractility. These actions are believed to result from the ability of 4-aminopyridine (8) to block membrane K^+ channels thereby prolonging the duration of nerve action potentials. With a pK_a of 9.25, the ring nitrogen is largely protonated at physiological pH and the cationic charge is delocalised about the molecule. Consider the following resonance forms:



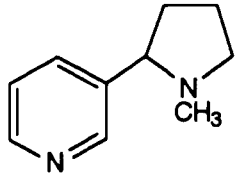
With an ionised moiety present, it would give a reduced penetration into the blood-brain barrier and clinical tests have shown limited success[42].

(e) Cholinergic Agonists

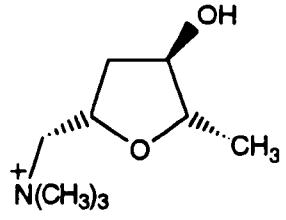
An agonist is defined as "a drug, hormone or transmitter substance that elicits a cellular response when it combines with a receptor"[43], therefore a cholinergic agonist will mimic the actions of ACh. As a therapeutic strategy,

stimulation of cholinergic receptors is the target of much on-going research, particularly into the structures of the cholinergic receptors themselves.

In 1914, Dale discovered that different drugs produced different actions at cholinergic junctions[44]. The molecules that produced these actions were the alkaloids nicotine (10) (from tobacco) and muscarine (11) (the active constituent of the poisonous mushroom *Amanita muscaria*).



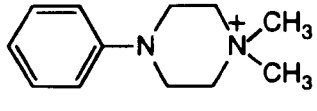
(10)



(11)

The cholinergic receptors were then divided into two broad categories termed **nicotinic** and **muscarinic** receptors which, when stimulated separately, could be shown to mediate different physiological effects.

Nicotinic actions include stimulation of all autonomic ganglia, voluntary muscle and secretion of adrenaline, and these receptors can be selectively stimulated by nicotine (10), 1,1-dimethyl-4-phenylpiperazinium (DMPP) (12) or phenyltrimethylammonium (13).



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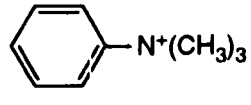
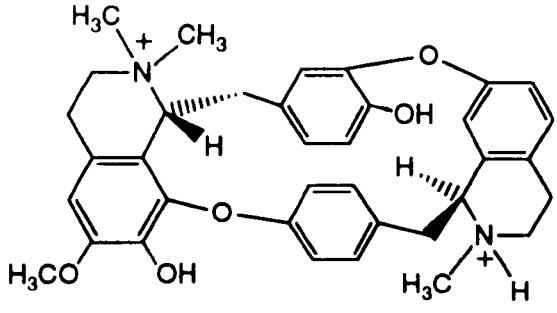


(13)

Nicotinic receptors are sensitive to blockade by (+)-tubocurarine (14), at the neuromuscular junction, or hexamethonium (15), at the ganglionic synapse.

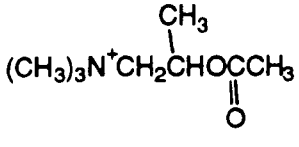


(14)

$(CH_3)_3N^+(CH_2)_6N^+(CH_3)_3$

(15)

Muscarinic actions tend to correspond to those of parasympathetic stimulation, controlling salivary and lacrimal glands, heart, lungs, gastrointestinal tract and bladder. Muscarinic receptors are selectively stimulated by agonists such as muscarine (11), methacholine (16), arecoline (17) or pilocarpine (18).



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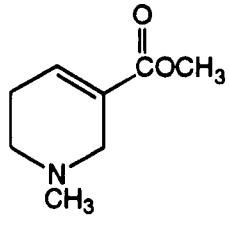

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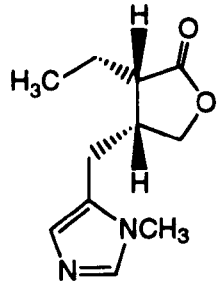
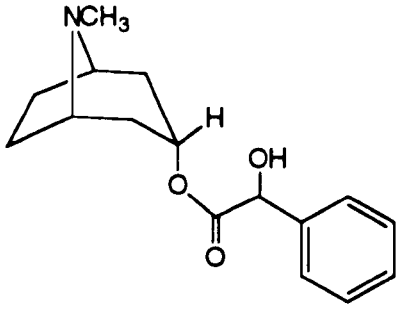


(18)

Blockade occurs with antagonists such as atropine (19), scopolamine (4) or 3-quinuclidinylbenzilate (20).



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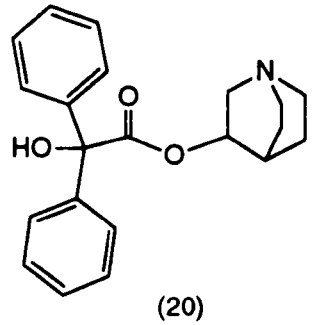
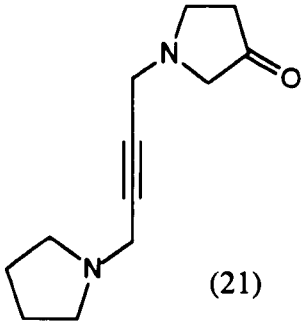


(20)

The predominant cholinergic receptor type in the brain is muscarinic, mediating most of the behavioural effects associated with cholinergic pathways. Nicotinic receptors do exist centrally but very little is known of their function. Enhancement of cognitive function, then, would be a strategy aimed at selectively stimulating central muscarinic receptors. However, clinical use of muscarinic agonists have in the past been hampered by unpleasant side-effects which include nausea, gastrointestinal cramps, slowing of the heart rate, hypothermia and tremor. The agonist arecoline (17) suffers from a very short duration of action due to its labile ester function and other muscarinic agonists such as oxotremorine (21) and RS-86 (22) have both shown disappointing clinical results[36,45,46] with peripheral



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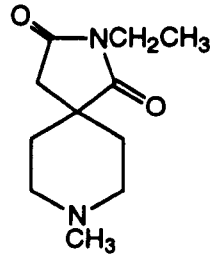
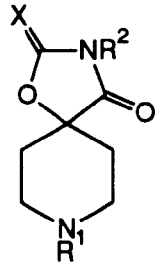


(22)

side-effects being a problem. To combat side-effects it was thought that a peripherally active antagonist could be administered simultaneously but the levels of both the agonist and antagonist would have to be titrated for each individual and even then, any physiological changes that might then occur could potentially alter the absorption rate of each drug, again producing side-effects.

So, investigation of the nature of the muscarinic receptors is the focus of much research today and molecular biology studies have already revealed the presence of five genetic products which code for five structurally distinct muscarinic receptors[47] - m_1, m_2, m_3, m_4 and m_5. Pharmacologically, the five receptors can be grouped into three types- M_1, M_2 and M_3[47,48]. M_1 is located on neurones located in the hippocampus and cerebral cortex, M_2 on cardiac muscle and M_3 on smooth muscle and glands. These pharmacologically distinct receptors account for the range of side-effects occurring with the use of non-selective agonists. M_1, located post-synaptically, is believed to be coupled to phosphoinositol hydrolysis[49,50] (a second messenger system) and is the receptor most closely associated with cognitive function[51].

With the elucidation of the M_1 receptor has come much drug development. Analogues of existing muscarinic agonists are now the target of much interest, for example, spiro-oxazolidine-2,4-diones[52] (23) from the drug RS-86 (22),



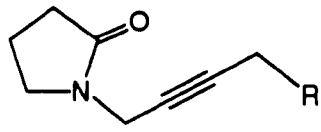
(23)

R^1 = Me, $PhCH_2$, $MeOCO(CH_2)_3$, *etc.*

R^2 = H, Me, Et, *t*-Bu, *etc.*

X = O, S

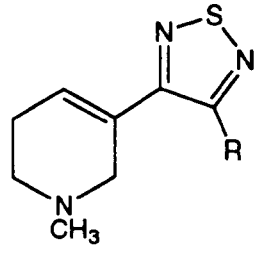
modified oxotremorine (21) derivatives[53] (24) and,



R = pyrroles, pyrimidines.

(24)

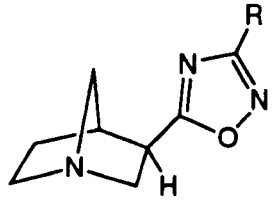
arecoline (17) derivatives[54] (25).



R = OR^1, SR^1, R^1, NHR^1 or H,
where R^1 = Me, other alkyl.

(25)

Other efforts include that of Baker *et al.* who are studying the properties of a range of azabicyclo analogues of the general structure (26) for possible stimulation of M_1 receptors with antagonism at the M_2 and M_3 receptors in the hope of negating the peripheral side-effects[55].



R = CH_3,NH_2, CH(OH)Ph,
Pr^n, Bu^n, *n*-pentyl.

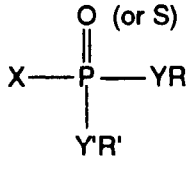
(26)

These examples are only representative of the vast amount of work being carried out world-wide into the therapy of AD in this way, but at the moment a definitive selective M_1 agonist has not yet been put forward.

(f) Anticholinesterase Drugs

The final important method of modifying cholinergic function is the inhibition of the cholinesterase enzyme. Cholinesterase inhibitors, or anticholinesterases, find use in agriculture as insecticides and in medicine for the treatment of glaucoma (a build-up of pressure in the eye, giving eye damage and possible blindness), myasthenia gravis (an autoimmune disease in which the body synthesises antibodies which block the cholinergic receptors at the neuromuscular junction, causing severe muscle weakness and paralysis), for the stimulation of bladder and bowel movements and to reverse the effects of curare-like muscle relaxants used during surgery.

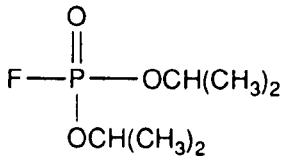
There exist many anticholinesterase drugs but they can be divided into two broad categories according to their chemical composition and the stability of the enzyme-drug complex, and they are termed irreversible and reversible anticholinesterases. Irreversible anticholinesterases are organophosphorus compounds with the following general structure (27).



(27)

R, R' = alkyl, aryl or H
Y,Y' = usually O, may be S, N, or C
X = labile group

e.g.



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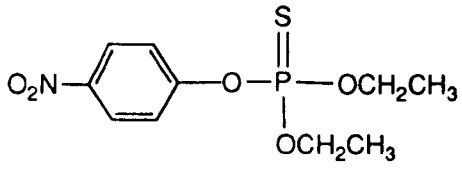
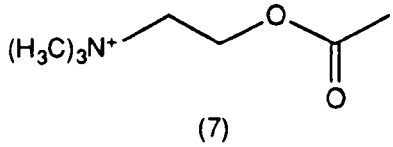


(29)

These types of compounds [*e.g.* Dyflos (28) and Parathion (29)] are used as insecticides and war gases and are extremely toxic. Being lipid soluble they readily penetrate the blood-brain barrier and exert serious toxic effects on the central nervous system causing nerve damage. Actions on the autonomic nervous system and at the neuromuscular junction cause severe bradycardia (slowing of the heartbeat), hypotension and respiratory depression, all of which may be life-threatening. These cumulative, widespread and long-lasting effects render them unsuitable for any kind of systemic administration and they have been abandoned as compounds for medicinal use.

Reversible anticholinesterases are organic compounds with particular structural features and are used in medicine, as mentioned before. Examples of the more clinically important drugs are edrophonium (30), neostigmine (31) and physostigmine (32).



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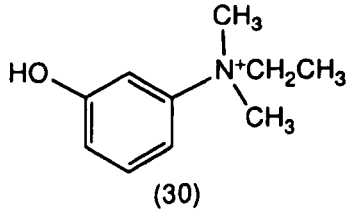
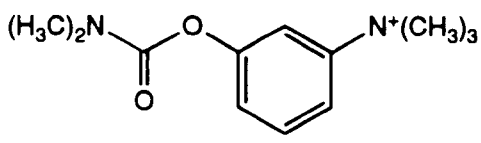
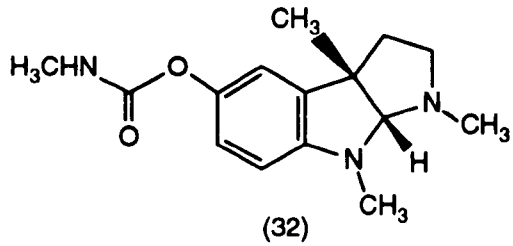


(30)



(31)



(32)

It can be seen that these compounds have several structural features in common with each other and with ACh (7). For example, edrophonium (30) and neostigmine (31) both have a quaternary centre, as does ACh (7). Neostigmine (31) and physostigmine (32) both possess the urethane linkage, and all three drugs have an aromatic portion. If these structural features are required for anticholinesterase properties then it would be useful to study the structure of the enzyme itself and the mechanisms by which these compounds interact with it.

Acetylcholinesterase was crystallised from the *Torpedo californica* electric organ by Sussman and coworkers[56,57]. The enzyme monomer belongs to the class of α/β proteins, and contains 537 amino acids and its structure consists of 12 stranded mixed β-sheets surrounded by 14 α-helices. The important feature of the enzyme is its active site, called the catalytic triad in reference to the three amino acids essential to the binding of the substrate. This site lies slightly above the base of a deep and narrow gorge which is about 20Å long and penetrates halfway into the enzyme's structure. Fourteen aromatic residues line almost half of this deep gorge, which may explain the hydrophobic sites (postulated by biochemical studies) which surround the active site, and may even bind aromatic cations. These studies into the enzyme structure call into question the nature of part of the binding site called the anionic site, which is negatively charged (and which will be described shortly) and hypothesise that the quaternary moiety of the ACh appears mainly to bind through interactions with the π-electrons of the aromatic amino acid residues

lining the gorge. However this binding occurs, the conclusion of this work is that with the extensive aromatic nature of the gorge there must be many ways and places in which a substrate may interact with the enzyme.

The first and obvious question concerns the binding of ACh (7) to the enzyme. Spectroscopic, kinetic and chemical modification studies have always indicated the presence of two areas within the binding site, one termed the anionic site, which contains glutamic acid and attracts the positively charged portion of the molecule, and an esteratic site, containing serine and histidine, the serine -OH being acylated by the ester function of ACh (7)[35b,57] (see *figure 3*), (see also *appendix 2* for amino acid structures).

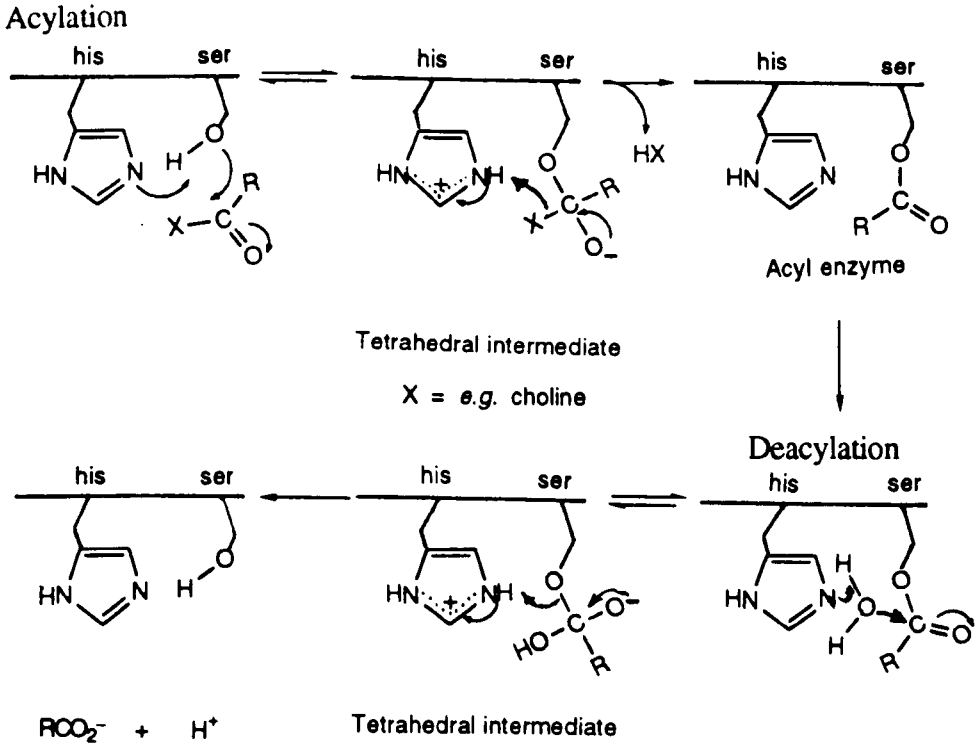


Figure 3: Mechanism of acylation of the acetylcholinesterase enzyme.

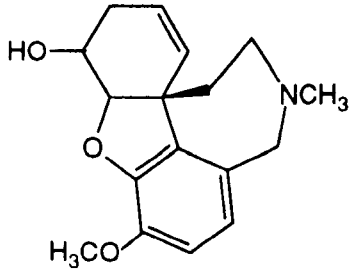
This acetylation and subsequently rapid hydrolysis is a mechanism common to other serine hydrolases, and the acetylcholinesterases hydrolyse over 10 000 molecules of ACh per second by a single active site.

Reversible anticholinesterases such as physostigmine (32) are presumed to bind similarly to ACh (*i.e.* by acylating the enzyme), the difference with the drug being in its rate of hydrolysis, which is slower. This means, of course, that the enzyme is bound for longer and is not free to hydrolyse the ACh molecules which can then further act upon their receptors and continue activating that tissue. Edrophonium (30) without a urethane group does not acylate the enzyme but interacts with the enzyme only with its positively charged nitrogen at the anionic site. It does this quickly and so the onset of action of the drug is rapid. It also dissociates rapidly, giving a short duration of action. This drug is therefore used mainly in the diagnosis of myasthenia gravis, the temporary reversal of symptoms being indicative of the disease.

Neostigmine (31) was the first synthetic anticholinesterase and is used to stimulate the gastrointestinal tract, as a treatment for myasthenia gravis and intravenously as an antagonist for tubocurarine and other anticholinergic drugs. It acts by the same mechanism as physostigmine (32). Physostigmine (32) is a naturally occurring alkaloid and, unlike neostigmine, is a tertiary amine. So, although it is ionised at physiological pH, its physical properties are sufficiently different from the quaternary compounds to give differences in absorption and distribution. This is an important point. Drug therapy for AD requires drugs to penetrate the blood-brain barrier. Ionised quaternary compounds cannot achieve this and so conventional anticholinesterases used for systemic disorders are not applicable for AD treatment. Physostigmine (32) does penetrate the blood-brain barrier and has been one of the most extensively studied drugs for use against

AD[36,58,59]. Difficulties with physostigmine (32) have occurred with its poor oral absorption, leading to intravenous administration, and although after several weeks some improvements in cognition were observed, there were several limiting factors. Physostigmine (32) has a very short half-life of about 30 mins in the body, and has a variable availability to the body[60] caused by rapid metabolism[61]. Further study of this drug is continuing with slow release formulations, and other physostigmine analogues[59].

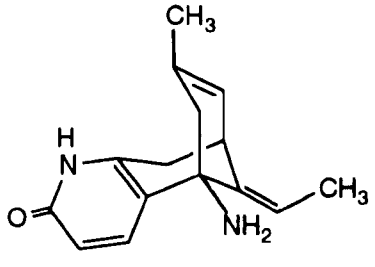
Another naturally occurring anticholinesterase drug is called galanthamine (33) and may be isolated from the bulbs of the Caucasian snowdrop, *Galanthus woronowii*.



(33)

Its potential application for AD has been studied by Joullie *et al.*, along with several derivatives which have been cited as having interesting biological responses[62].

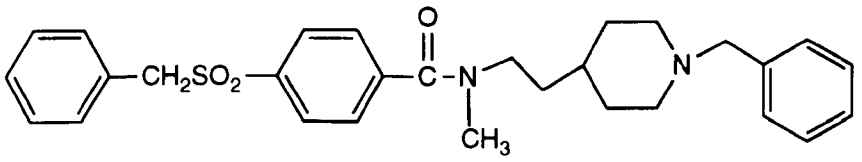
(-)-Huperzine A (34) is a natural product originally isolated in China from the moss *Huperzia serrata*, and its synthesis has been investigated[63].



(34)

Originally considered to be a nootropic[61,64] (enhancer of brain metabolism), (-)-huperzine A and its analogues have been found to possess anticholinesterase properties[63] although no clinical trials for AD have yet been reported in the literature.

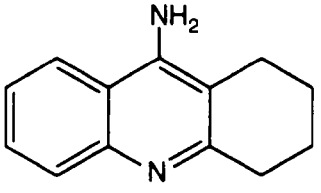
Some novel compounds of the 4-substituted-1-benzylpiperidine type that have been synthesised in recent years have shown potent and long-lasting central anticholinesterase activity. These types of drugs appear to act in a non-competitive manner and do not acylate the enzyme. An example of this work (35) comes from Japanese workers[65].



(35)

Although a number of these compounds have been patented none appear to have advanced through clinical testing.

The final anticholinesterase that will be discussed is the lead compound of this project, namely tacrine (9-amino-1,2,3,4-tetrahydroacridine) (36).



(36)

Tacrine is a reversible anticholinesterase that is proposed to bind close to, but not at, the active site of the acetylcholinesterase enzyme. It also displays many other biological activities. However, a full discussion of its history and biological activities has been reserved for the next chapter. Various analogues will also be reviewed and discussed.

(g) Non-cholinergic Treatments

As the abnormalities in the cholinergic system are only part of the changes occurring in AD, albeit very significant, some attention has focussed on the other chemical deficits in the brain. The problem with this method of studying AD is that the levels of the deficits seem to be individual to each patient. 5-HT (3) and its receptors seem to be the only other neurotransmitter system that is consistently reduced in AD[10]. Also, the neuropeptide somatostatin (2) shows reduced levels in the temporal lobe of the cerebral cortex, correlating with the loss of the choline acetyltransferase enzyme (ChAT) (see *figure 2*). However, it is not known how significant these findings are to the study of AD therapy. Several vasodilator substances and CNS stimulants have been studied as alternatives to cholinergic treatments[66] without any particular success. This seems to be because the abnormalities are too diverse and serious to be reversed by, say, increasing blood flow in the brain even if such vasodilator drugs give occasional minor improvements in cognition and behaviour.

(h) Conclusion

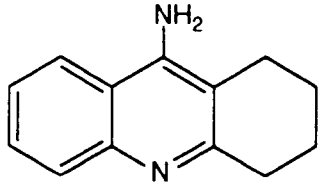
The conclusion that may be drawn from this chapter is that many more biological studies at a molecular level are required to describe AD from its cause to its effects on the brain before **definitive** treatments can be formulated. At the moment, the best that can be provided is a drug regime which will delay or reduce

the symptoms as much as possible before the inevitable conclusion of the disease. This project has set out to provide a treatment in one of these areas - that of using an anticholinesterase drug to enhance existing cholinergic function. The lead structure of the study - tacrine (36) - itself has biological functions other than that of an anticholinesterase and so some attention will be given to these actions and how the novel compounds produced by this work could potentially act on other aspects of brain chemistry. Firstly, a description will be given of tacrine itself, the diversity of its functions and some of its analogues. This will give an insight into tetrahydroacridines and their potential for further development.

Chapter 3

THE PHARMACOLOGY OF TACRINE

(a) Pharmacology



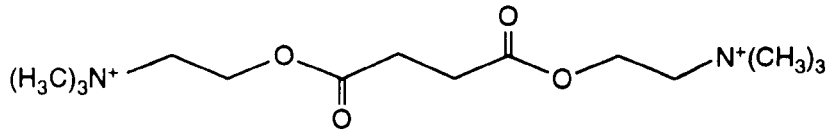
(36)

Tacrine (36), or 9-amino-1,2,3,4-tetrahydroacridine, was first synthesised in 1945 by Albert and Gledhill[67] who had been investigating the synthesis and anti-bacterial properties of acridines. Unlike many other acridine (and quinoline) derivatives, tacrine itself does not possess any anti-bacterial activity, but since its initial synthesis it has been shown to display an astonishing array of other biological activities.

In 1949, Shaw and Bentley developed tacrine as a partial antagonist of morphine[68] where it potentiated the analgesia but antagonised the respiratory depression produced by the opiate. It was later, in 1953, that the same workers first demonstrated that tacrine was a potent inhibitor of the enzyme acetylcholinesterase[69]. This finding was later expanded upon by Heilbronn who

showed that tacrine was in fact an even more potent inhibitor of butyrylcholinesterase[70,71]. Because of this action on the cholinergic system Gershon and Shaw, in 1958, used tacrine as a decurarising agent *i.e.* to reverse the blockade of nicotinic receptors at the neuromuscular junction produced by curare[72], or its most active constituent, tubocurarine (14)[73] (a muscle relaxant). However, since then more effective anti-curare agents have been found and tacrine was withdrawn for this purpose from the UK market in 1982. In 1961, tacrine found clinical use again as a partial morphine antagonist when it was given in conjunction with morphine in the treatment of the intractable pain of terminal cancer[74], and 1975 saw tacrine being cited as a new treatment for the auto-immune disorder myasthenia gravis[75] [see *chapter 2, section (f)*], utilising its action as an anticholinesterase. Additionally, tacrine's anticholinesterase properties were proposed for the therapy of movement disorders caused by prolonged usage of centrally active drugs in disorders such as tardive dyskinesia[76]. This consists of involuntary movements often of the face and tongue, but may also be of the trunk and limbs resulting in the adoption of abnormal postures. (This is due to tonic contractions of the muscles and can be both very disabling and frightening for the patient.)

An important use of anticholinesterases occurs during surgical anæsthesia when it is sometimes necessary to use muscle relaxants without which a more dangerous level of anæsthesia would have to be induced to achieve the same level of relaxation of the muscles. One type of compound used as a muscle relaxant is suxemethonium (succinyldicholine) (37) which is the structural equivalent of two molecules of ACh (7). It acts by blocking transmission after first depolarising the membrane and is normally much less rapidly hydrolysed by acetylcholinesterase than ACh, but rapidly hydrolysed by butyrylcholinesterase.

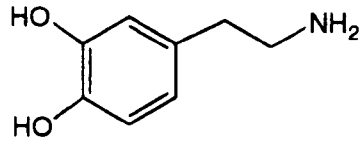


(37)

Inhibition of the cholinesterases with tacrine greatly prolongs the action of suxemethonium and this has been utilised by anæsthesiologists[77].

Another pharmacological action of tacrine has been much examined in the search to define its pharmacological profile. Tacrine is a structural analogue of 4-aminopyridine (8) which itself blocks potassium channels in a wide range of membranes, and tacrine was subsequently shown to possess this activity also[78]. However, it is thought that the serum concentration of tacrine required to affect potassium channels is much higher than that required for a therapeutic effect in the treatment of AD[79]. Recent work on voltage-dependant potassium channels has identified an 18 amino acid sequence which lines the mouth of the pore[80] and possibly contains some aromatic residues[81]. This has been cited as a plausible site of interaction for the tacrine molecule[82].

A further significant finding came when tacrine was found to inhibit the uptake process for the catecholamine neurotransmitters noradrenaline (1) and dopamine (38) and in particular for the monoamine 5-HT (3) (serotonin)[83, 84].



(38)

It has been much discussed if both of these actions account for the overall effect of tacrine on Alzheimer patients. In addition, tacrine has shown an ability to inhibit monoamine oxidase[85,86], one of the enzymes responsible for the metabolic degradation of noradrenaline (1) and dopamine (38) and this may also contribute to the effect on aminergic transmission. Augmentation of 5-HT transmission would confer an antidepressant effect on the patient and such a two-fold action would be beneficial to a confused patient, especially if depression was a contributory factor to the dementia, as is sometimes the case. Enhancement of catecholamine transmission would also be of benefit in correcting the deficits of these neurotransmitters that are sometimes manifest in AD.

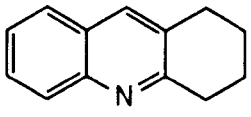
One of the primary reasons for elucidating these activities of tacrine is due to the fact that although it has been shown that physostigmine (32) is a more potent anticholinesterase than tacrine[87], tacrine shows greater efficacy with AD. It was proposed by Dutar *et al.* [88] that tacrine's other direct effects on central neurones (which are unrelated to its anticholinesterase function) were not observed with physostigmine and many other researchers have now also concluded that the multiple activities of tacrine in addition to its cholinergic activity seem to have significance in Alzheimer therapy.

(b) Proposed Mode of Action of Tacrine

We must return to the fact that AD manifests itself as a profound cholinergic deficit and the tacrine has its primary action as an acetylcholinesterase inhibitor. Probing the mechanism of this inhibition has been the focus of much research and many biochemical studies have proposed that tacrine is a selective ligand for a hydrophobic region adjacent to the active site of the enzyme[89] and that it acts as a reversible and non-competitive inhibitor[90]. Patocka *et al.* discussed the fact that as butyrylcholinesterase has more hydrophobic domains than acetylcholinesterase

and that this may explain the higher affinity of tacrine for the butyrylcholinesterase[70]. Patocka's results also provided evidence that tacrine binds to these hydrophobic domains altering the conformation of the enzyme, tacrine thereby acting through an allosteric effect with the enzyme. Recently, the acetylcholinesterase enzyme was crystallised by Sussman *et al.*[57] and then subsequently co-crystallised with the two inhibitors edrophonium (30) and tacrine (36)[82]. The crystal structure of the tacrine-enzyme complex showed that the tacrine molecule was stacked against the tryptophan-84 residue with the tacrine ring nitrogen forming a hydrogen bond with the main-chain carbonyl of histidine-440. The amino nitrogen was found to form a hydrogen bond with a water molecule but the most pronounced difference between the drug-enzyme complex lay in the position of certain aromatic residues. The phenyl ring of phenylalanine-330 swung away from its original position so as to make a better interaction with the aromatic system of the inhibitor. Also, the tacrine structure was stacked opposite the indole ring of the tryptophan-84 making strong aromatic contact in a sandwich between tryptophan-84 and phenylalanine-330. This indicates that the interaction between tacrine and the enzyme manifests itself through the aromaticity of tacrine and certain aromatic amino acid residues lining the gorge around the active site. The inference from this is that aromaticity is the requirement for an anticholinesterase action, but it was questioned if tacrine was the optimum structure for this action to occur.

In 1962, Kaul investigated experimentally the structure-activity relationship of key features of tacrine by comparison of its activity, structure and basicity compared with tetrahydroacridine (39), 4-aminoquinoline (40) , 4-aminopyridine (8) [see *chapter 2, section (d)*] and 9-(N-butyl)-amino-1,2,3,4-tetrahydroacidine (41) for inhibition of acetylcholinesterase[85].



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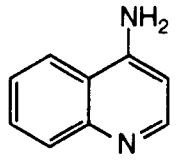

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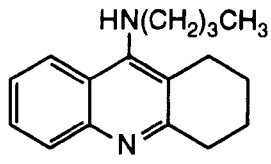


(41)

Kaul's results indicated that the N-butyl derivative had a hindered interaction with the enzyme and was consequently found to be less active. 4-aminoquinoline (40) and 4-aminopyridine (8) were almost equal in basicity to tacrine but had a very weak cholinesterase inhibition. Interestingly, tetrahydroacridine (39), a much weaker base than tacrine, had an anticholinesterase activity almost equal to it. Tacrine was, however, concluded to be the optimum structure for cholinesterase inhibition without Kaul proving that it was specifically due to either its basicity or bulk. However, it was Steinberg *et al.* who carried out a study into the effects of ring substitution and variation[91]. Bulky substitution did not appear to reduce activity very much and the first conclusion from both of these works was that if substitution on the amino nitrogen was not a factor then the enzyme must interact principally with the planar ring system. Complete aromatisation to the 9-aminoacridine had no effect on binding and changing the size of the saturated ring to both 5- and 7-membered rings also gave no major changes. Pendant methyl groups on the benzene ring of tacrine also had no real effect, nor saturation of the benzene ring to the octahydroacridine. Activity only really changed significantly when the saturated ring was changed to small alkyl chains or methyl substituents. This suggested that the constraint of having a cyclohexyl group fused to the aromatic quinoline moiety, hence giving a more rigid and planar structure, was desirable.

The final conclusions, then, from all of this research were that a fairly rigid and near planar molecule was required for enzyme inhibition with some mystery surrounding the rôle of the cyclohexyl ring. If the planar aromatic ring system was required for binding, but 4-aminoquinoline (40) was almost inactive, then the cyclohexyl ring must be of importance, although not associated with binding. To elucidate this, the crystal structure of acetylcholinesterase must again be referred to in order to define the way in which the ACh molecule binds with the enzyme[57] *(figure 4)*, (see also *appendix 2* for amino acid structures).

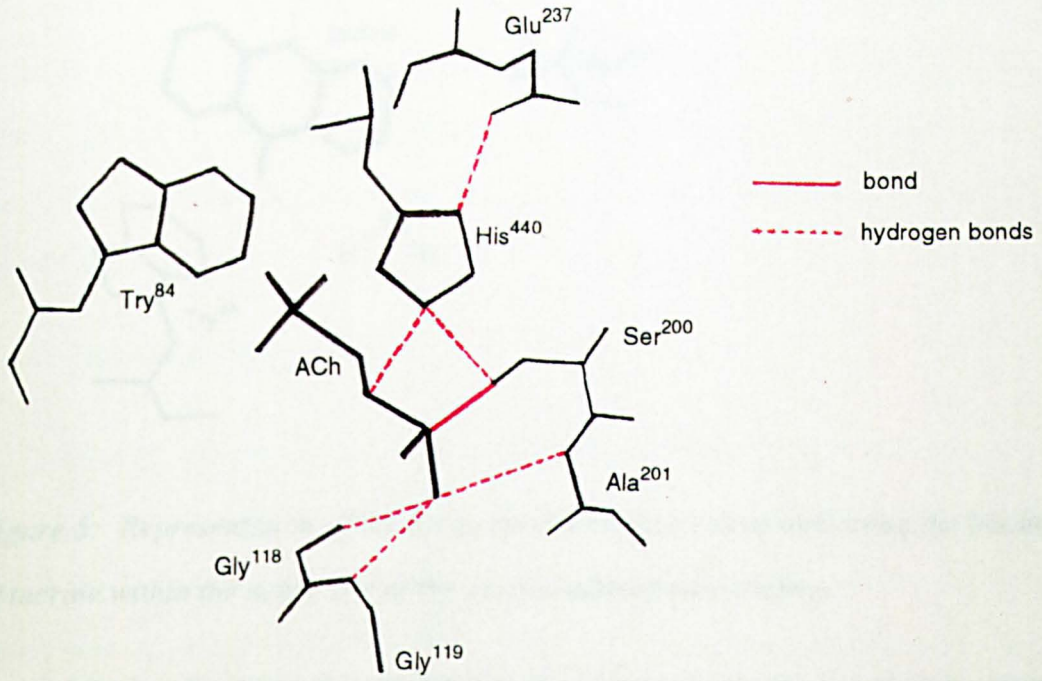


Figure 4: Representation of the proposed binding of ACh within the active site of the acetylcholinesterase enzyme.

A bond is proposed between the serine-200 residue and the carbonyl carbon of the ACh, hydrogen bonding (dashed line) between glycines-118 and 119 with the histidine-440 residue also tethering the molecule. This is a simple description of the interaction of ACh and the active site of the enzyme. Tacrine, as indicated

before, is held between the aromatic indole group of tryptophan-84 and phenylalanine-330, hydrogen bonding occurring between the ring nitrogen and an oxygen from histidine-440 (*figure 5*), (see also *appendix 2* for amino acid structures).

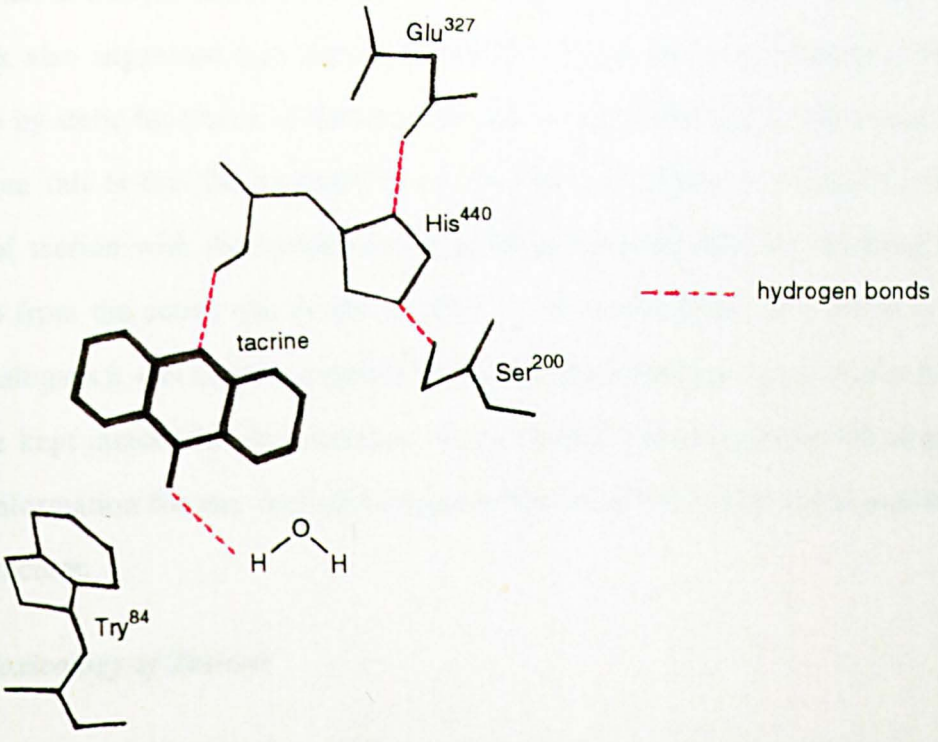


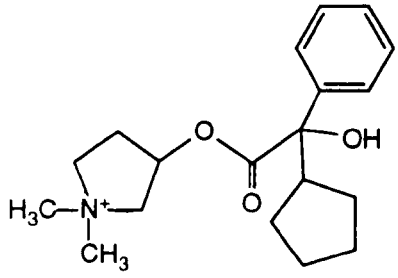
Figure 5: Representation of the X-ray crystal structure data indicating the binding of tacrine within the active site of the acetylcholinesterase enzyme.

It can therefore be stated that the binding of tacrine requires the use of some amino acid residues also used in the binding of ACh, with the 4-aminoquinoline part of tacrine being involved in all of the interactions concerned. So, the conclusion from this may be that the presence of the cyclohexyl ring blocks any other potential substrate for the enzyme reaching the active site. The difference in anticholinesterase activity between 4-aminoquinoline and tacrine would seem to support this. Further evidence comes from the discovery that tacrine protects the acetylcholinesterase and butyrylcholinesterase enzymes from "inactivating agents"

such as the organophosphorus compound dyflos [see *chapter 2, section (f)*][92]. This protective action also correlated with the ability of certain drugs to displace tacrine from the enzyme - drugs which bind at the anionic site did not displace tacrine [such as edrophonium (30)] but drugs which bind at peripheral sites did[93]. This work also suggested that tacrine protected a substrate from binding at the active site by steric hindrance of the esteratic site. The conclusions that could be drawn from this is that the aromatic 4-aminoquinoline portion is utilised in the binding of tacrine with the cyclohexyl ring being essential for the blocking of substrates from the active site of the enzyme. Consequently, for a study into tacrine analogues it was initially proposed for this project that this 4-aminoquinoline moiety be kept intact with the alteration of the cyclohexyl ring giving structure-activity information for any desirable features that could be incorporated into the tacrine structure.

(c) The Toxicology of Tacrine

Tacrine has so far been shown to possess the optimum structure for acetylcholinesterase inhibition **and** beneficial effects in AD. The necessity for finding other, equally active compounds lies with the fact that tacrine has been shown to have some toxicity. This is obviously an important consideration with the chronic administration of a drug as would be the case with Alzheimer therapy. As tacrine is primarily an anticholinesterase, many of its side-effects can be predicted. Due to the excessive build-up of ACh at peripheral synapses gastrointestinal disturbances, bradycardia, nausea, excessive salivation and muscular stiffness can become apparent. These symptoms are variable and are reduced with lowered doses. However, side-effects are still manifest even when tacrine is administered with the peripheral muscarinic antagonist glycopyrrolate (42).



(42)

The side-effect that has given most concern is the hepatotoxicity of tacrine and although many clinical trials have been carried out to try to define this toxicity[94,95], the level of liver damage seems to vary from trial to trial. However, elevated liver enzymes in plasma were found and are indicative of impaired liver function, although this was usually asymptomatic and reversible on cessation of tacrine administration.

(d) Other Actions of Tacrine

Until now, only the major and hence the most important activities of tacrine have been discussed, but such is the interest in this molecule that many other actions have been discovered. However, it should be noted that tacrine does act primarily as an anticholinesterase at levels of about 0.1μM[96] whereas all other actions become significant in concentrations above this. A weak binding to muscarinic receptors occurs around 1μM and the previously described blocking of potassium channels also occurs at these levels or higher[78]. It has also been described by Harvey *et al.* that the facilitation of ACh release may be apparent at these levels[98], along with the already discussed effects of tacrine on noradrenaline (1), 5-HT (3) and dopamine (39) uptake[83]. After these activities, all occurring at relatively low concentrations, several other actions can be demonstrated at higher levels of tacrine. The slowing of sodium channel inactivation (10μM)[99], the blocking of the PCP receptor

(15μM)[100], blocking of GABA release (56μM)[101], blocking of cardiac potassium channels (100μM)[102], and binding to adenosine receptors (640μM)[103] are all manifest at much higher concentrations and probably have no significance in Alzheimer therapy.

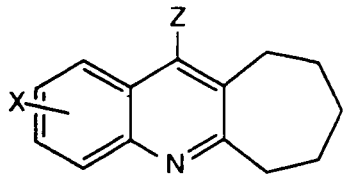
More recent work with tacrine has shown even further diversity of action. Krishnaraj has shown, for the first time, that tacrine is an immunosuppressant of normal human lymphocytes (*in vitro*) in microgram quantities[104]. This again raises the question of the safety of chronic administration of the drug, suppression of the immune system in the elderly obviously being undesirable.

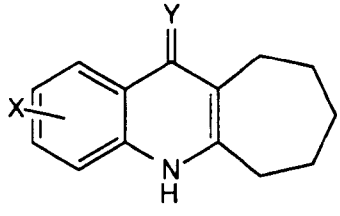
In 1990, it was announced that platelet abnormalities could be discovered in AD and this could be used as a diagnostic aid[105]. Subsequently it was shown that tacrine possessed a potent antiplatelet activity and could disperse platelet aggregates (>10μM)[106]. This work by Lui *et al.* also discussed the proposal that the abnormal cleavage of amyloid precursor protein to give the β-amyloid fragment associated with AD (see *chapter 1*) had its source of the amyloid precursor from platelets during their activation and aggregation[107]. This raised the interesting questions of whether or not platelets have a rôle in the pathogenesis of AD, therefore indicating antiplatelet therapy, and if this was a contributory factor to tacrine's efficacy in AD.

It can be seen that there is still much to be elucidated of the pharmacological profile of tacrine. Tacrine has many diverse effects, some of them desirable in Alzheimer therapy, some less relevant due to the clinical concentrations used, and some, such as hepatotoxicity, clearly undesirable. Much research has been carried out into the synthesis of structural analogues of tacrine in the hope that another compound may be found which possesses tacrine's desired actions with none of its toxic effects.

(e) Some Tacrine Analogues

Due to the nature of tacrine-like molecules with their diverse biological activities, intense interest has focussed on their synthesis and design. In particular, reversal of respiratory depression and local anæsthetic properties have led to the development of many interesting ranges of compounds. One example of this is from Plotnikoff *et al.* in 1963[108].

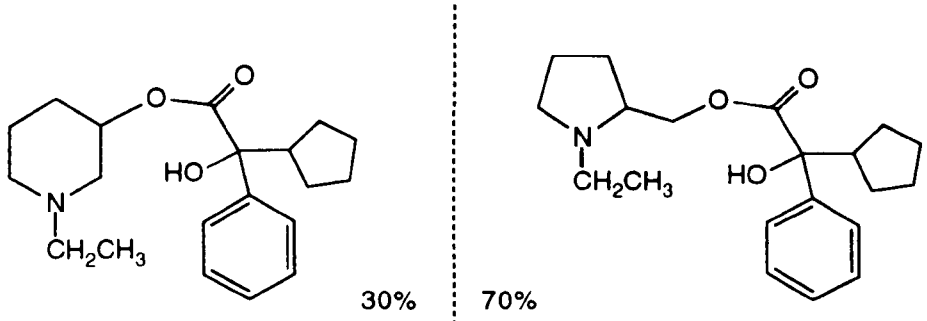




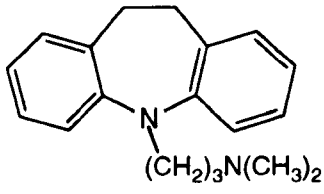
Range of compounds (43)

Z = NH_2, Cl
X = H, Cl, CH_3, C_2H_5, OCH_3
Y = O, S

This range of compounds was developed in an attempt to mimic the respiratory stimulant properties of tacrine (demonstrated on patients under morphine sedation) and the antagonism of a hallucinogenic drug named Ditran® (a cholinergic antagonist). Ditran® (44) produced similar peripheral side-effects to that of a cholinergic antagonist (dry mouth, flushing, tachycardia) and marked disturbances of the central nervous system, hallucinations in the visual auditory, olfactory and tactile senses, confusion, emotional lability and paranoia and, finally, a period of amnesia. After this ends, there is an improvement in mood, particularly with depressed patients. These actions are reversed by tacrine which suggests that they are mediated by the central cholinergic system, but there is no other evidence to support this. The study also pointed out the structural similarity between tacrine and the tricyclic antidepressant imipramine (45) and for these reasons, Plotnikoff investigated structural-activity relationships of these ranges. Various central effects (not of relevance to dementia) were discussed.

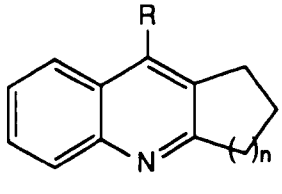


Ditran® (44)



(45)

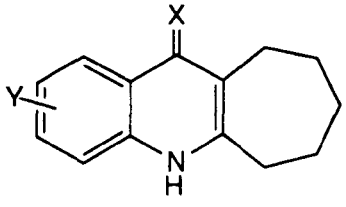
Patnaik *et al.* in 1966 synthesised a range of tacrine analogues (46) to investigate anticholinesterase action, local anæsthetic activity, analeptic and other respiratory stimulant action and analgesia[109]. Several compounds with weak actions were discovered. Structural variations concentrated on the substitution of R with heterocyclic rings.



Range of compounds (46)

n = 1 - 3,
I R = - N *e.g.* morpholino, pyrrolyl *etc.*
II R = - CON

In 1968, Lee investigated the antidepressant activity if 2,3,4,5-tetrahydro-[*1H*]-cyclohepta-[*b*]-quinolin-11-ones and -11-thiones (47) but with no obvious success[110].

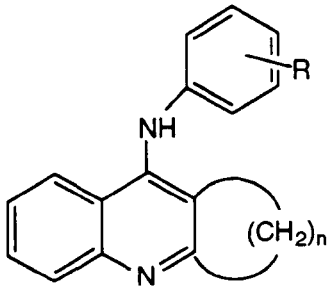


Range of compounds (47)

X = O, Y = 2-Cl, 3-Cl, 4-Cl, 3-OCH_3, 3-NO_2
3-CF_3, 2,4-$(Cl)_2$, 2,3,4-$(OCH_3)_3$

X = S, Y = H, 2-Cl, 3-Cl

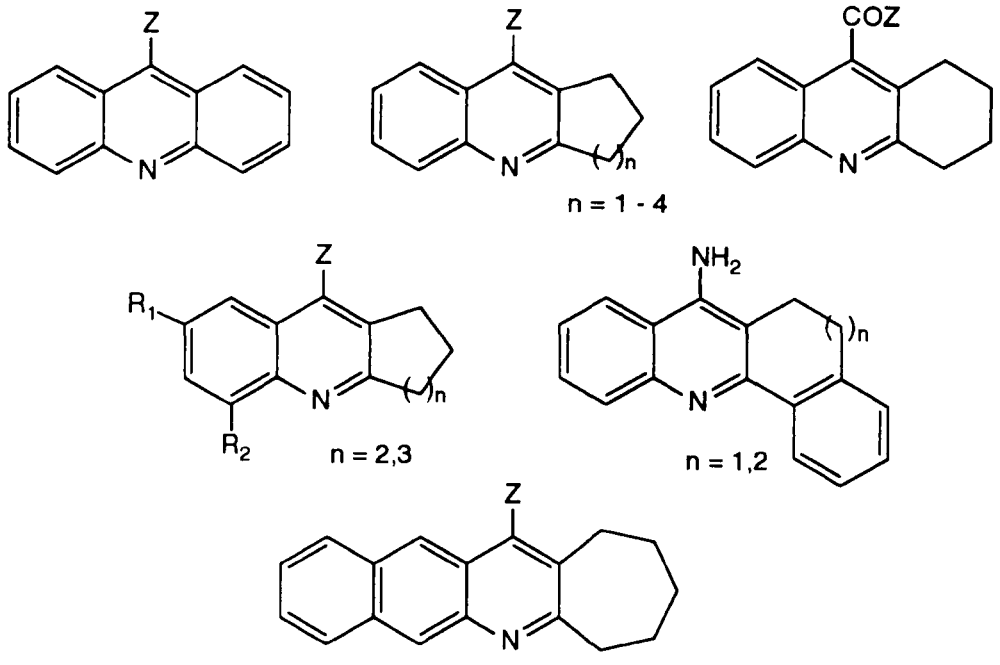
Investigating the synthesis of polymethylene quinolines with aryl substitution, Pedersen *et al.* in 1983 synthesised a range of compounds substituted on this aryl ring (48)[111]. Antifungal and insecticidal activities were studied.



Range of compounds (48)

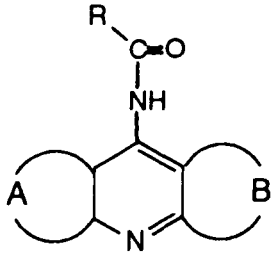
n = 4, R = H, 3-CH_3, 4-CH_3, 4-CH_2CH_3, 4-F
n = 5, R = H, 3-CH_3, 4-CH_3
n = 6, R = H, 4-CH_3
n = 10, R = H

Bindra *et al.* in 1987 produced an extensive series of tacrine analogues (49) in an attempt to produce prototype molecules for various biological activities. The pharmacological results were interesting though no potent anticholinesterases were discovered[112].

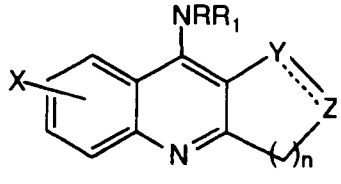


Range of compounds (49), Z = OH, Cl, various amino groups

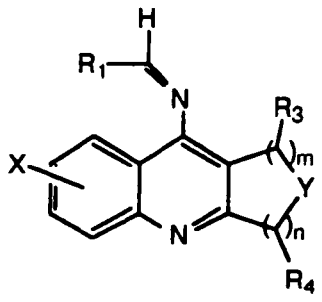
More recently, researchers have been narrowing their study for further modifications with the aim of producing anticholinesterase drugs. Again, interesting ranges of compounds have been produced and many are subject to patent protection. Below is a brief summary of recent work in this area as it is beyond the scope of this thesis to discuss each group of work in detail.



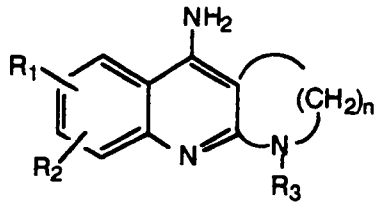
Morita *et al.*[113] (50)



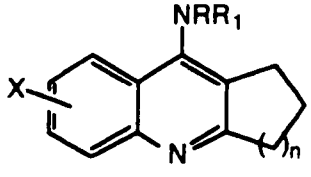
Shutske *et al.*[114] (51)



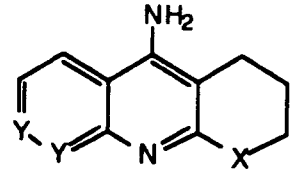
Shutske *et al.*[115,116] (52)



Kuroki *et al.*[117] (53)



Shutske *et al.*[118] (54)



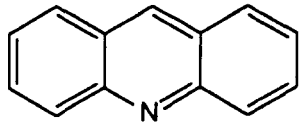
Desai *et al.*[119] (55)

These ranges exemplify the type of work that has been carried out for the treatment of cognitive disorders such as AD. Manipulation of the tacrine nucleus consisted of various substitutions of the amino nitrogen, substitution of the aromatic ring, changing the ring size of the saturated ring and incorporation of heterocyclic atoms into the rings. Such manipulations can be seen to have little value in the search for a potent anticholinesterase. For the purposes of this project, it was felt that maintaining the presence of the 4-aminoquinoline moiety would confer upon the compounds at least the potential for binding to the anticholinesterase enzyme, in a manner similar to tacrine. Therefore, the search for an alternative to the cyclohexyl ring was proposed for the initial stages of this project.

Chapter 4

THE SYNTHESES AND BIOLOGICAL ACTIVITIES OF ACRIDINES

Tacrine (36) belongs to a range of compounds derived from a basic structure called acridine (56). This substance was discovered in 1870 by Graebe *et al.* who had isolated it from the anthracene fraction of coal tar and had named it for its acrid smell and ability to irritate the skin and mucous membranes[120].

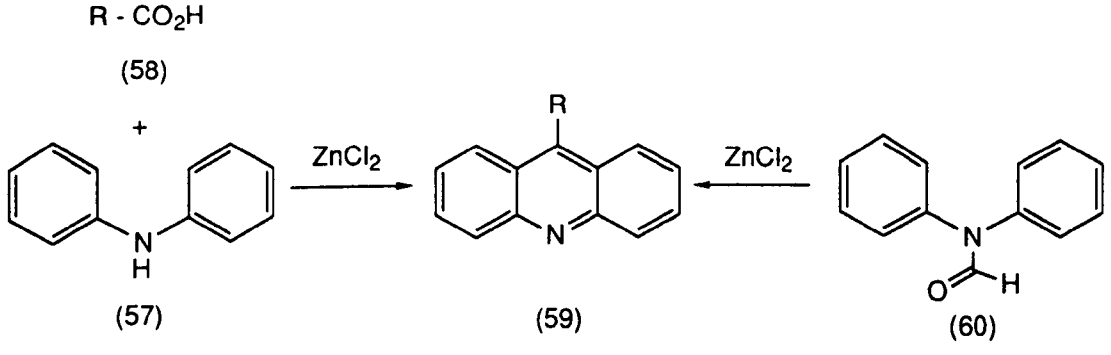


(56)

Many acridines and, in particular, aminoacridines exhibit pronounced biological activities or find other useful applications, for example, as dyestuffs. However, much of the initial syntheses were discovered in the first half of the century due to the increased public awareness of hygiene and consequent demand for antiseptics, and the necessity to produce antimalarials for the forces fighting overseas. To indicate some strategies towards these types of compounds, some of the syntheses will be described and compared to the modern day synthesis of tacrine-like structures.

(a) The Bernthsen Reaction

One of the earliest known reactions for the synthesis of acridines is the Bernthsen reaction[121b] which consisted of heating a mixture of an aromatic or aliphatic carboxylic acid with diphenylamine (57) and zinc chloride (in the absence of solvent) at 200 - 270°C for 20 hours. This work extended the previous studies on the earliest recorded synthesis of acridine (59, R = H) from N-formyldiphenylamine (58) and zinc chloride[121a] *(Scheme 1)*. This method was later modified by the replacement of zinc chloride with polyphosphoric acid (PPA) at a temperature of 200°C for 15 minutes, with a ratio of 2:1 amine to acid[122]. This gave a more convenient method albeit with reduction in yields.

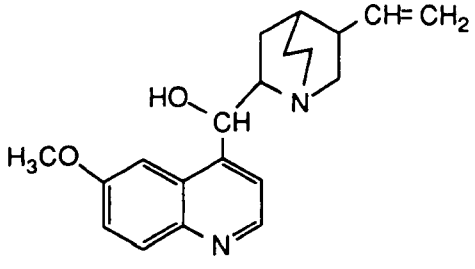


Scheme 1

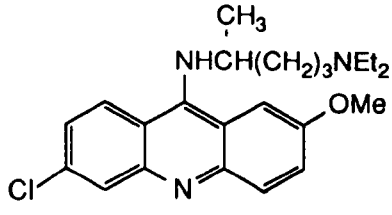
Variations on these procedures have yielded many acridine compounds, for example, by using the corresponding acid anhydrides or trichloro compounds instead of the acid itself, or by changing the Lewis acid[123].

(b) Acridines as Antimalarials

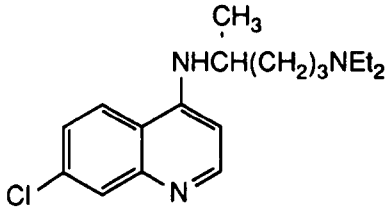
Acridines have found use as antimalarials since the initial research into a substitute for the conventionally used quinine (61) in the 1930's. In 1932, the acridine antimalarial drug quinacrine[124] (62) was developed and went on to replace the dwindling stocks of quinine (61) during World War II. Quinacrine (62), formerly named atebrin, was itself replaced by chloroquine (63), a close analogue possessing greater potency than quinacrine (62) and fewer side effects.



(61)



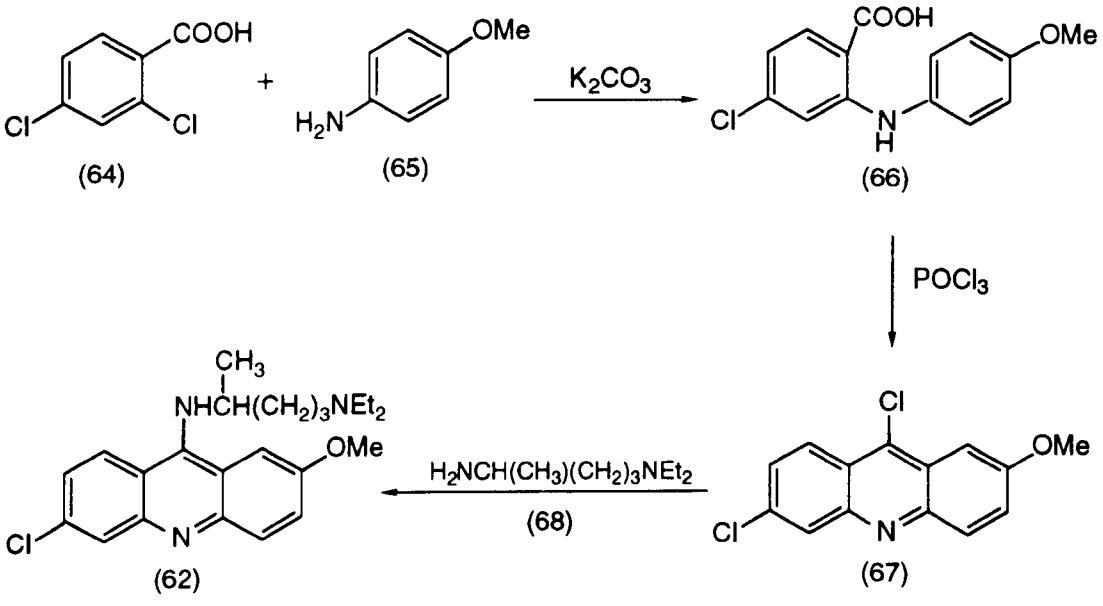
(62)



(63)

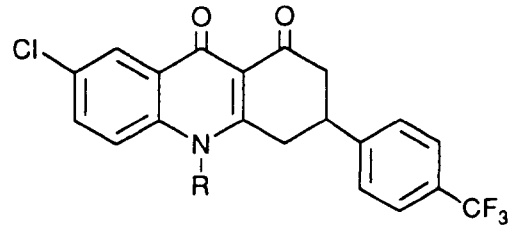
Antimalarial properties were conferred to the acridine by the insertion of the diamino side chain in the 9-position. The compounds lost activity when the 9-amino group bore an additional substituent, probably due to its relative chemical instability as 9-dialkylaminoacridines readily hydrolyse to the 9-acridones. The terminal amine was also found to be essential, as was its substitution with only alkyl

groups. Quinacrine (62) can be synthesised from the starting materials 2,4-dichlorobenzoic acid (64) and *p*-anisidine (4-methoxyaniline) (65) which, when condensed with potassium carbonate, gave a substituted diphenylamine intermediate (66)[123]. This was subsequently cyclised with phosphorus oxychloride to give the 9-chloroacridine (67). To obtain the final product, 9-chloroacridine was then reacted with 4-amino-1-(*N,N*-diethyl)aminopentane (68) in phenol and the product quinacrine (62) was isolated after basification of the reaction mixture *(Scheme 2).*



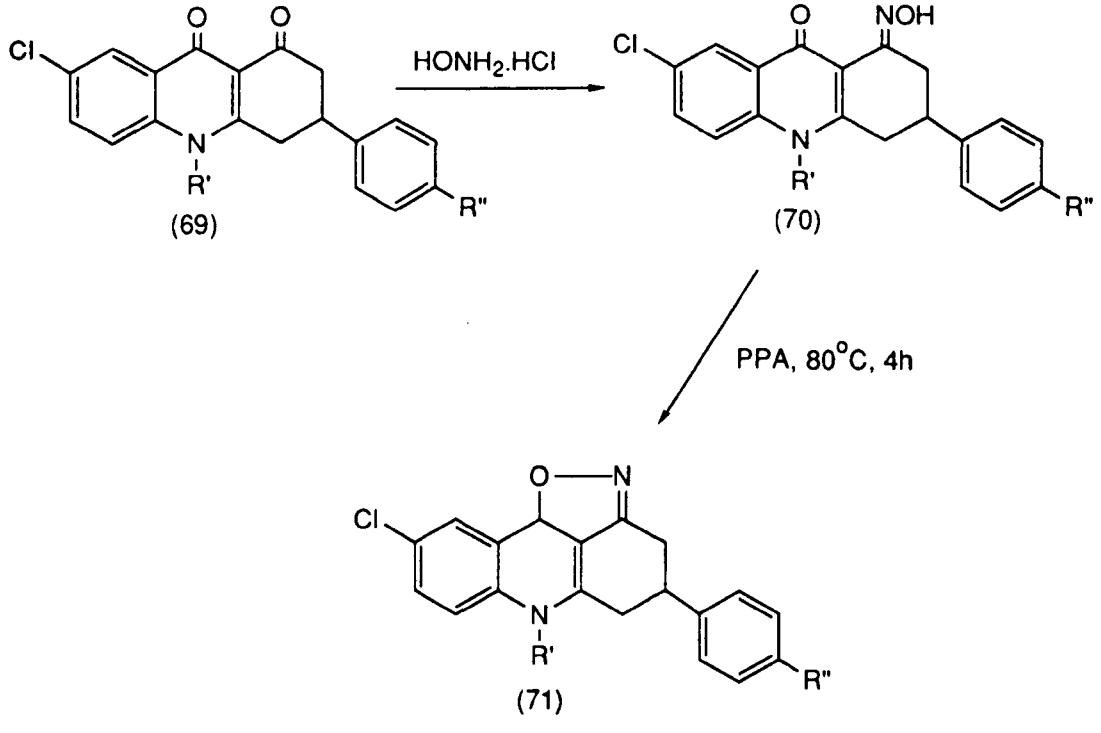
Scheme 2

An example of more recent work in this area is derived from the acridinones (69a,b) in which some novel isoxazolo-[3,4,5-*kl*]-acridines (71) were synthesised from the diones themselves[125] by formation of the oximes (70) and cyclisation with polyphosphoric acid at 80°C (*Scheme 3*).



(69a) R = OH, Floxacrine

(69b) R = H, Deoxyfloxacrine

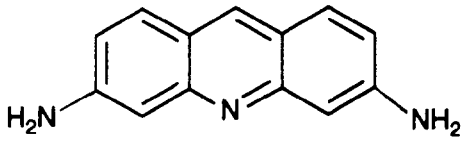


(69) (70) (71)

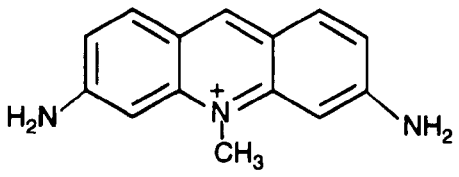
Scheme 3

(c) Acridines as Antibacterials

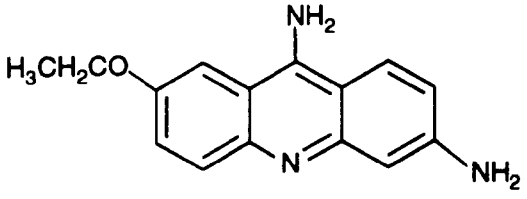
Discovering that certain acridine compounds possessed an antibacterial action around 1913, interest in the potential of these compounds began to grow. The major antibacterials derived from the acridine nucleus are proflavin[126] (72), acriflavin (73) and rivanol (ethacridine)[127] Both proflavin (72) and acriflavin (73) found use as wound disinfectants during both World Wars although they began to be superceded by 9-aminoacridine (75), a more potent antibacterial.(74). Rivanol (74) was used as a surgical antiseptic[128].



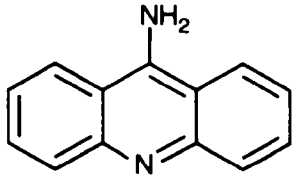
(72)



(73)



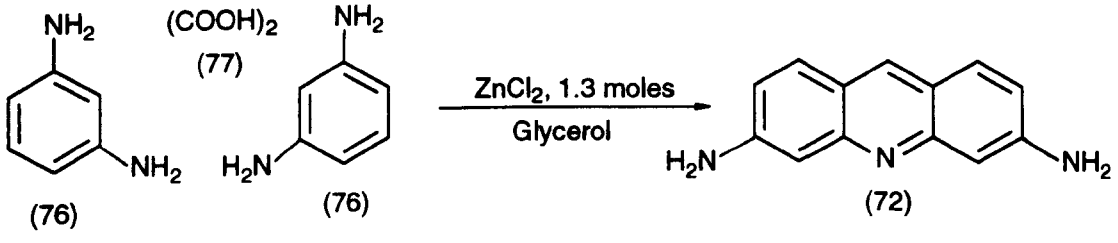
(74)



(75)

Antibacterial properties have been shown to be dependant on the presence of amino groups, and the completely aromatic acridine system[123b]. 2-Aminopyridine and 4-aminoquinoline (40) have very little antibacterial activity, similarly to tacrine (9-amino-1,2,3,4-tetrahydroacridine) (36) itself.

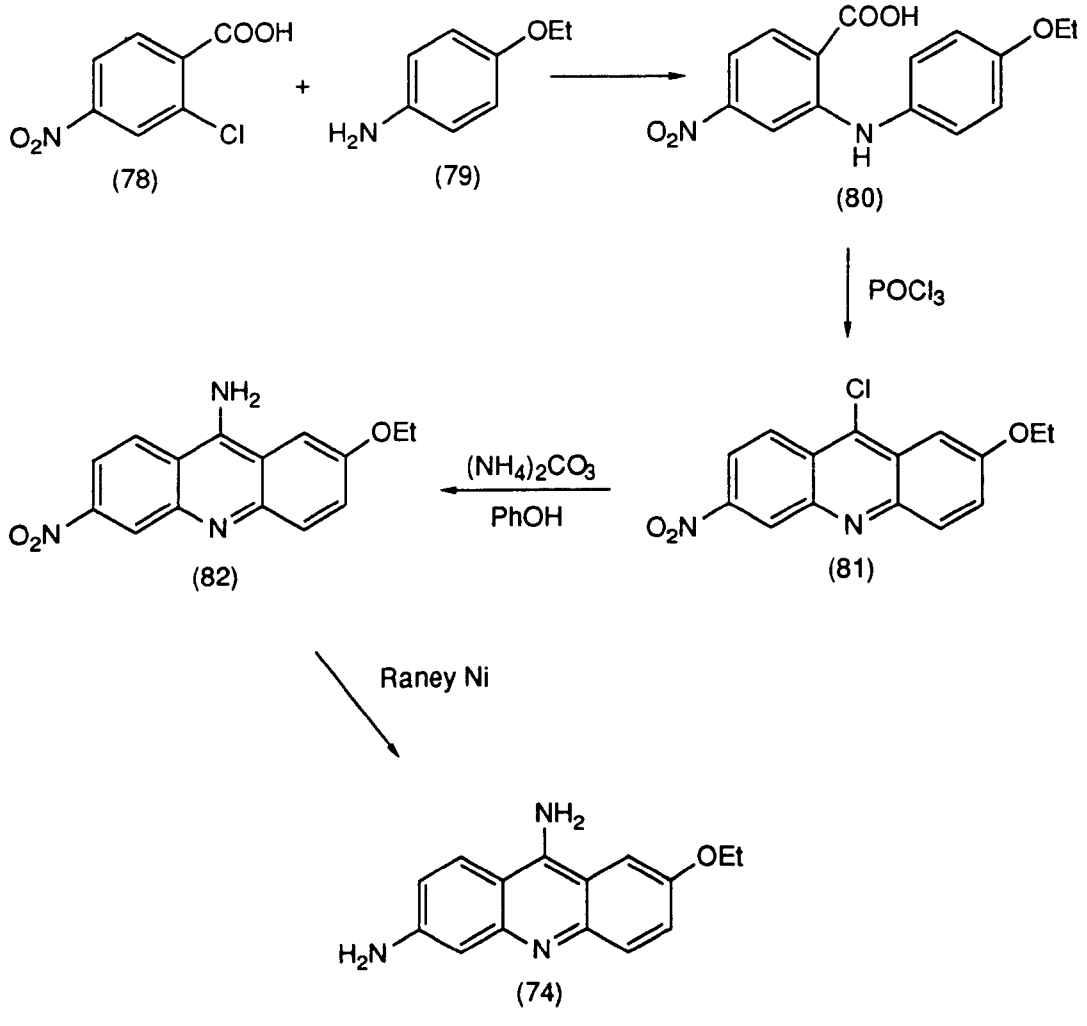
Proflavin (72) may be synthesised by heating oxalic acid (77) and 2 equivalents of *m*-phenylenediamine (76) with zinc chloride at 155°C *(Scheme 4).*



(76) (77) (76) (72)

Scheme 4

Acriflavin (73) is produced by methylation of proflavin (72) with either dimethyl sulphate or methyl *p*-toluenesulphonate[123c]. Rivanol (74) was synthesised from the 2-chloro-4-nitrobenzoic acid (78) and the substituted aniline (79). Heating the resultant diphenylamine (80) with phosphorus oxychloride gave the cyclised, fully aromatic chloro acridine (81). When reacted with ammonium carbonate in phenol the 9-aminoacridine (82) was produced and subsequently reduced with Raney nickel to the diamino product (74) *(Scheme 5)*.

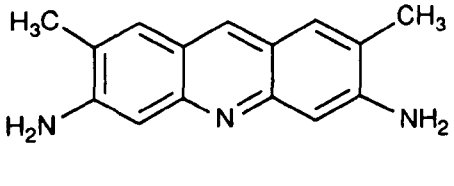


Scheme 5

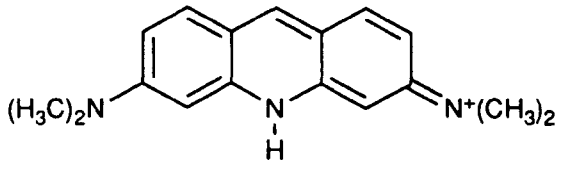
9-Aminoacridine (75) was reported to be synthesised in good yield from the 9-chloroacridine and ammonium chloride in phenol[129]. (The 9-chloroacridine is itself conventionally synthesised by reaction with 9-acridone and phosphorus oxychloride)

(d) Acridines as Dyestuffs

Proflavin (72), better known for its antibacterial properties, is the simplest acridine dye and many other dyes are its structural analogues. Acridine yellow (83) is synthesised in a similar manner to that of proflavin (72) and was once used in coloured prints.



(83)



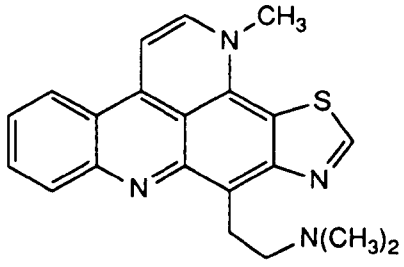
(84)

Other simple acridine dyes include acridine orange (84) which itself may be brominated to the 2,7-dibromo derivative, acridine scarlet. Many acridine dyes are inferior in light fastness to most other dyes and are now obsolete. They also encompass 9-acridone compounds and complex polycyclic compounds derived from them[123d].

(e) Acridines as Antiviral/Antitumour Agents

Polycyclic, fused ring alkaloids isolated from plant sources and containing an acridine nucleus have been shown to diplay antitumour and antiviral activities[130].

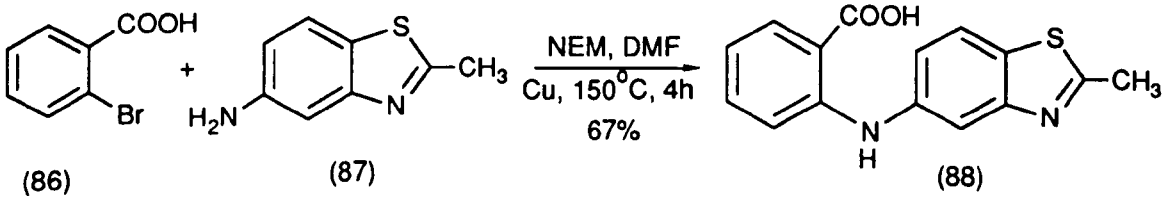
For example, the pentacyclic alkaloid dercitin[130a] (85) isolated from the sponge *Dercitus* has a range of activity against tumour cells and RNA/DNA viruses.



(85)

Strategies towards this type of acridine commenced with the synthesis of acridones containing thiazole rings. These were provided by utilising the Ullman-Jourdan coupling reactions, reactions which consist of two types and constitute major routes towards the synthesis of acridines.

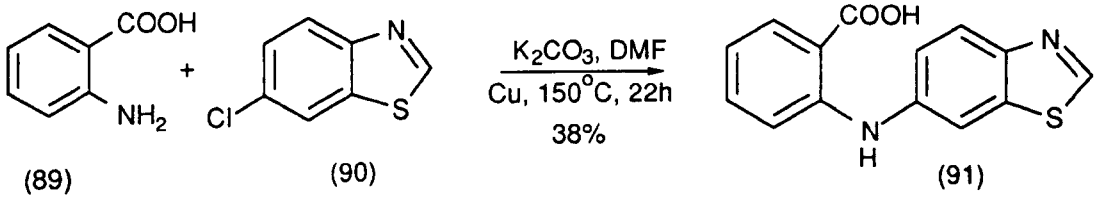
A Type I Ullman-Jourdan reaction consists of a copper catalysed coupling between a 2-halobenzoic acid and aniline compounds. In an example from the *Dercitin* work, 2-bromobenzoic acid (86) was condensed with 5-amino-2-methylbenzothiazole[130b] (87) to give a 67% yield of N-(2-methylbenzothiazol-5-yl)-anthranilic acid (88) *(Scheme 6).*



(86) (87) (88)

Scheme 6

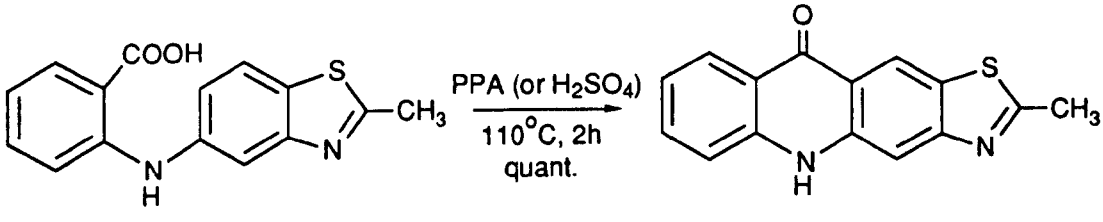
A Type II Ullman-Jourdan reaction occurs between an anthranilic acid and a halobenzene. A second example from the work described above utilises anthranilic acid (89) amd 6-chlorobenzothiazole[130b] (90), again with copper catalysis, to give the N-(benzothiazol-6-yl)-anthranilic acid (91) *(Scheme 7)*.



(89) (90) (91)

Scheme 7

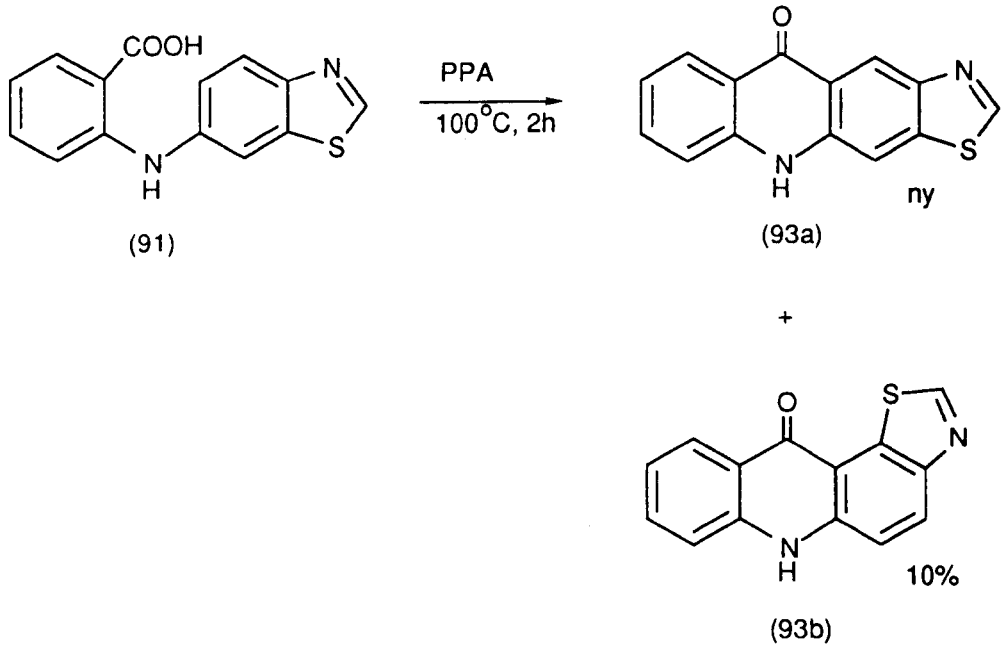
These two intermediates (88) and (91) were subsequently cyclised to the respective acridones using PPA as the reagent for the cyclodehydration *(Schemes 8a, 8b)*.



(88) (92)

single isomer

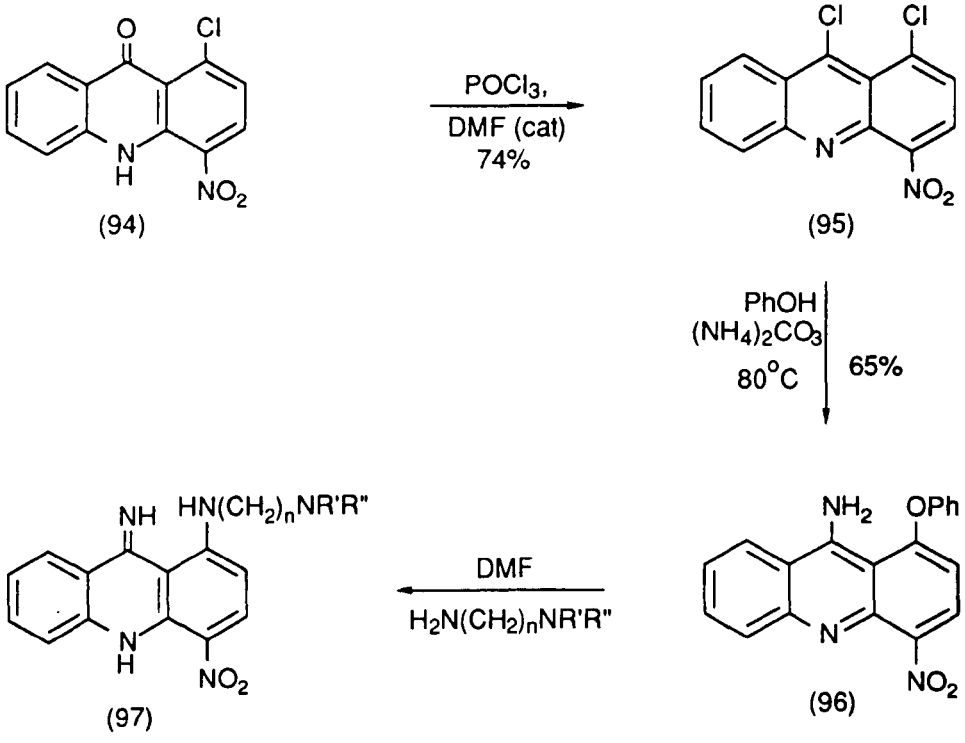
Scheme 8a



Scheme 8b

The Ullman-Jourdan reactions typify a facile entry to acridine nuclei, and the above work describes the previously unknown route towards thiazolo-[5,4-*b*]-acridines (92) as well as a preparation for the known thiazolo-[4,5-*b*]-acridines (93a), for the purposes of providing non-nucleoside antiviral and antitumour agents.

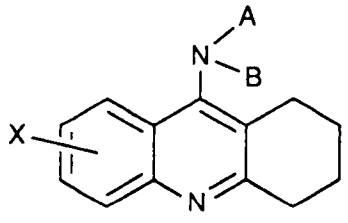
Studies into the syntheses of other types of acridine-based compounds were also carried out for the purpose of investigating antitumour properties. Also synthesised from acridones (94), functional group transformations were performed to give the 1-amino-9-imino-4-nitro-9-10-dihydroacridines (97), a new class of compound[131] *(Scheme 9)*.



Scheme 9

The acridone (94) was heated at reflux with an excess of phosphorus oxychloride and catalytic DMF to yield the 1,4-dichloro-4-nitroacridine (95). Sensitive to hydrolysis, this compound was utilised immediately by heating with phenol and ammonium carbonate which gave the 9-amino-4-nitro-1-phenoxyacridine (96) intermediate for the synthesis of a range of compounds with the general structure of (97). Condensation of the 9-amino-4-nitro-1-phenoxyacridine (96) with a three- to four-fold excess of amine in DMF gave the target acridines in good yields.

Pharmaceutical compositions for use against retroviral infections and in particular the AIDS virus have also found use for acridines. One example contains the derivatives (98) of 9-amino-1,2,3,4-tetrahydroacridine (tacrine) and its reported inhibition of virus RNA polymerase[132].



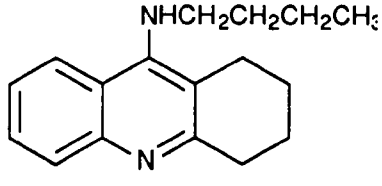
A,B, = acyl of aliph. or arom. carboxylic acids, lower alkyl, alkylidene, arylidine

X = H, C_{1-6}alkyl, C_{1-6}alkoxy halo,OH, Ph *etc.*

(98)

(f) The Local Anaesthetic Properties of Acridines

A study carried out in 1987[112] into various substituted polymethylene quinolines discovered the local anæsthetic activity of several compounds. One in particular was especially potent and had been reported by the same workers earlier in 1982. Named 4-N-*n*-butylamino-2,3-tetramethylenequinoline (9-N-*n*-butylamino-1,2,3,4-tetrahydroacridine) (99), it has the trivial name "centbucridine" and has been subjected to clinical trials as a local anæsthetic. Structure-activity relationship studies[112] indicate several important features: branching of the alkyl chain reduces activity; alkyl amino compounds are more active than their amino analogues; increasing the length of the chain seemed to improve safety.



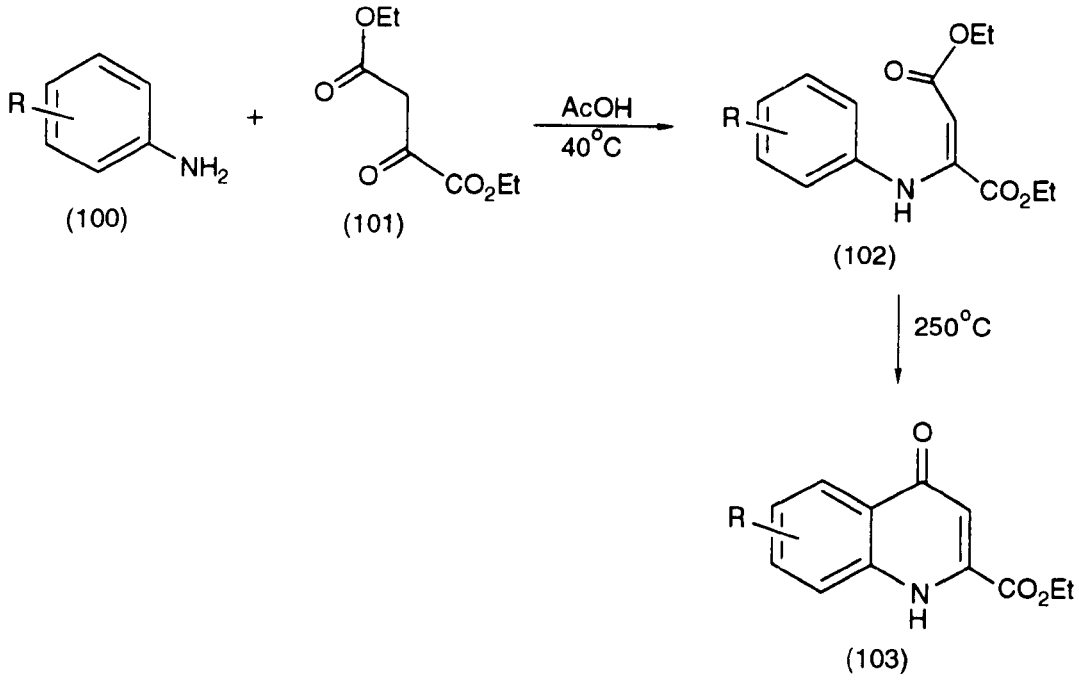
(99)

(g) 1,2,3,4-Tetrahydroacridines, Polymethylenequinolines

To summarise further all the possible methods for the formation of acridines is beyond the scope of this thesis but a succinct review of some syntheses more pertinent to this work is worthwhile.

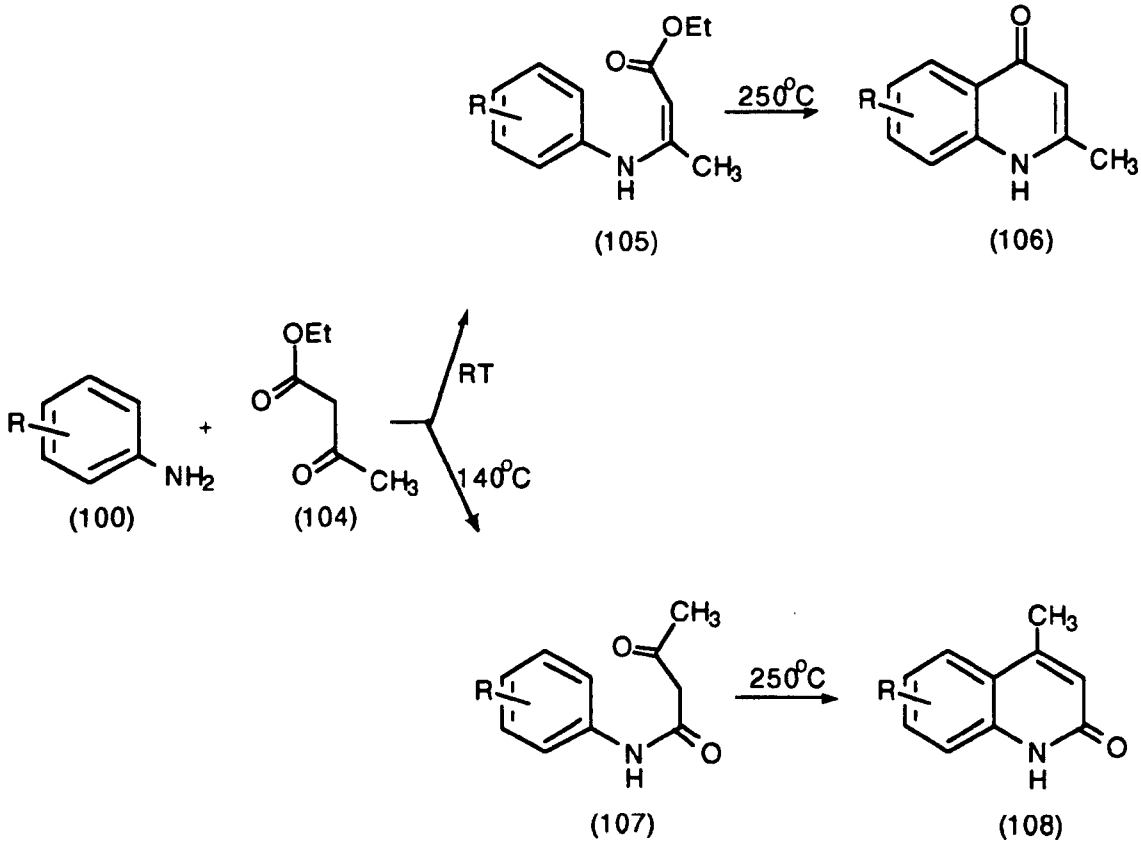
Tacrine (36) is a tetrahydroacridine, but its structure may be described as a "tetramethylenequinoline" or a "cyclohexa-[*b*]-quinoline", and as such the range of available syntheses towards tacrine analogues can be extended. Indeed, 1,2,3,4-tetrahydroacridines closely resemble the 2,3-dialkylquinolines in physical and chemical properties[133]. Considering tacrine as a derivative of 4-aminoquinoline we may now study the synthesis of quinolines, of which there are three types important to this work. These methods can construct the quinoline ring system conveniently from non-heterocyclic starting materials.

The first type of reaction entails the use of (substituted) anilines and 1,3-dicarbonyl compounds for which there are two main reactions - the Combes synthesis and the Conrad-Limpach-Knorr synthesis. Of particular relevance is the Conrad-Limpach-Knorr method which has been used extensively to produce tacrine analogues. This procedure utilised anilines (100) as the aromatic starting material and β-keto esters (101) as the 1,3-dicarbonyl component. The amine group attacks the more reactive ketone carbonyl first, giving a β-aminoacrylic ester (102) which was subsequently cyclised to give a quinolone (103) *(Scheme 10)*.



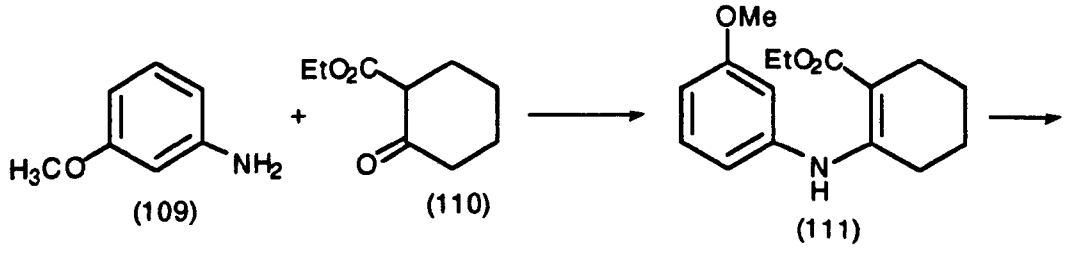
Scheme 10

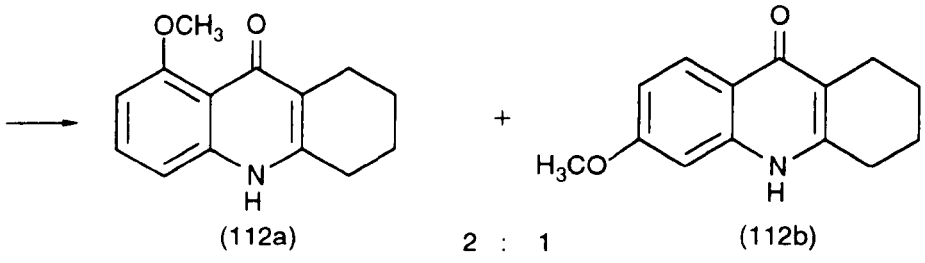
The reaction of the amino group with the ketonic carbonyl occurs at lower temperatures and is the product of kinetic control but as the reaction is reversible at higher temperatures, the thermodynamic product may be formed *via* the more stable amide (107). Subsequent cyclisation of these isomeric intermediates (105), (107) leads to isomeric quinolones (106), (108) *(Scheme 11).*



Scheme 11

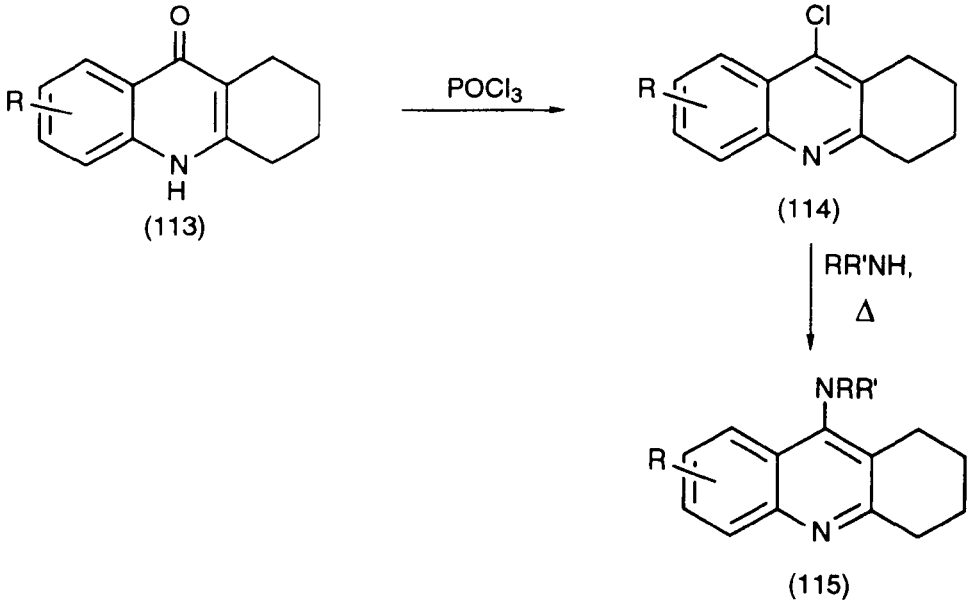
An early example of this is the work by Stephen *et al.* in 1947 to give substituted acridones (112a,b) from *m*-anisidine (109) and a cyclic β-keto ester (110)[134] *(Scheme 12).*





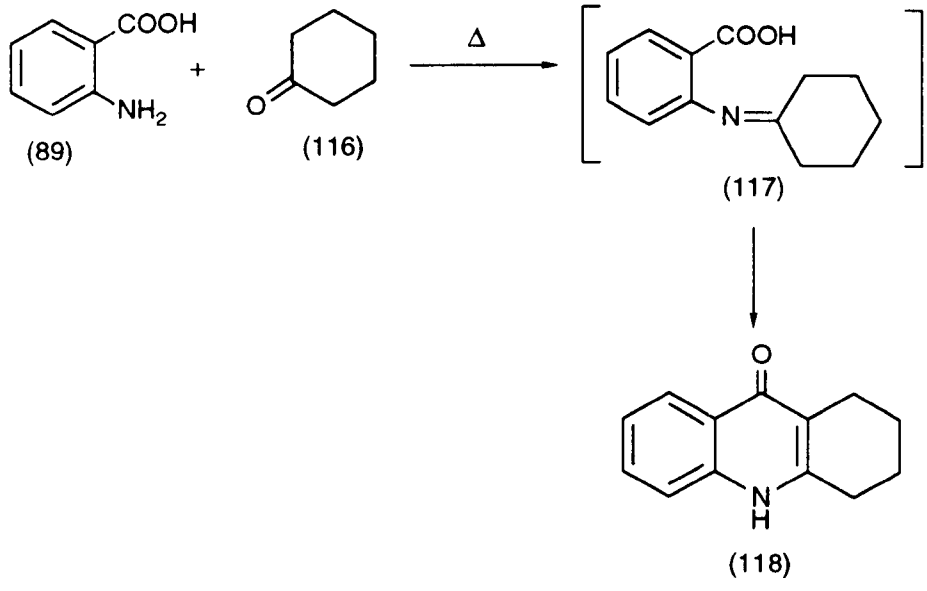
Scheme 12

To produce the tetrahydroacridines the acridones are then chlorinated with phosphorus oxychloride[109,135,136,137,138] and further functional group transformation occurs by heating at reflux the chloro compounds in phenol with the required amine[112,138,139] *(Scheme13).*



Scheme 13

The first synthesis of a 1,2,3,4-tetrahydroacridone (118) was accomplished in 1909 by Tiedke[140] using anthranilic acid (89) and cyclohexanone (116) by a proposed imine intermediate (117) *(Scheme 14)*.

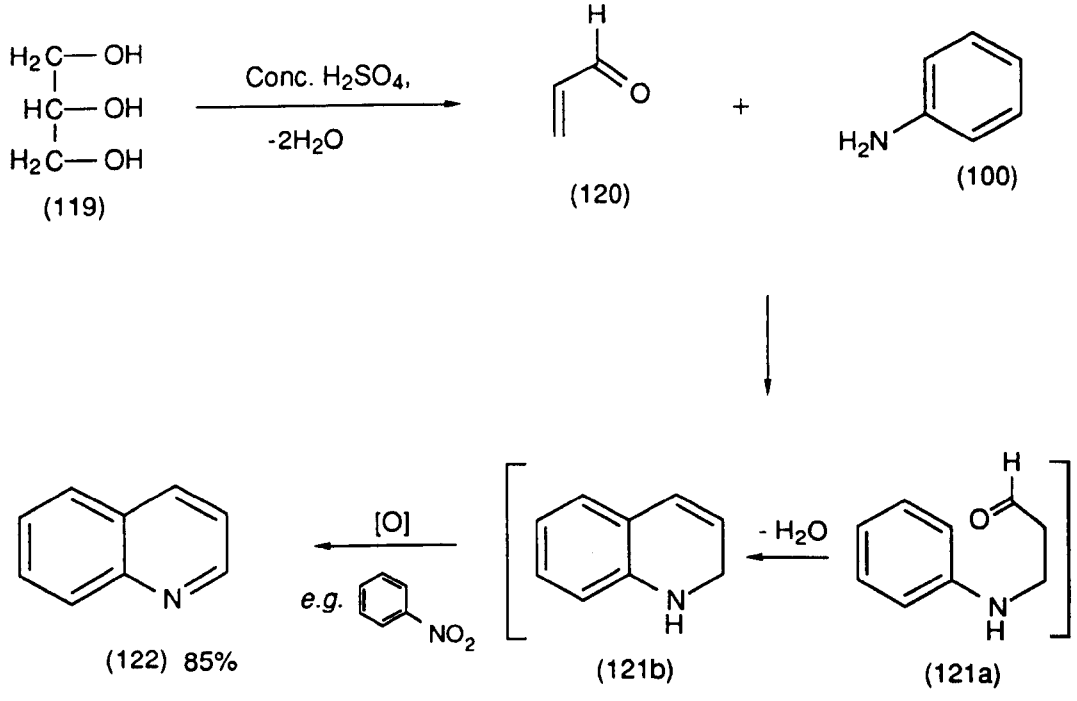


Scheme 14

In choosing between this and the Conrad method, the availability of the required aromatic starting materials must be taken into consideration, substituted anilines being more commonly available than substituted anthranilic acids.

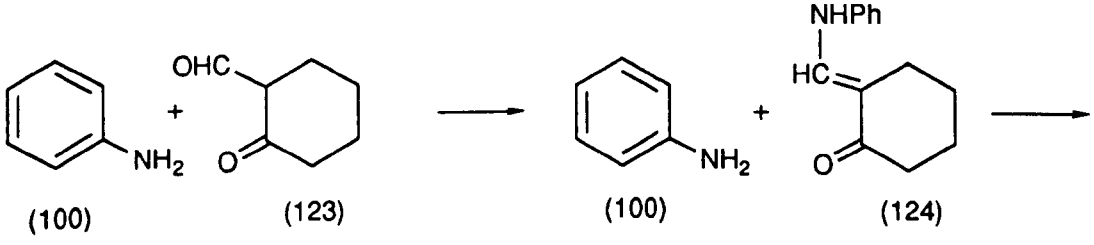
The second type of reaction concerning the synthesis of quinolines requires, again, aniline compounds and α,β-unsaturated carbonyl compounds. This reaction is termed the Skraup synthesis and the classical method uses glycerol (119) which is dehydrated to acrolein (120) with concentrated sulphuric acid. Reaction with aniline (100) then proceeds to form the secondary amine (121a) and electrophilic addition to the aromatic ring and loss of water to give the

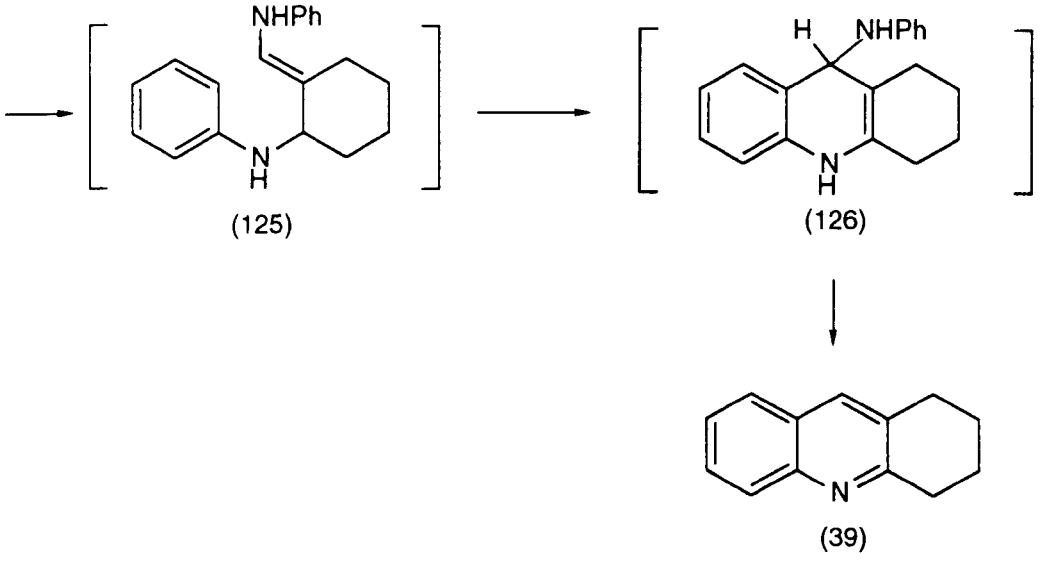
dihydroquinoline (121b). This is subsequently oxidised to the fully aromatic quinoline (122) *(Scheme 15).*



Scheme 15

Petrow, in 1942[141], produced 1,2,3,4-tetrahydroacridine (39) from aniline 100) and formylcyclohexanone (123) which proceeded via an α,β-unsaturated carbonyl compound (124) and, in a Skraup-type reaction, gave an second intermediate enamine (125) which cyclised (126) and on aromatisation with loss of aniline, gave the product (39) *(Scheme 16).*

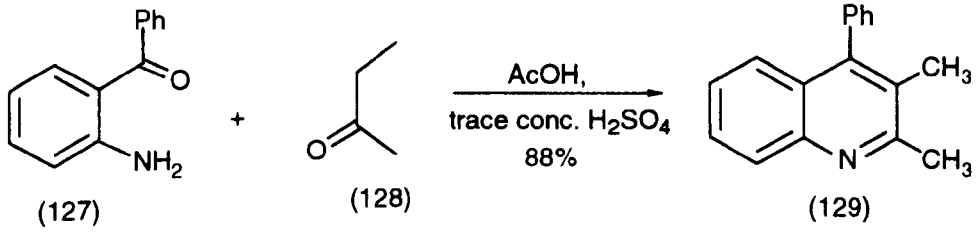




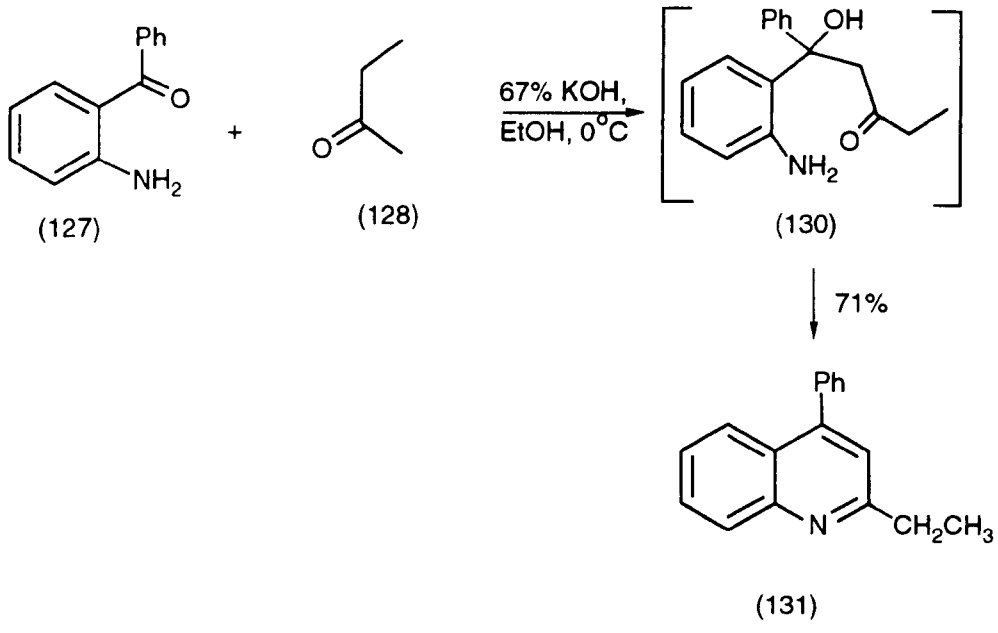
Scheme 16

The third type of reaction involves the use of anilines with an acyl group in the ortho position and carbonyl compounds with a methylene group in the α-position. There are two named reactions that follow this general description - the Friedländer synthesis and the Pfitzinger synthesis.

The Friedländer synthesis can proceed with either acid or base catalysis, each method giving a different intermediate, and hence different products (129), (131) *(Schemes 17a, 17b).*

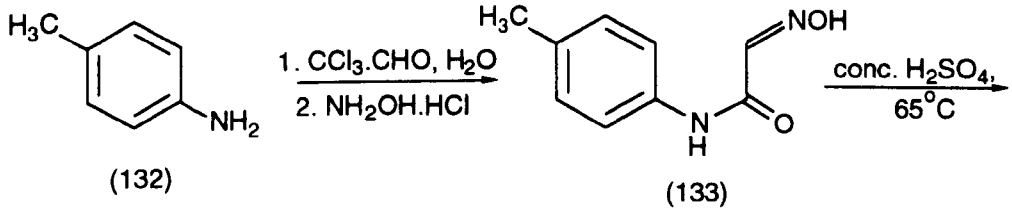


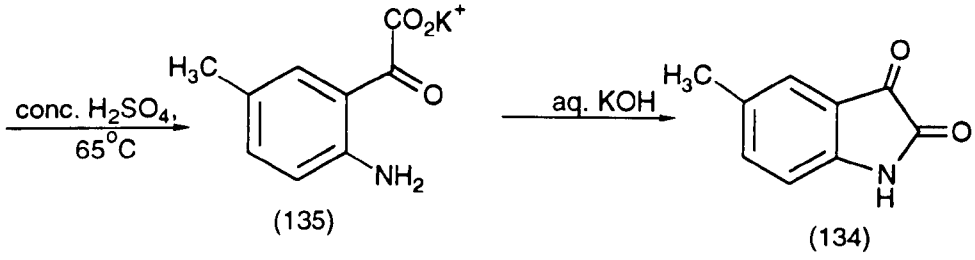
Scheme 17a



Scheme 17b

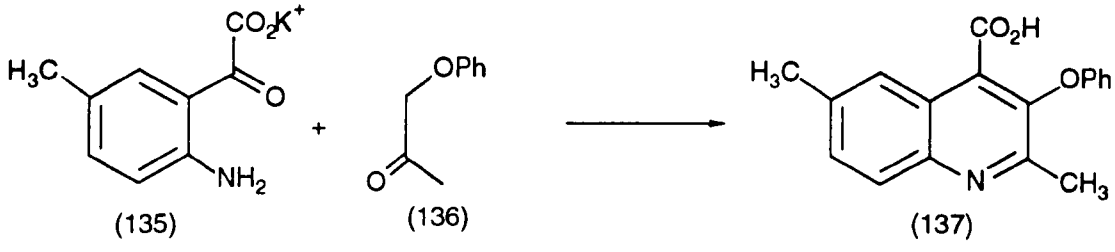
In a modification of the Friedländer reaction, the Pfitzinger synthesis requires the presence of an aldehyde group ortho to the amino group but these compounds are not readily available. Isatins (134) are therefore synthesised and introduced to the required carbonyl compound (136) *(Schemes 18a, 18b).*





(135) (134)

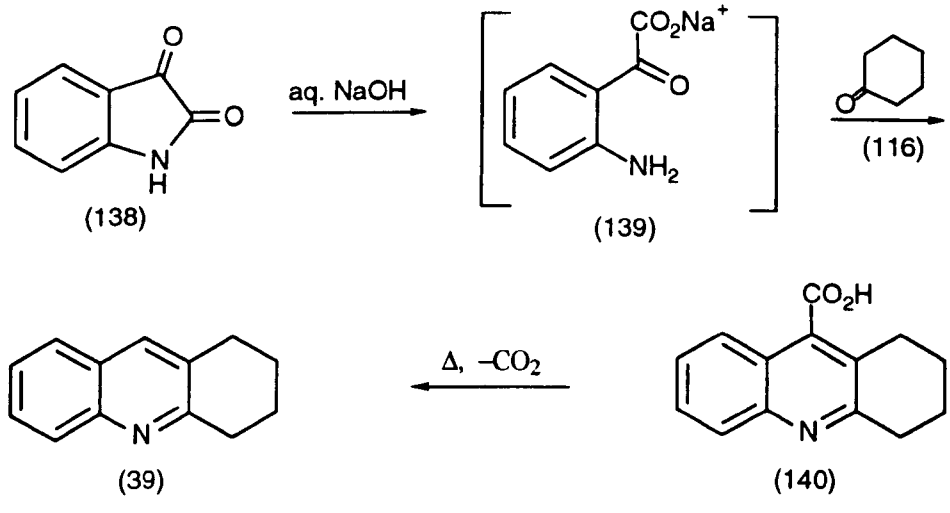
Scheme 18a



(135) (136) (137)

Scheme 18b

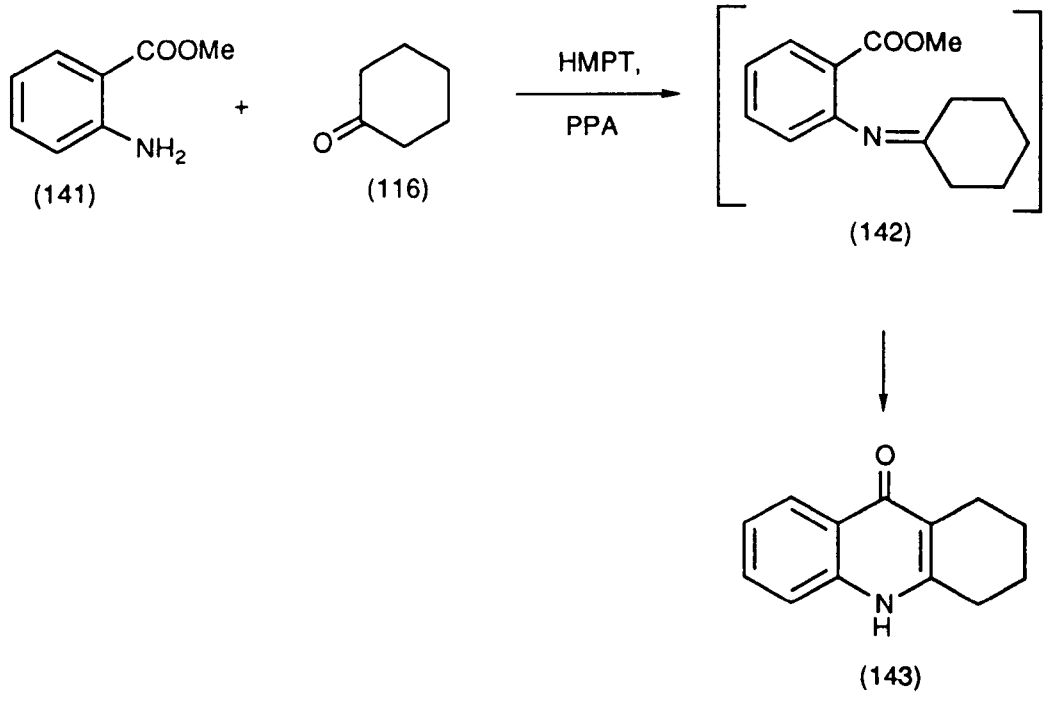
This sequence of reactions was utilised in 1924 by Perkin and Sedgewick to produce the unsubstituted 1,2,3,4-tetrahydroacridine (39)[142] *(Scheme 19).*



(138) (139) (39) (140)

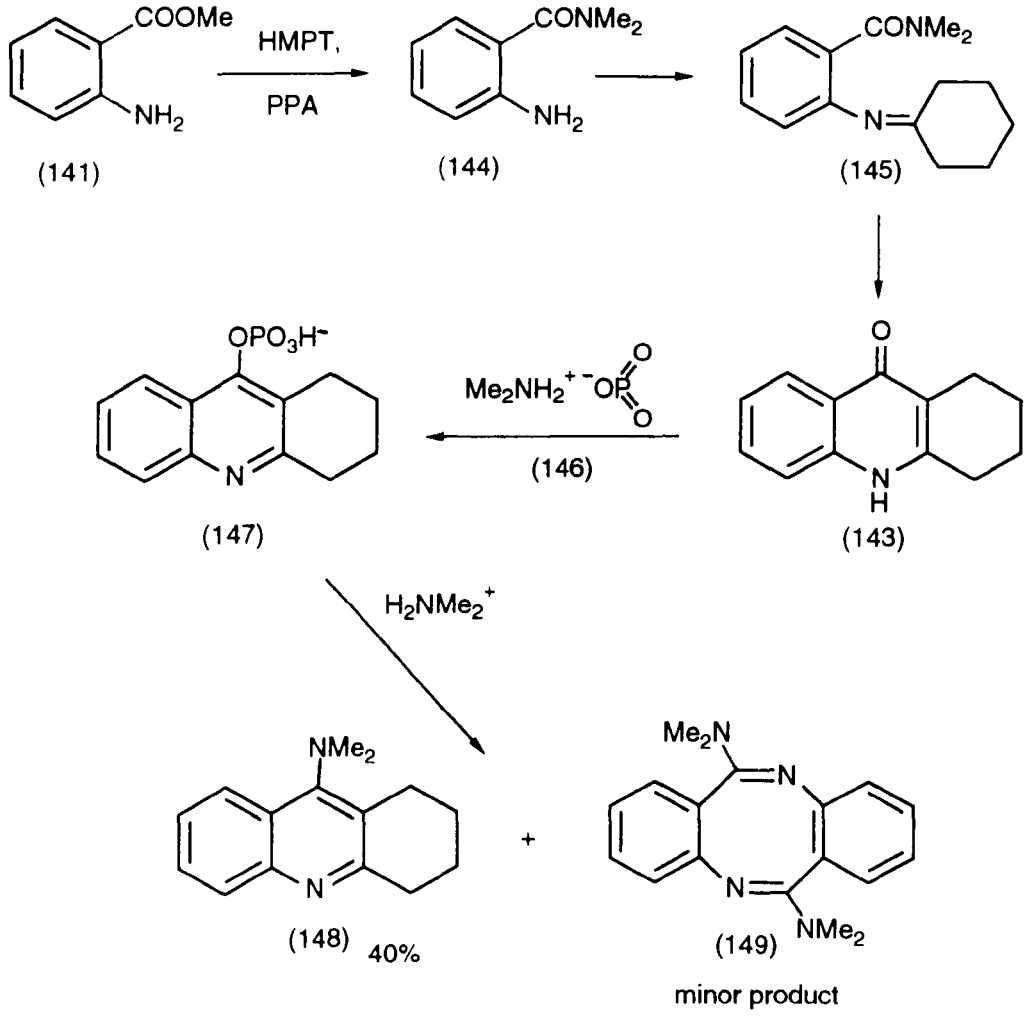
Scheme 19

More recently, a Friedländer type reaction employs hexamethylphosphoric triamide (HMPT) as a ring closing and dimethylaminating agent[143]. At lower temperatures it was found that quinolones (143) were formed, for example from methyl anthranilate (141) and cyclohexanone (116), presumably through an imine intermediate (142) which was not isolated *(Scheme 20).*



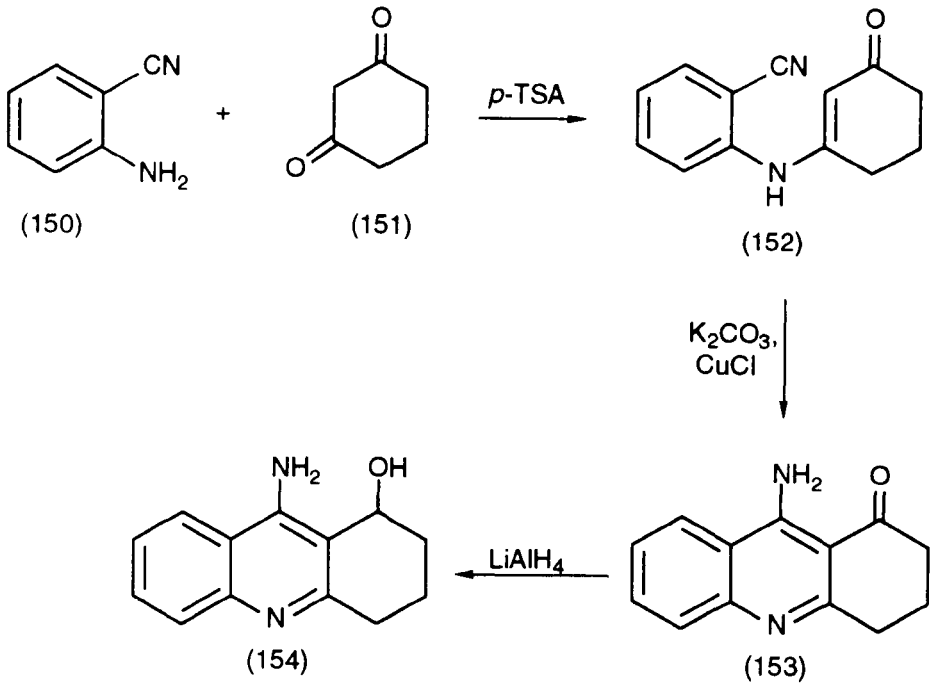
Scheme 20

Raising the temperature to around 260°C, the HMPT acted as a reagent rather than as a solvent and the dimethylamino compounds were formed *via* the amide (144) to give the quinolone (143) as before but this then reacted further with a metaphosphite ion (146) (transiently formed at the higher temperature[147]) to give (147). The resultant phosphate group is replaced with the dimethylamino group to give the product (148) and small amounts of a dibenzazocine compound (149) *(Scheme 21).*



Scheme 21

Shutske and co-workers[145] synthesised a great number of analogues using cyclic diones to stabilise this enamine intermediate. Anthranilonitrile (150) was condensed with cyclohexanedione (151) in the presence of *p*-toluene sulphonic acid, and the resultant enamine (152) cyclised with potassium carbonate and a catalytic amount of cuprous chloride. Reduction of the ketone (153) with lithium aluminium hydride gave the desired analogue (154) *(Scheme 22).*

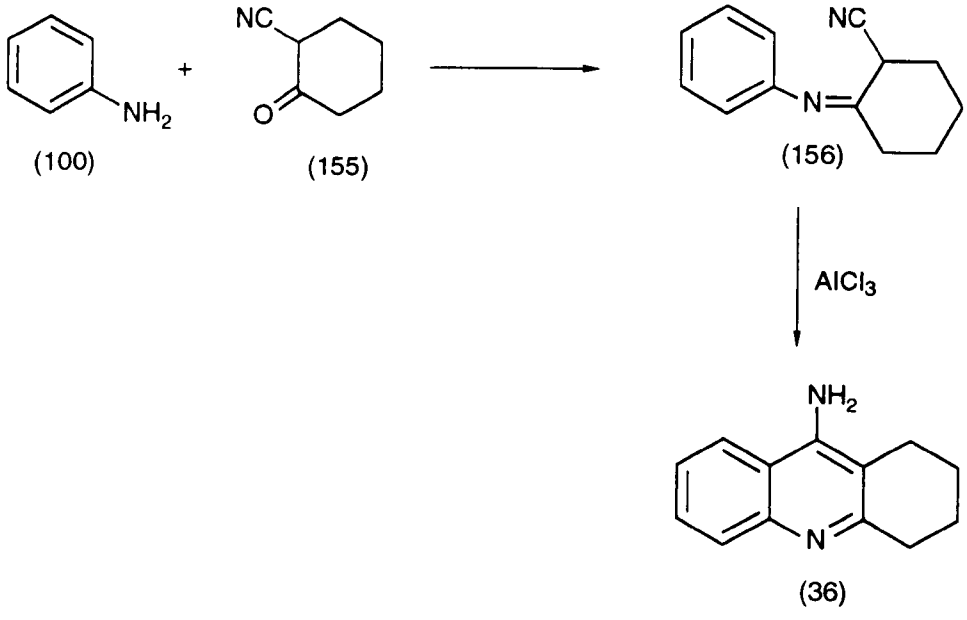


(150) (151) (152) (154) (153)

Scheme 22

Indeed, many analogues have been synthesised in this way by Shutske and co-workers in the search for effective tacrine analogues.

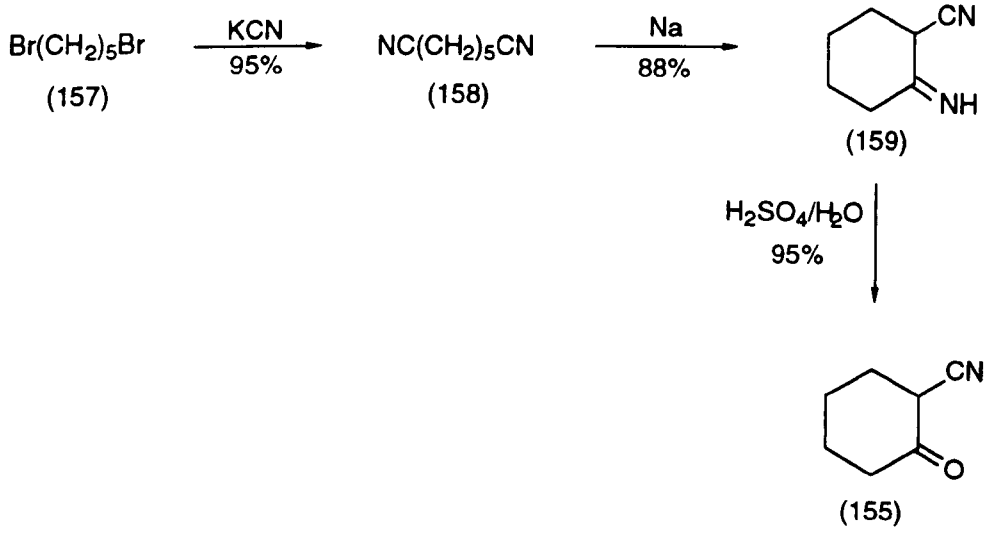
A little discussed method of synthesising tacrine (36) was first discovered in 1959 by Lamant who reacted aniline (100) with 2-cyanocyclohexanone (155) to give an imine intermediate (156) which was then cyclised with aluminium chloride to give tacrine (36)[146] *(Scheme 23).* The efficiency of this reaction was not commented upon.



(100) (155) (156) (36)

Scheme 23

The cyanoketone was synthesised by the transformation of 1,5-dibromopentane (157) into 1,5-dicyanopentane (158) with potassium cyanide and then with the use of sodium metal, induced a Thorpe-Ziegler cyclisation[147] reaction to give the cyano-imine (159). Acid hydrolysis subsequently produced the ketone (155) in good yield *(Scheme 24)*.



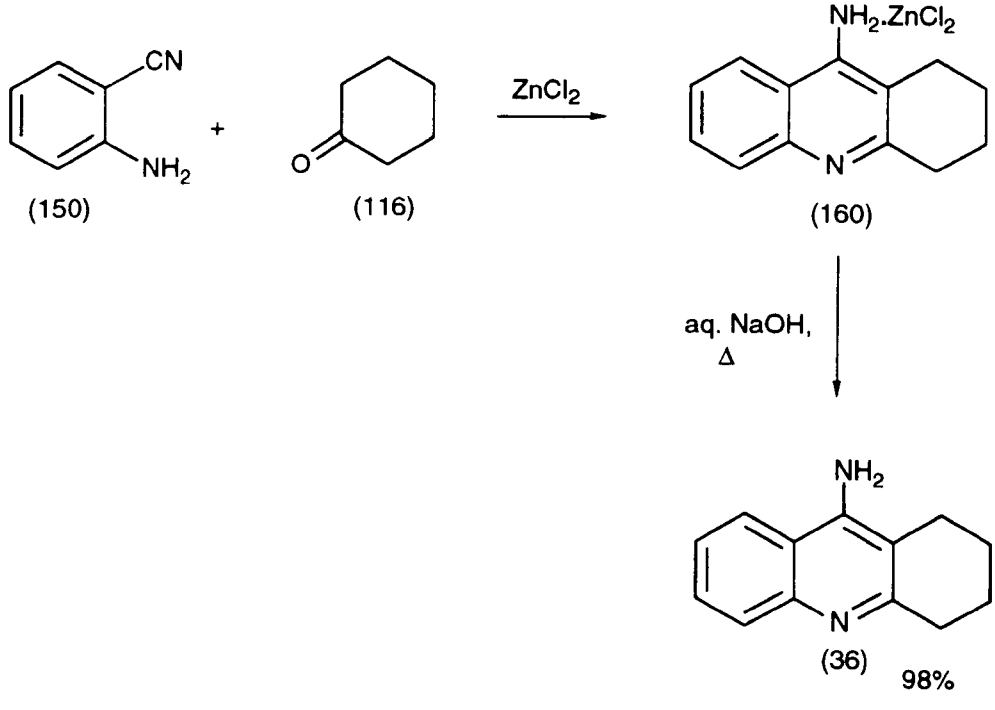
(157) (158) (159) (155)

Scheme 24

The Thorpe-Ziegler reaction may also be effected with potassium hydride[148] and will be discussed later in chapter 5.

(h) A Lewis Acid-Mediated Cyclodehydration Reaction

In 1963, co-workers Moore and Kornreich were searching for a facile synthesis towards 4-aminoquinoline type molecules[149]. They discovered that they could produce tacrine in one step using anthranilonitrile (150) and cyclohexanone (116) in a cyclodehydration reaction, using the Lewis acid zinc chloride *(Scheme 25).*



Scheme 25

This cyclodehydration reaction represents a convenient and speedy method towards tacrine (36). Investigation of the scope of this reaction was therefore the starting point of the project. Substitution of the cyclohexanone (116) with other cyclic

ketones was envisaged to give rapid access to ranges of related analogues for pharmacological evaluation. In particular, the 4-aminoquinoline moiety would remain unchanged as desired, and manipulation of the saturated ring using a selection of ketones was planned to give related ranges of molecules in an efficient manner.

RESULTS AND DISCUSSION

Chapter 5

SYNTHESIS OF SOME NOVEL TACRINE ANALOGUES

(a) The Initial Aims of the Project

At the outset, the aim of this research was to produce related ranges of tacrine analogues and subject them to pharmacological assay. From these results, the design and synthesis of further novel tacrine-related structures was to be undertaken along with subsequent testing for biological activity. The overall aims were (a) to find biologically active molecules and (b) to develop the chemistry to the point where further novel syntheses could be achieved.

At the beginning of the project, definitive information on the binding of tacrine was not known. Initial strategies had to be based both on the biological results from other research and various biochemical studies. Concerning the activities of the other molecules, for example those discussed in *Chapter 3*, no single structural feature could be cited as increasing anticholinesterase action, even although there were very many chemical variations. Straightforward substitution about the ring system had shown no particular benefit, similarly with extensive substitution on the amino nitrogen (although certainly other biological activities were exhibited). Incorporating heteroatoms into the ring system itself again gave no clear indications of an enhancement of biological activity but these types of

molecules were clearly of interest[119] because of their scope for manipulating the electronic properties of the tacrine nucleus.

A significant study was felt to be that of Kaul who investigated the anticholinesterase activities of tacrine, 4-aminoquinoline (40), 4-aminopyridine (8), tetrahydroacridine (39) and 9-(N-butyl)-amino-1,2,3,4-tetrahydroacridine (41)[85] as discussed in *Chapter 3*. 4-Aminopyridine (8) and 4-aminoquinoline (40) had shown little anticholinesterase activity although closer to tacrine in their chemical properties than tetrahydroacridine which exhibited an anticholinesterase action remarkably similar to tacrine. Therefore, the first condition for this project was that the 4-aminoquinoline portion of tacrine would be maintained because of the implications of this part of the molecule in binding. Along with the X-ray data for the co-crystallisation of acetylcholinesterase with tacrine, published in 1992[82], seemed to come a confirmation of the proposed binding model and hence of our strategy for the design of tacrine analogues.

Having decided upon this initial condition for design, synthetic approaches towards tacrine analogues had to be considered. To produce an extensive range of analogues which would demonstrate the effects of stepwise changes (*e.g.* changing ring size) there had to be a method whereby the saturated ring could be readily altered, ideally without extensive chemical manipulation. As described in the previous chapter, a number of approaches towards such structures exist (when tacrine is considered as a derivative of 4-aminoquinoline) but after some consideration the convenient and high-yielding one-pot synthesis of tacrine developed by Moore and Kornreich[149] became the favoured route (*chapter 4, scheme 25*). The benefit of investigating this reaction was that it allowed access to a large number of tacrine analogues with the only limitation seeming to be, at this

time, ready availability, either commercially or synthetically, of the requisite cyclic ketones.

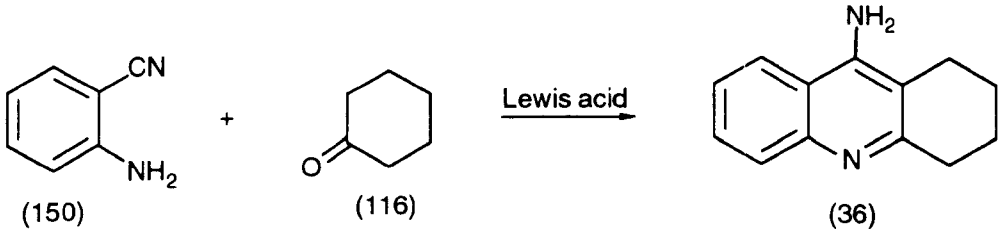
In previous years, a great deal of research has been carried out in this laboratory into the synthesis of cyclic ketone intermediates (*e.g.* benzosuberones and benzazepinones) and it was felt that this methodology would be ideal for utilisation in the tacrine project. The use of commercially available ketones would therefore be supplemented by these more functionalised ketones and lead to more extensive ranges of novel tacrine analogues.

(b) Reagents for the Cyclodehydration Reaction

In order to utilise the Lewis acid-mediated cyclodehydration reaction to its fullest, it was essential to investigate the relative merits of a range of suitable Lewis acids, the ideal reagent having the combined properties of easy handling and successful reaction. It was deemed to be essential that the Lewis acid would give reliable results in order that the ketones synthesised in the laboratory would not be destroyed in unsuccessful reaction thus requiring the repeated synthesis of starting materials.

The reaction between anthranilonitrile (150) and cyclohexanone (116) was assumed to be illustrative and a small series of Lewis acids was applied to effect the cyclodehydration reaction *(Scheme 26).* Following the literature protocol[149] for this particular reaction, anthranilonitrile (150) and cyclohexanone (116) were placed in toluene and anhydrous zinc chloride was added. The mixture was heated to reflux and the tacrine-zinc chloride complex precipitation was observed after about 10 minutes. The liquors were decanted and the tacrine liberated from the complex by heating the remaining solids at reflux in 2M sodium hydroxide for 1 hour. From this reaction an excellent 81% yield of crude tacrine was extracted.

Although this procedure was effective and proceeded at a convenient rate, anhydrous zinc chloride suffered from a propensity to form large aggregates which interfered in the mechanical agitation of the reaction and, after a few attempts, was demonstrated to cause a fluctuation in yields. The was due presumably to the decreased surface area of the Lewis acid, aggregation giving less contact with the reactants. So, this first reagent, although excellent at times, did not give reproducible results. It was difficult to handle both before reaction, where it tended to deliquesce rapidly when exposed to air and during reaction when it tended to aggregate.



(150) (116) (36)

Scheme 26

Zinc chloride diethyl etherate solution is commercially available as a more convenient reagent for the handling of zinc chloride. In an attempt to overcome the difficulties of using the solid, this solution was applied to the reaction following the same experimental procedure. Unfortunately, despite the advantages of handling a liquid, the crude yield of tacrine fell disappointingly to 52%.

Use of zinc chloride diethyl etherate had introduced the idea of using "liquid" Lewis acids and so tin(iv) chloride, titanium(iv) chloride and boron trifluoride diethyl etherate complex were investigated. Unfortunately use of tin(iv) chloride gave only a 39% yield and titanium(iv) chloride gave no reaction at all. This latter result was surprising as titanium(iv) chloride is a standard Lewis acids for the synthesis of enamines and imines[150,151,152] and the cyclodehydration

was presumed to go through an imine intermediate[140]. Also, titanium(iv) chloride is a good water scavenger.

Following on from this, boron trifluoride diethyl etherate was utilised in the reaction. As before the liquid was readily manipulated, and slow introduction of the complex to the suspension of anthranilonitrile (150) and cyclohexanone (116) resulted in a steady formation of a precipitate. After heating at reflux overnight and subsequent hydrolysis of the tacrine-BF_3 complex with aqueous alkali, tacrine was isolated from the reaction mixture in an excellent 89% yield.

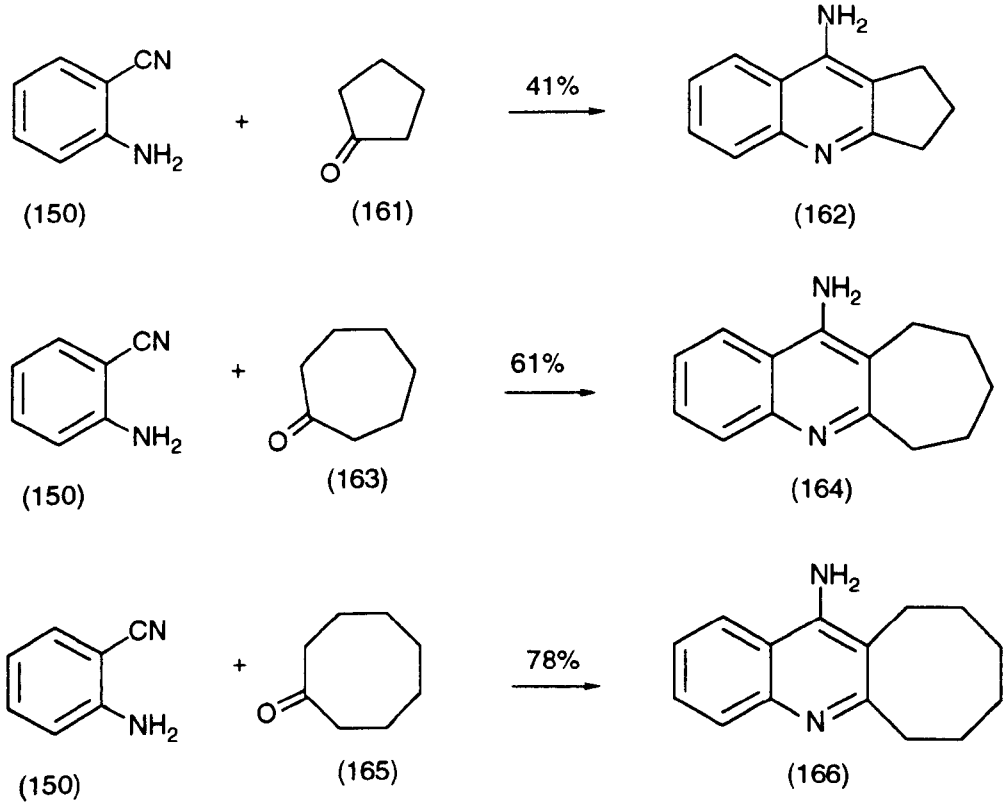
Finally, a cyclodehydration reaction was attempted with the use of polyphosphoric acid, a common reagent for cyclodehydrations[153] (*e.g.* intramolecular Friedel-Crafts acylations). The polyphosphoric acid was made by stirring phosphorus pentoxide and orthophosphoric acid from which the clear viscous reagent was formed after 30 minutes. On addition of the anthranilonitrile (150) and cyclohexanone (116) the expected colour change characteristic of this reagent did not develop, even over a range of temperatures. On hydrolysis of the reaction with ice water, tacrine could not be isolated from the reaction mixture at any stage.

From these results it was concluded the the reagent of choice for bringing about the cyclodehydration between anthranilonitrile (150) and a series of cyclic ketones was the boron trifluoride diethyl etherate complex, due to its efficiency and ease of handling.

(c) Use of Some Cyclic Ketones

(i) Changing Ring Size

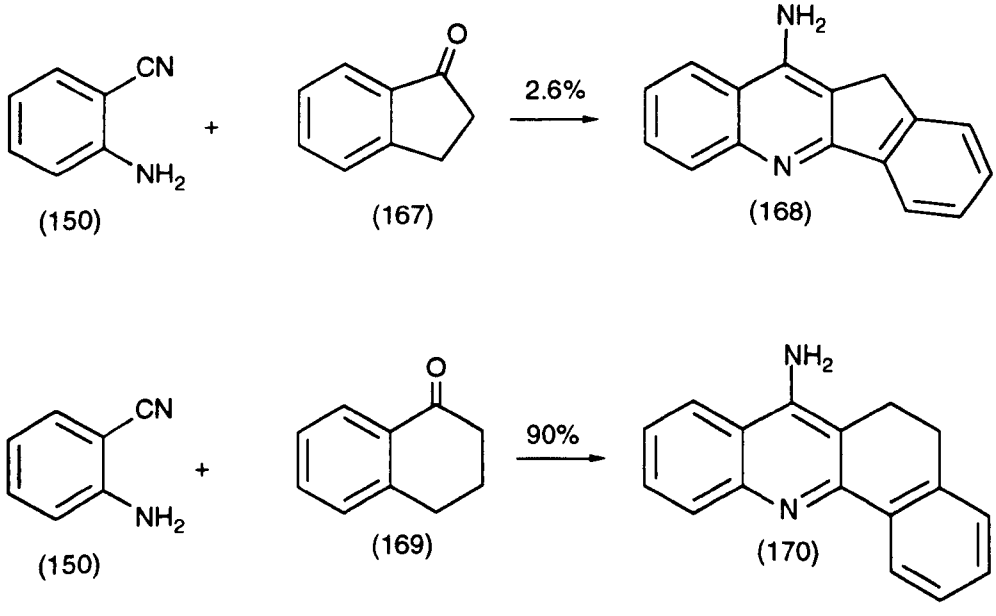
To begin with, it was proposed that cyclohexanone would be substituted in the cyclodehydration reaction by its cyclopentyl, cycloheptyl and cyclooctyl analogues to produce tacrine analogues with differing ring size. It was known that these compounds were not novel[91,112] but their anticholinesterase activities in relation to tacrine were unknown. Synthesis, then, of these analogues (162), (164) and (166) would allow their biological activity to be quantified and an opinion on the effects of ring size to be formed. The cyclodehydration reaction was carried out under standard conditions between anthranilonitrile (150) and cyclopentanone (161), cycloheptanone (163) and cyclooctanone (165) to give moderate to good yields of their respective analogues *(Scheme 27)*.

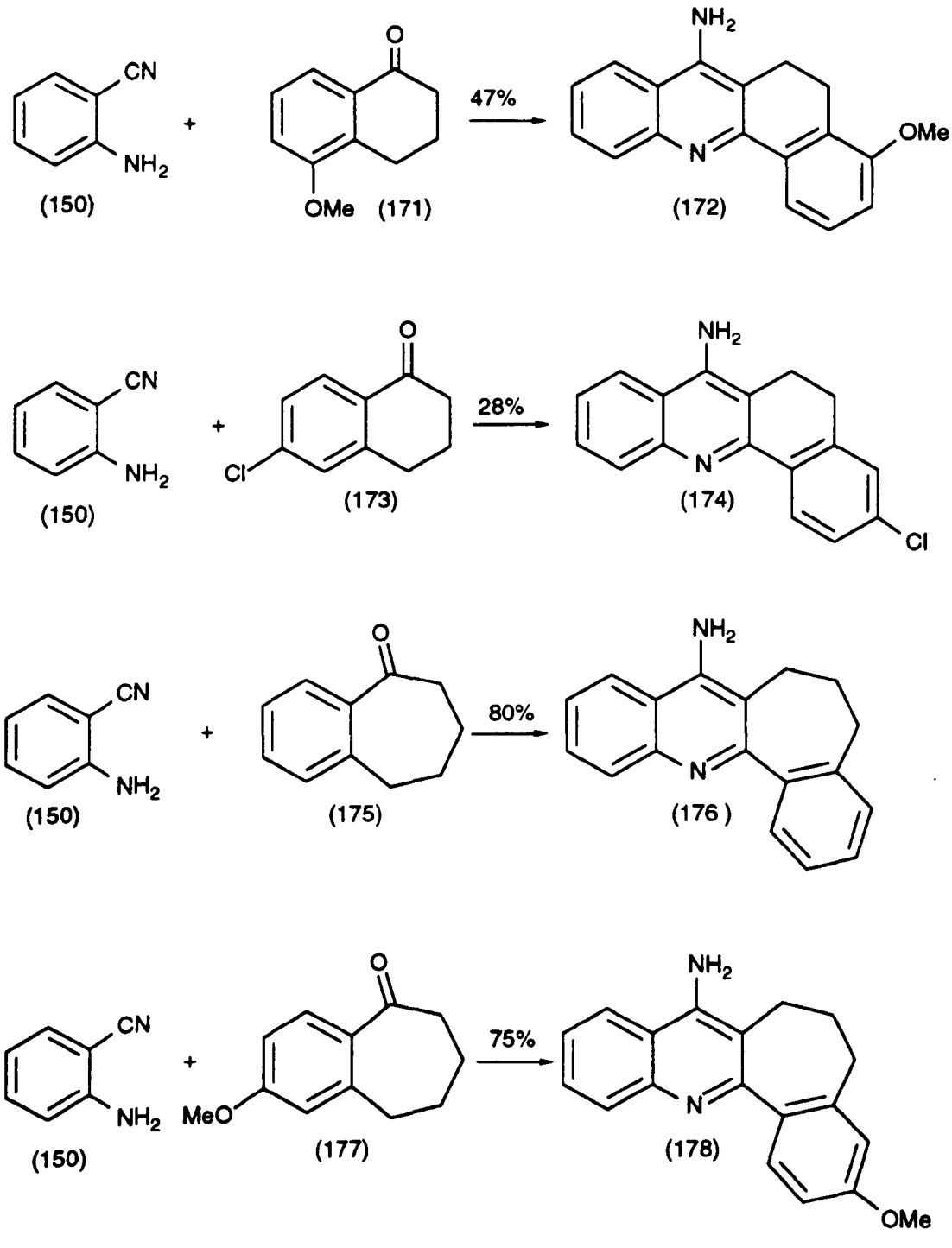


Scheme 27

(ii) Incorporating a Fused Benzene Ring

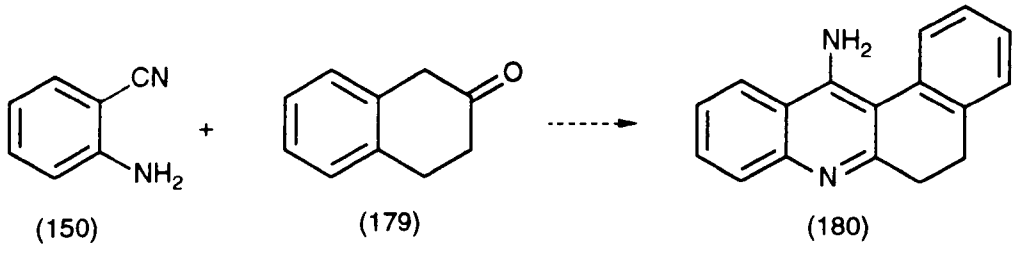
The next range of compounds to be synthesised extended the study on ring size by the attachment of a fused benzene ring to the nuclei of the tacrine analogues described above. Specifically, the bicyclic ketones 1-indanone (167), 1-tetralone (1,2,3,4-tetrahydronaphthalen-1-one) (169) and 1-benzosuberone (2,3,4,5-[*1H*]-tetrahydrobenzocyclohept-1-one) (175) were visualised as offering products with an increased lipophilicity and thus investigating the possibility of increasing π-stacking interactions with the aromatic residues lining the enzyme's gorge containing the active site. The substituted 1-tetralones 5-methoxy-1-tetralone (171) and 6-chloro-1-tetralone (173) and 7-methoxy-1-benzosuberone (177), synthesised in this laboratory by previous workers, were also utilised. Again with the use of boron trifluoride diethyl etherate to mediate the cyclodehydrations with anthranilonitrile, the required products were obtained. Unexpectedly, indanone (167) gave only a very poor yield of product in contrast to the excellent yield from the 1-tetralone (169) but the majority of yields were satisfactory. *(Scheme 28).*




CN
NH2
(150)
+
O
OMe
(171)
47%
NH2
N
OMe
(172)
CN
NH2
(150)
+
O
Cl
(173)
28%
NH2
N
(174)
Cl
CN
NH2
(150)
+
O
(175)
80%
NH2
N
(176)
CN
NH2
(150)
+
O
MeO
(177)
75%
NH2
N
(178)
OMe

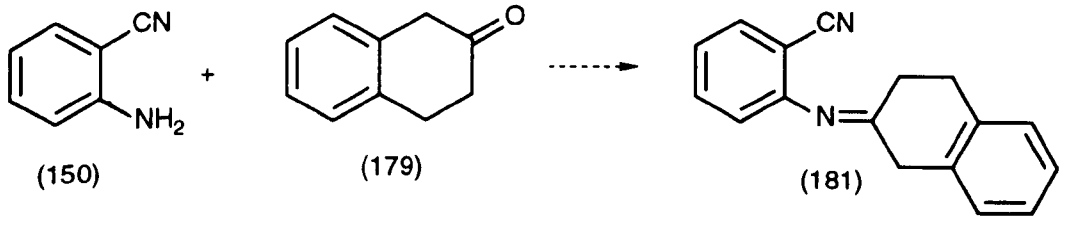
Scheme 28

In an attempt to further define any effects of the fused benzo group, the reaction was attempted with freshly distilled 2-tetralone (1,2,3,4-tetrahydronaphthalen-2-one) (179) *(Scheme 29).*



Scheme 29

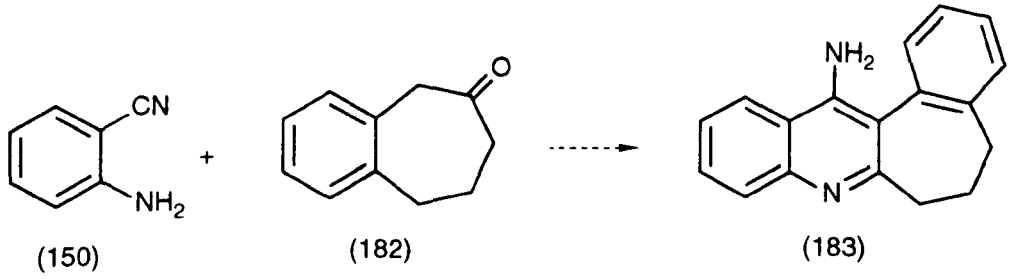
It was anticipated that the product (180) would be isolated in only modest yield because of the propensity for 2-tetralone to self-condense. Unfortunately, the reaction mixture contained only black solids from which no amino compounds could be extracted with dilute hydrochloric acid. Shifting the benzene ring to a different face of the tacrine nucleus could have given more information concerning the structure-activity relationships of these compounds. It was subsequently reasoned that should the other tetralone products produce good pharmacological results that this line of chemistry would be further investigated by attempting to synthesise the intermediate imine (181) before cyclisation *(Scheme 30).*



Scheme 30

(d) Synthesis of 2-Benzosuberone

Producing the ring-expanded analogues of various ring systems has been the subject of much study in this laboratory in recent years following the discovery that a combination of silver nitrate and iodine in aqueous methanol gave a facile and relatively non-toxic method of ring expansion[154,155]. In particular the synthesis of 2-benzosuberone (182) was desired in order that the effects of shifting the benzene ring around the nucleus could be investigated in the following proposed manner *(Scheme 31)*.

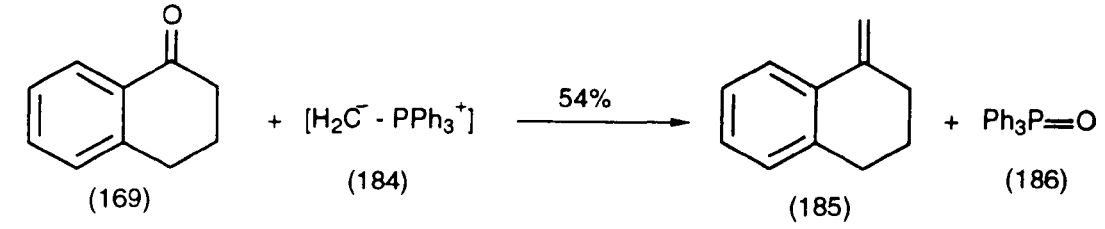


(150) (182) (183)

Scheme 31

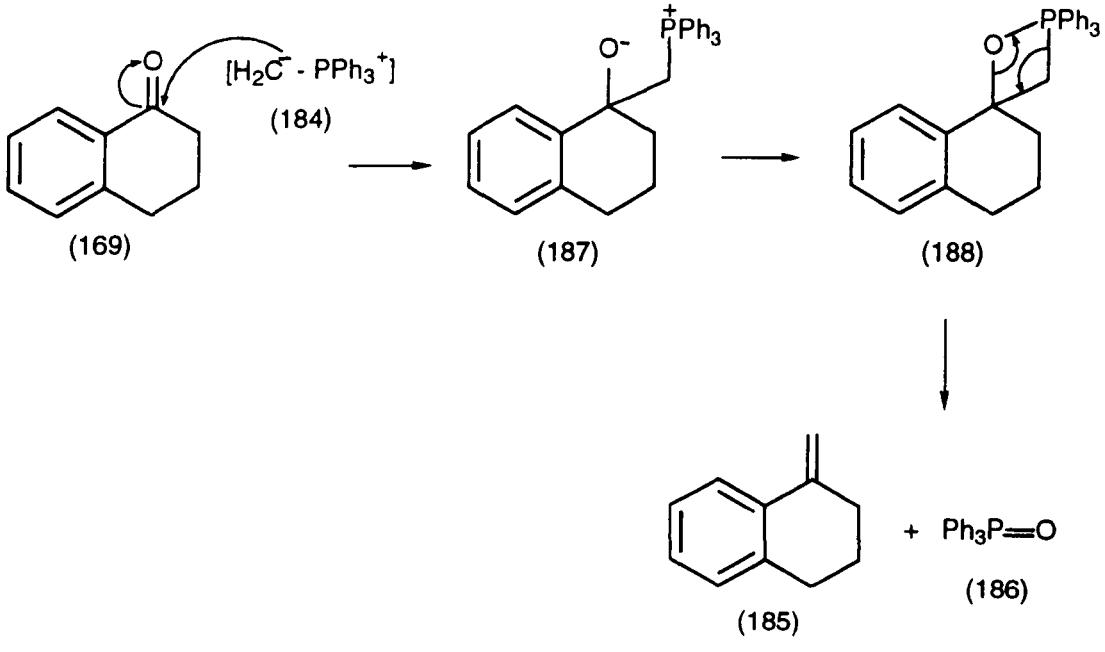
(i) Methylenation of 1-Tetralone

The first step in the synthesis of 2-benzosuberone (182) was to methylenate 1-tetralone (169) to the exocyclic alkene (185). The is conventionally carried out in the laboratory by use of the Wittig reaction[156]. In this reaction, an aldehyde or ketone is reacted with a triphenylphosphonium ylide to give an alkene and triphenylphosphine oxide (186). The scheme below shows the exocyclic alkene (185) obtained in a 54% yield from 1-tetralone, for use in this project*(Scheme 32a)*.



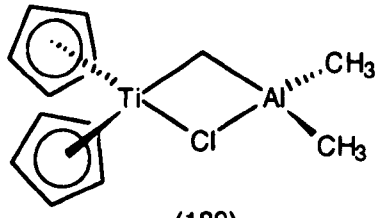
Scheme 32a

The highly reactive phosphonium ylide (184) is formed by the deprotonation of the appropriate alkyltriphenylphosphonium salt with a strong base, such as *n*-butyllithium, enabling it to react with the carbonyl group. The mechanism of this reaction is traditionally thought of as a nucleophilic attack on the carbonyl group giving a zwitterionic intermediate betaine (187) which cyclises to the oxaphosphetane (188) and collapses to the alkene, liberating triphenylphosphine oxide *(Scheme 32b).*



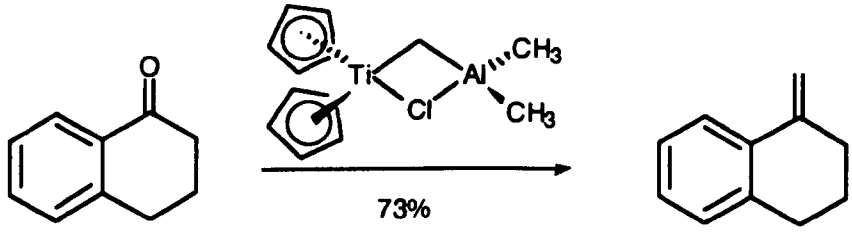
Scheme 32b

Since there are many cases in which use of the Wittig reagent is not appropriate (*e.g.* because of steric hindrance or base-sensitive starting materials) many reagents have been developed over the years to effect methylenation of carbonyl groups[157]. One such reagent is Tebbe's reagent[158], (μ-chloro)(μ-methylene)bis(cyclopentadienyl)(dimethylaluminium)titanium, a metallocycle with the following structure (189).



(189)

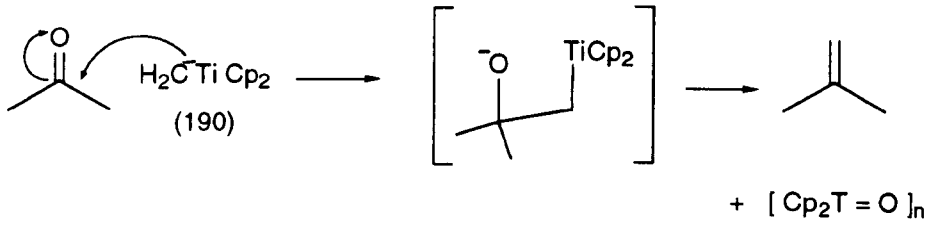
Tebbe's reagent is a versatile methylenation reagent for the production of alkenes from ketones, vinyl enol ethers from esters, and enamines from amides[159,160,161]. It may also be used with base-sensitive ketones in a way that the Wittig reaction may not. It can be utilised for the methylenation of 1-tetralone (169) in the following manner giving a reported 73% yield of the exocyclic alkene *(Scheme 32c).*



(169) (184)

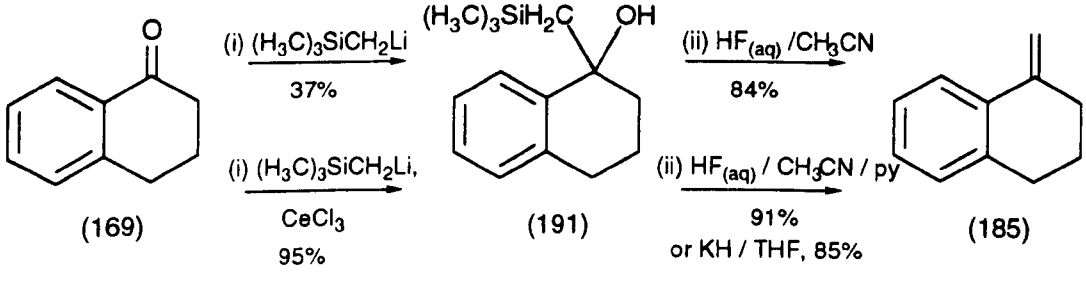
Scheme 32c

The reactive fragment of the reagent is [Cp_2TiCH_2] (190) which is trapped by the carbonyl as shown *(Scheme 32d).*



Scheme 32d

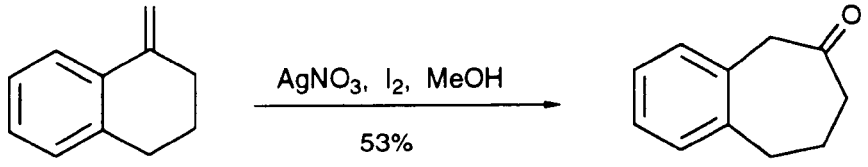
Optimum yields of the methylenation of 1-tetralone to the exocyclic alkene may be obtained by a modification of the Peterson Olefination[162,163]. Used solely, the Peterson reagent trimethylsilylmethyllithium reacts with 1-tetralone to give a yield of 37% of the desired product (185). Use of anhydrous cerium trichloride to produce a cerium species, presumed to be $RCeCl_2$ (where R = trimethylsilylmethyl in this case)[164] gave excellent yields (95%) of the intermediate β-hydroxysilane. Elimination to the alkene is carried out with either aqueous hydrofluoric acid in acetonitrile or potassium hydride in THF and the literature results are described below[165]*(Scheme 32e).*



Scheme 32e

(ii) Ring Expansion Reaction

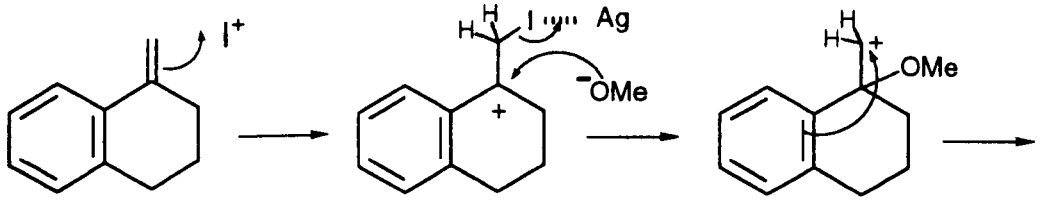
Having obtained the exocyclic methylene compound (185) by the Wittig method, it was then subject to the "Prevost" conditions of silver nitrate and iodine[166] (silver nitrate : iodine, 2:1) in aqueous methanol to produce the ring expanded product (192) in 53% yield *(Scheme 33)*. The silver nitrate was dissolved in aqueous methanol and a solution of the alkene in THF was added in one portion followed immediately by the iodine.



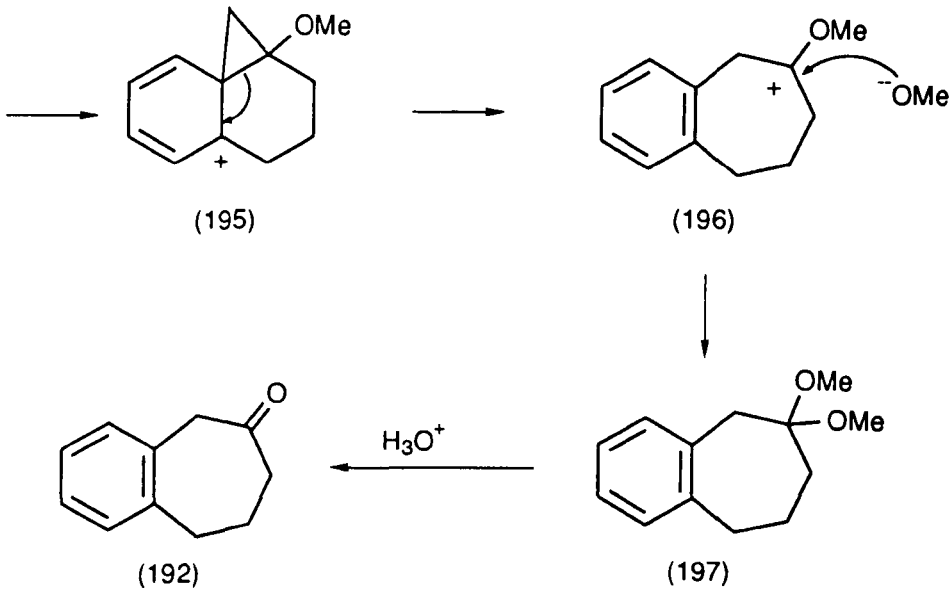
(185) (192)

Scheme 33

This reaction cleanly effects the ring expansion and the mechanism is believed to proceed by the following mechanism[154] *(Scheme 34)*.



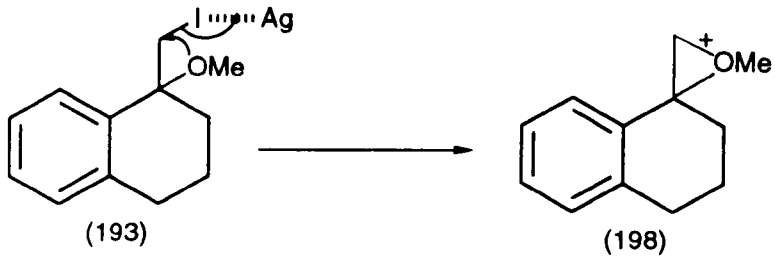
(185) (193) (194)



Scheme 34

The iodine and silver nitrate combine to produce silver iodide and an $I^+NO_3^-$ species. The iodonium cation is captured by the double bond to form the tertiary cation (193) which is then attacked by a methoxy anion. The iodine is then abstracted as the iodide by the silver ion, leaving the primary cation (194). It is proposed that this cation is attacked by the benzene ring by disrupting its aromaticity to form a cyclopropane ring and a new cationic centre (195). The site of the cation is moved again when the aromaticity is restored to reform the benzene ring, forming a seven-membered carbocycle, the cation again being attacked by a methoxy group to give the acetal (197). Under the acidic conditions, the acetal is hydrolysed to the ketone (192).

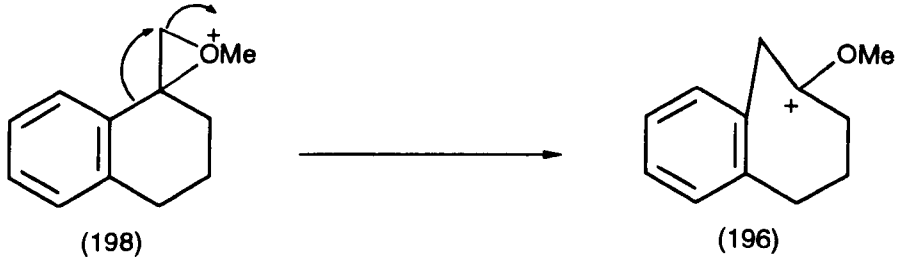
However, this mechanism does not take into account any possible neighbouring group effect involving the oxygen atom of the methoxy group, giving a relatively stable oxonium ion *(Scheme 35)*.



(193) (198)

Scheme 35

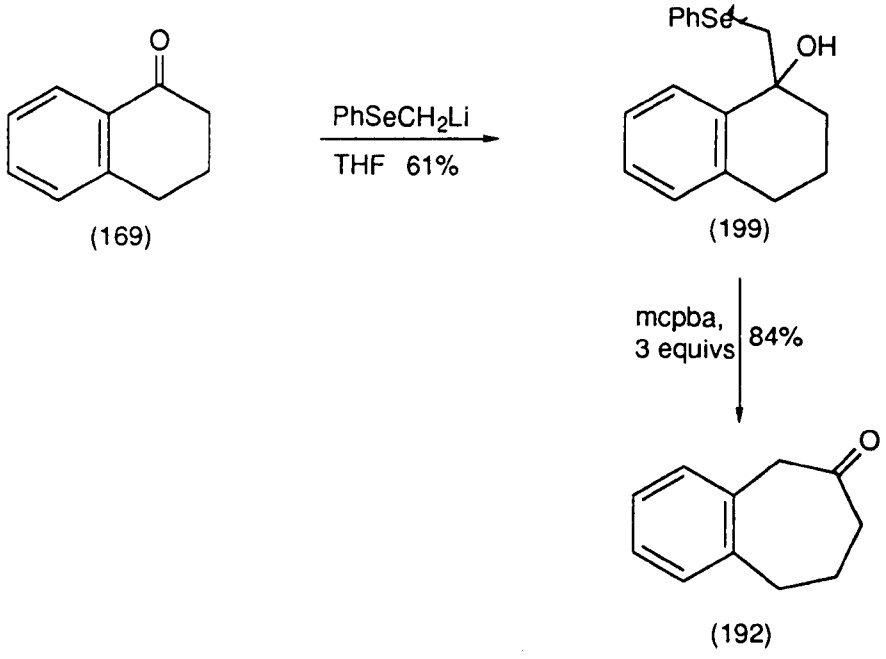
Also, the question arises of whether or not the benzene ring is nucleophilic enough to disrupt its aromaticity in order to quench the cation. Other methods of ring expansion cite migration of the aryl group as the mechanism of action in these types of systems *(Scheme 36).*



(198) (196)

Scheme 36

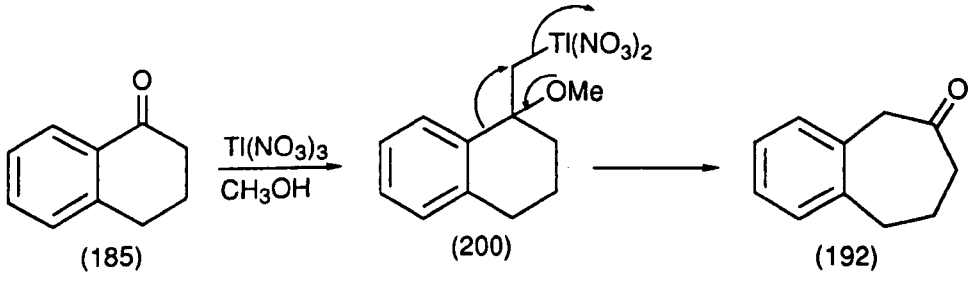
The use of selenides is illustrative of this mechanism. 2-Benzosuberone (192) is produced from 1-tetralone (169) by the use of phenylselenide methyllithium to give the β-hydroxy selenide (199), which on oxidative work-up with *m*-chloroperbenzoic acid produces the ring expanded product (192) in a method described by Uemura *et al.*[167] *(Scheme 37).* The epoxide may be achieved with acyclic systems.



(169) (199) (192)

Scheme 37

Thallium trinitrate is also reported to ring expand the exocyclic alkene[168] with a proposed mechanism of aryl migration which could be seen to be formally similar to the silver nitrate method *(Scheme 38).*

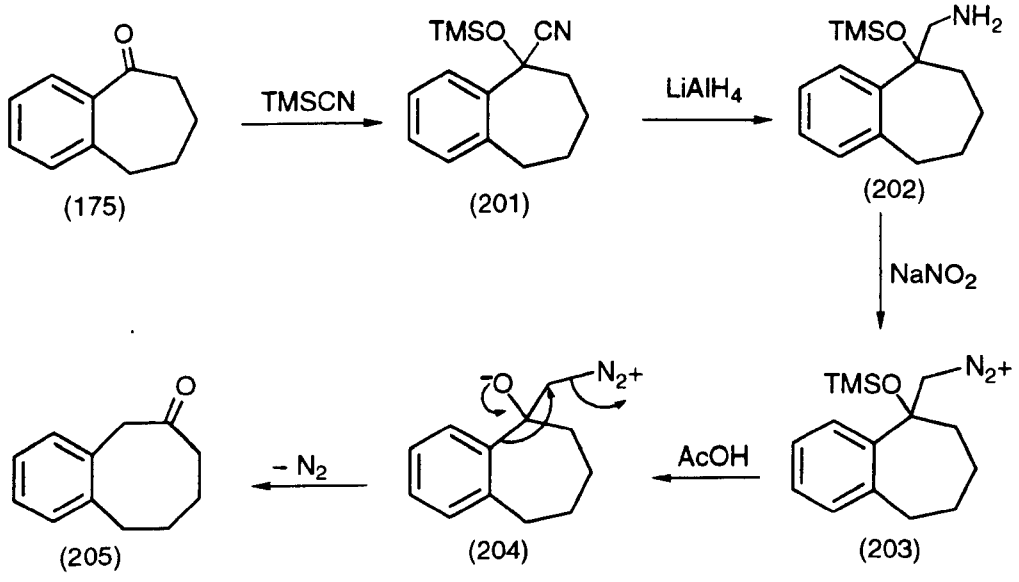


(185) (200) (192)

Scheme 38

The Tiffaneau-Demjanov ring expansion reaction[169] is a series of standard organic steps which is known to give the ring expanded product of 1-benzosuberone

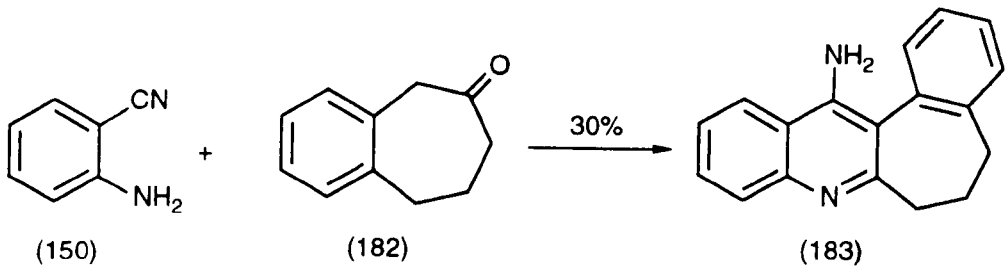
The Tiffaneau-Demjanov ring expansion reaction[169] is a series of standard organic steps which is known to give the ring expanded product of 1-benzosuberone (175) in the following way, with aryl migration giving the final product (205) *(Scheme 39)*.



Scheme 39

So, there is much literature precedent for aryl migration to propose this mechanism for the silver nitrate / iodine ring expansion. It should be noted that this reaction is a convenient alternative to the thallium and selenium methods of ring expansion as these compounds are extremely toxic, whereas silver nitrate and iodine are relatively innocuous routine laboratory reagents.

2-Benzosuberone was successfully condensed with anthranilonitrile (150) in a 30% yield using the standard method of cyclodehydration mediated by boron trifluoride diethyl etherate*(Scheme 31)*.

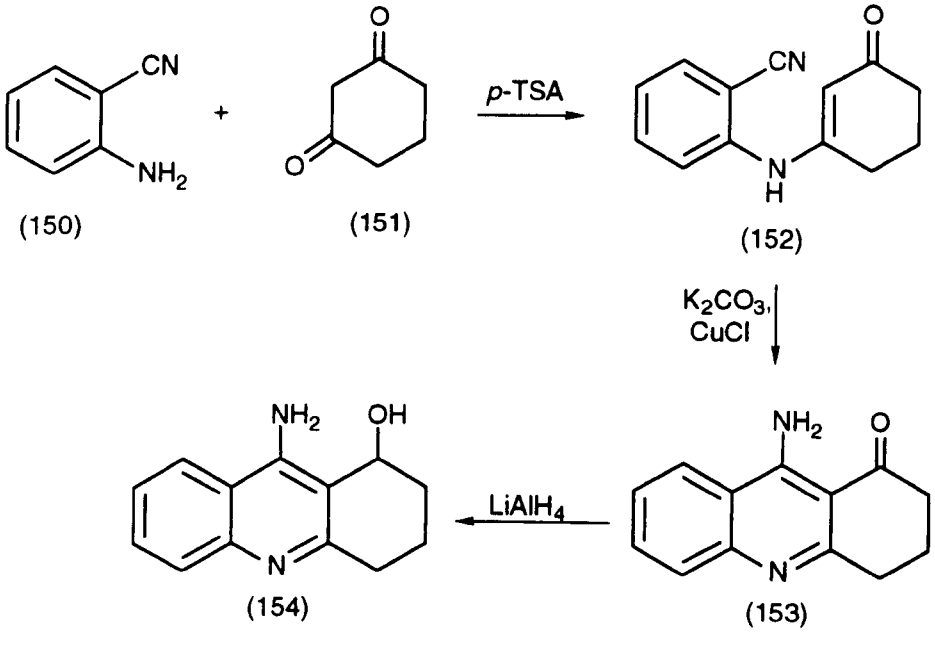


(150) (182) (183)

Scheme 31

(e) Attempted Syntheses of Some Known Drugs

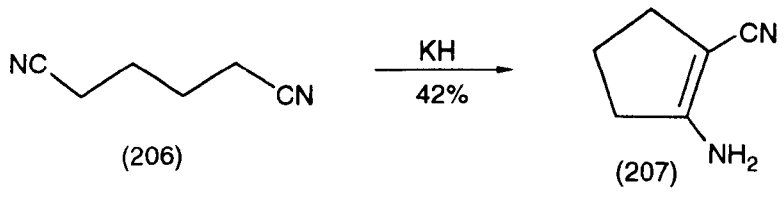
In order to have a pharmacological comparison for the novel compounds produced by this project, in addition to tacrine, the Hoescht-Roussel drug Velnacrine (154) was synthesised in the manner described before *(Chapter 4, Scheme 22)* from anthranilonitrile (150) and cyclohexan-1,3-dione (151). Formation of the enamine and subsequent basic cyclisation produced the 1-acridone which was reduced to the alcohol product (154).



(150) (151) (152) (154) (153)

Scheme 22

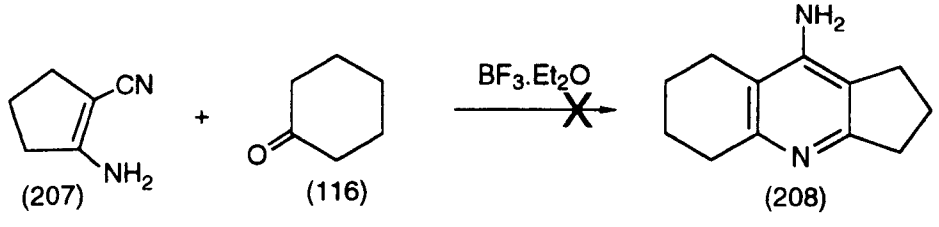
There exists another drug, synthesised by Japanese workers called amiridin[170] (9-amino-2,3,4,5,6,7-hexahydro-[*1H*]-cyclopenta-[*b*]-quinoline (208) which was to be included in this study. Its synthesis required the compound 1-amino-2-cyano-cyclopent-1-ene (207) to replace anthranilonitrile in the cyclodehydration reaction.. This α,β-unsaturated nitrile was made by the Thorpe-Ziegler cyclisation of 1,4-dicyanobutane (206). The reaction is an intramolecular version of the Thorpe reaction where the α-carbon of one nitrile molecule is added to the nitrile carbon of another[147]. 1,4-Dicyanobutane (206) was cyclised in this case using potassium hydride[148] to give the desired amino nitrile (207) *(Scheme 40).*



(206) (207)

Scheme 40

The cyclodehydration was attempted in the usual manner with cyclohexanone (116) *(Scheme 41).* Unfortunately, this reaction was unsuccessful and produced only decomposition products. The 1-amino-2-cyano-cyclopent-1-ene (207) is obviously lacking the stability of the aromatic anthranilonitrile (150).

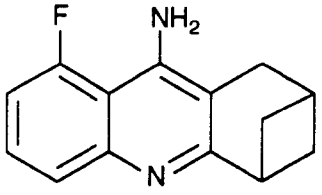


(207) (116) (208)

Scheme 41

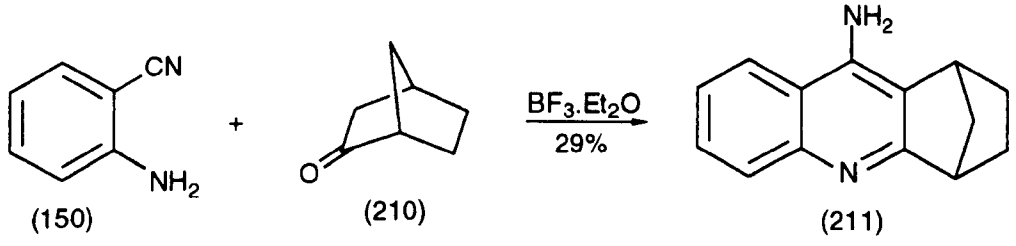
(f) Bridged Analogues

In reference to a bridged compound synthesised by Japanese workers[171], 9-amino-8-fluoro-1,2,3,4-tetrahydro-2,4-methanoacridine (209) which was cited as having a greater selectivity for the CNS than tacrine (36), physostigmine (32) or amiridin (208). It was therefore decided to attempt the synthesis of some bridged analogues.



(209)

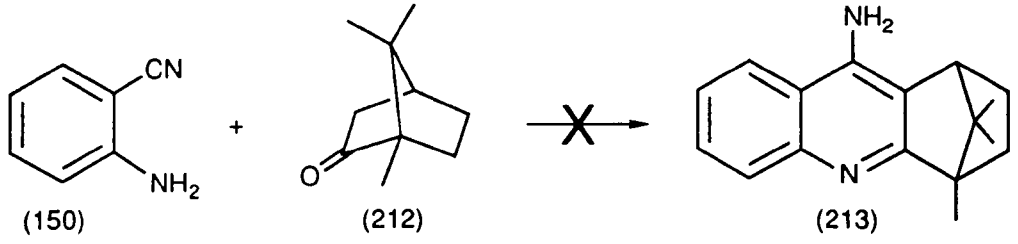
The first bridged ketone that was selected to take part in the cyclodehydration reaction was norcamphor (210). After many attempts the reaction finally yielded an optimum 29% yield of product was achieved *(Scheme 42).*



(150) (210) (211)

Scheme 42

The tacrine analogue so made was then found to constitute part of a patent held by Hoescht-Roussel[172] and therefore was not novel. But, if the reaction could be extended to include camphor there would exist two bridged compounds which could be compared to tacrine to investigate the effects of increasing bridge size. The effect on biological activity of the tacrine nucleus having a bridge could then be more defined. Unfortunately, the cyclodehydration reaction between anthranilonitrile (150) and R(+)-camphor (212) *(Scheme 43)* could not be achieved and an alternative method had to be developed (see *Chapter 7*).



(150) (212) (213)

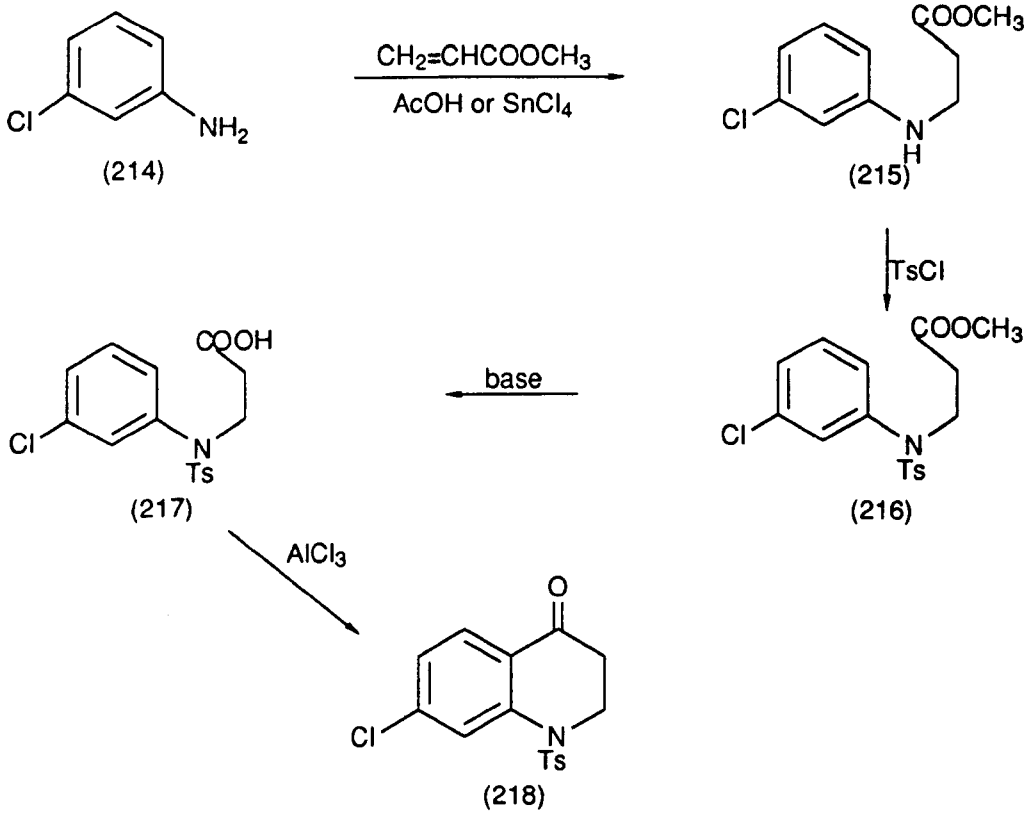
Scheme 43

(g) Some Heterocyclic Ketones: Quinolones, Benzazepinones and Benzazocinones.

As discussed previously, methodology from this laboratory was planned to be incorporated into this project, as illustrated by the use of 2-benzosuberone (192). This was now to be expanded to include the use of some heterocyclic ketones, namely quinolines, benzazepinones and benzazocinones. It was hoped that if these ketones could successfully take part in the cyclodehydration reaction, and the biological results of the products showed promise then, from previous work in these laboratories, there would be access to an extensive range of these heterocycles for further investigation. Firstly though, the syntheses of a few products were to be

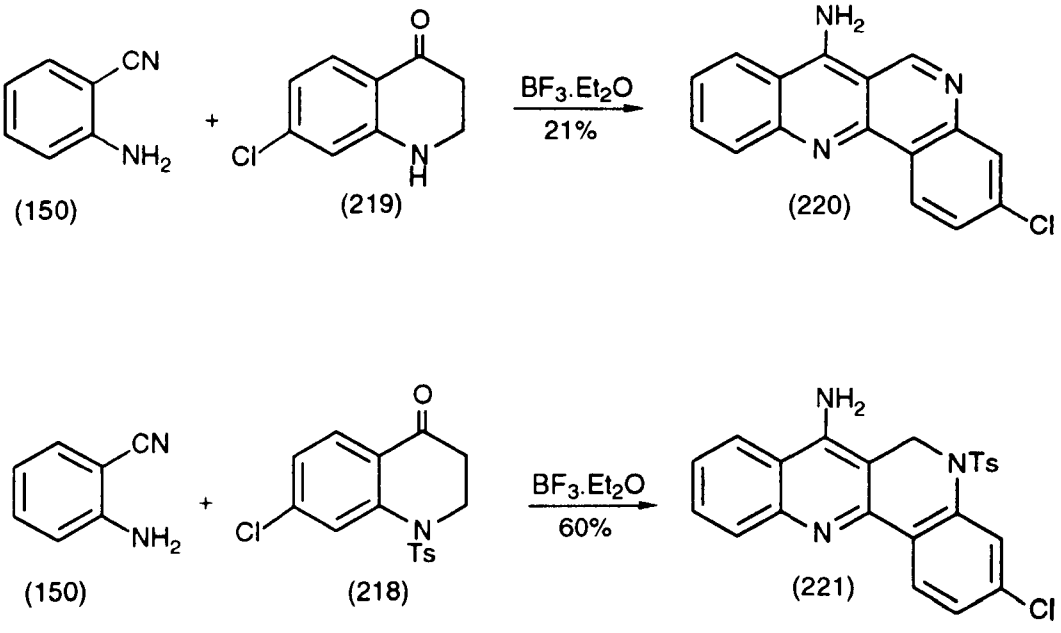
attempted in order to gain knowledge as to the facility of reaction shown by these heterocycles, and to obtain a general picture of their biological activities.

The six-membered heterocycles were examined in the first instance. The quinolones to hand were the 7-chloro-1,2,3,4-tetrahydroquinolone (219) and 7-chloro-N-tosyl-1,2,3,4-tetrahydroquinolone (218). These had been previously synthesised in this laboratory in the manner of Johnson *et al.* [173] *(Scheme 44)*. For example, alkylation of the amino group of 2-chloroaniline (214) with methyl acrylate, protection of the nitrogen with tosyl chloride, base hydrolysis of the ester and an aluminium chloride-mediated intramolecular Friedel-Crafts acylation gave the 7-chloro-1,2,3,4-tetrahydro-N-tosyl-quinolone (218).



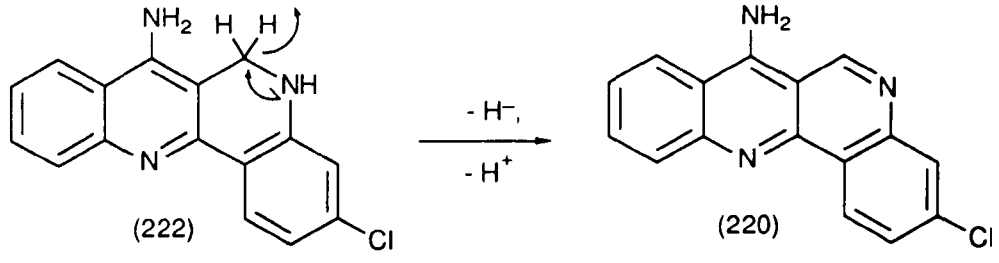
Scheme 44

The two quinolones were subjected to the cyclodehydration reaction with anthranilonitrile and produced two quite different products *(Scheme 45)*.



Scheme 45

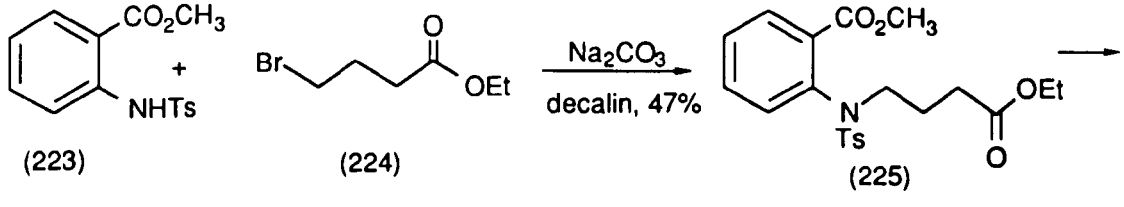
The unprotected quinolone (219) unexpectedly gave the fully aromatic dibenz-[1,6]-[*b*,*h*]-naphthyridine (220). Aromatisation of the expected dihydro compound requires the presence of an oxidising agent to bring about removal of a hydride and subsequent loss of a proton to give naphthyridine (220). This was probably due to air oxidation[174] *(Scheme 46)*.

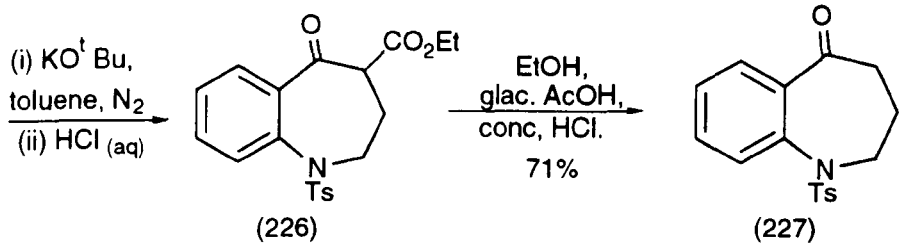


Scheme 46

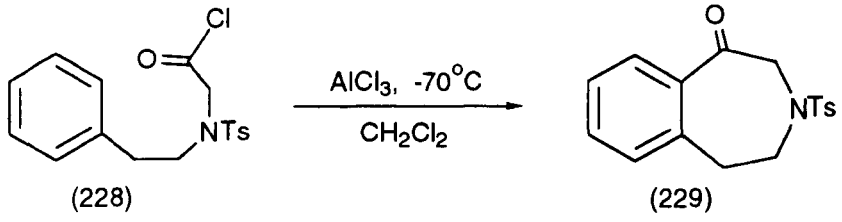
On the other hand, the protected quinolone (218) gave the 1,2-dihydro product (221) as expected, the tosyl group conferring the stability required for the product to exist through its strong electron withdrawing nature.

A range of benzazepinones previously studied in the laboratory became the next ketones to be substituted into the cyclodehydration reaction. The two successful reactions occurred with benzazepinones 1,2,3,4-tetrahydro-1-N-tosyl-[*5H*]-benzazepin-5-one[175] (227) and 1,2,3,4-tetrahydro-3-N-tosyl-[*5H*]-benzazepin-5-one[176] (229) both of which were synthesised in the following manner with methods developed by Proctor and co-workers*(Schemes 47 and 48)*.



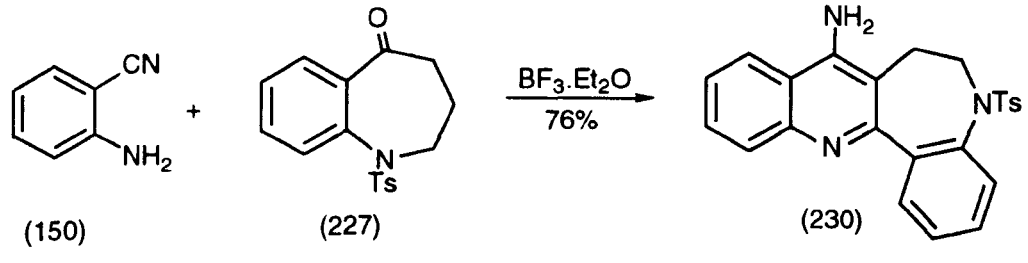


Scheme 47

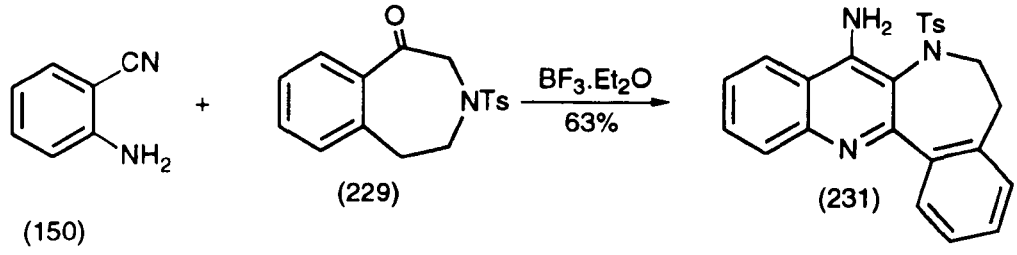


Scheme 48

Using the benzazepinones in the cyclodehydration reaction with anthranilonitrile (150) in the usual manner gave the following products (230) and (231) in good yield*(Schemes 49a, 49b)*.

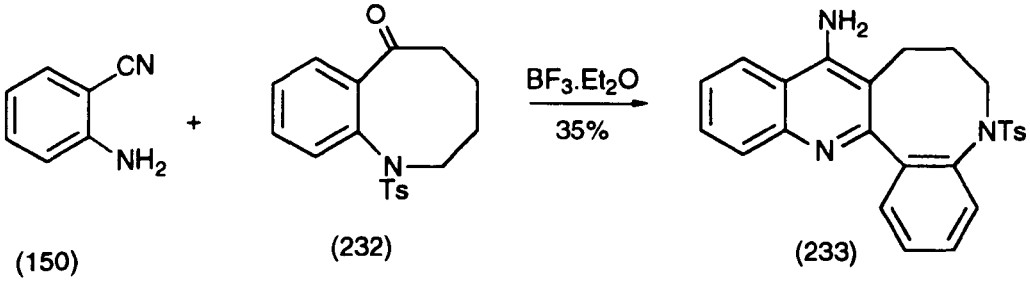


Scheme 49a



Scheme 49b

One eight-membered heterocycle was included in this study; 1,2,3,4-tetrahydro-1-N-tosyl-[5*H*]-benzazocin-6-one (232)[177], the synthesis of this molecule being analogous to that of the 1,2,3,4-tetrahydro-1-N-tosyl-[5*H*]-benzazepin-5-one[175] (227) The benzazocine successfully reacted with anthranilonitrile (150) to give the product 9-amino-5,6,7,8-tetrahydro-5-N-tosyl-[3,2-*b*]-quinolino-[5,6-*e*]-1-benzazocine (233) in 35% yield *(Scheme 50).*

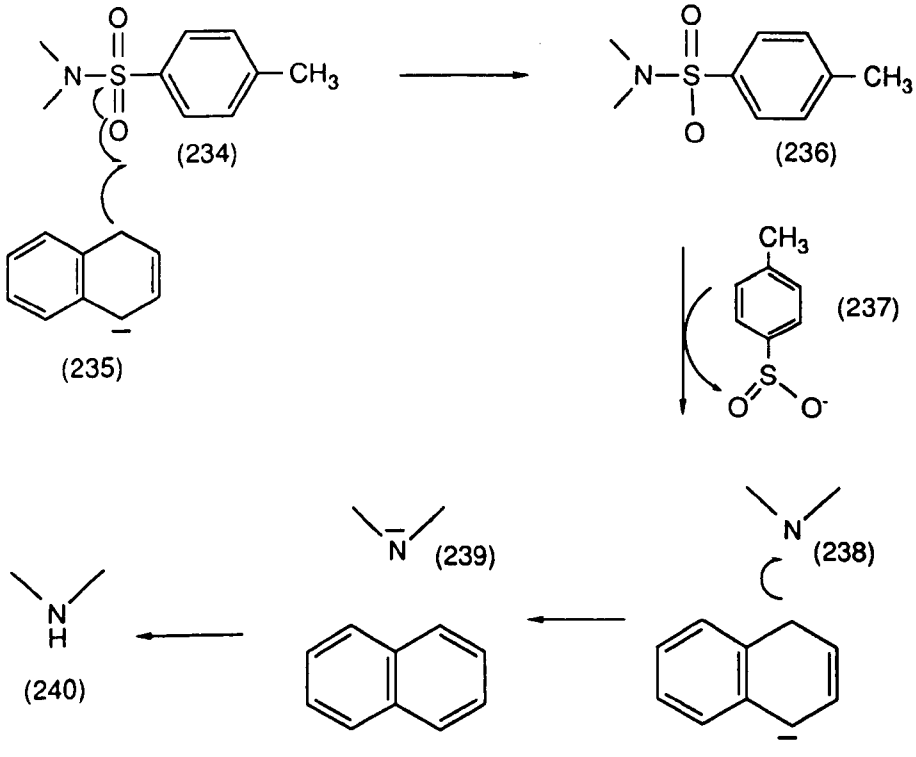


Scheme 50

(h) Detosylation with Sodium Naphthalenide

Previous experience in this laboratory led to the realisation that tosyl substitution on amino moieties conferred too much bulk upon molecules for them to

possess any biological activity so it was decided to remove this protecting tosyl group using sodium naphthalenide. Sodium naphthalenide[178] was conveniently prepared by the addition of pieces of sodium metal to a solution of naphthalene in degassed dimethoxyethane (DME). Stirring under a nitrogen atmosphere produced the radical anion sodium naphthalenide to which was added the tosylated amine, also in a solution of degassed DME. The protected compounds were proposed to be detosylated by the following mechanism *(Scheme 51)*.

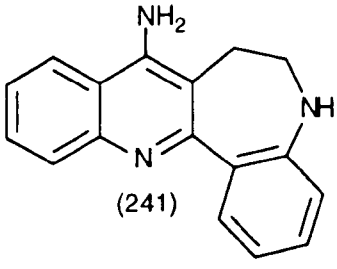


Scheme 51

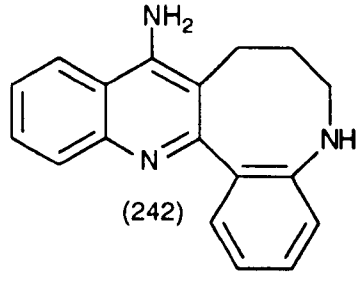
Donation of an electron from the radical anion (235) produces the radical anion of the tosyl moiety (236). This then cleaves to give the toluenesulphinate (237) and the radical becomes resident on the nitrogen (235). Accepting another electron

from the naphthalenide then gives an anion (239) which is protonated to the secondary amine on acidic work-up (240). Sodium naphthalenide is also used in this manner to deprotect tosylated hydroxyl groups, *e.g.* in carbohydrate chemistry[179], although diprotected vicinal diols may give the corresponding alkene on this treatment[180].

By treating the heterocyclic products with sodium naphthalenide the following amines were obtained (241) and (242). Detosylation could not be achieved with compound (229) which returned only starting material.

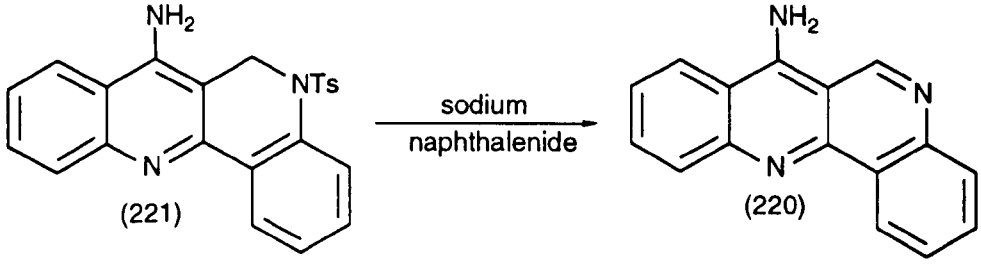


(241)



(242)

Interestingly, when detosylation was attempted with the 1,2-dihydrodibenznaphthyridine (221) the product was again the fully aromatic dibenznaphthyridine (220). Loss of the stabilising influence of the tosyl group obviously promoted the aromatisation *(Scheme 52)*.



Scheme 52

Chapter 6

PHARMACOLOGICAL RESULTS AND CONCLUSIONS

(a) Methods of Determining a Pharmacological Action on Cholinergic Transmission

(i) The Neuromuscular Junction

There exist many methods of assay which investigate the effects of drugs on cholinergic function and the preliminary findings on the compounds synthesised by this project were obtained using the following standard pharmacological experiments. One of the most straightforward ways to determine effects on the cholinergic system is to study the neuromuscular junction. Transmission between nerve and skeletal muscle utilises acetylcholine and although the receptor type present on the muscle membrane is nicotinic, this was not too important as the study was mainly interested in the inhibition of acetylcholinesterase.

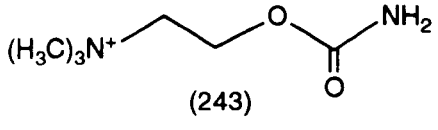
The muscle used for these assays was the chick biventer cervicis which was connected to a pen recorder and placed in an organ bath containing a physiological salt solution. Any response due to induced stimulation would then be recorded by a trace on graph paper as the muscle responded accordingly. The muscle was stimulated in four different ways in the presence of each of the compounds in order

to be able to compare the control results where responses to the stimuli are recorded with no drugs present.

1. The first assay concerns the application of a current which stimulates the nerve to release its neurotransmitter, causing the muscle to contract. This is termed indirect stimulation and mimics the effects of an action potential. Any action that a drug has on the release or action of ACh can then be monitored by this assay. An anticholinesterase action would show as an augmentation of the response of the muscle.

2. The second assay uses administration of ACh (10^{-3} M) to produce a response from the tissue. Again, an anticholinesterase action would augment the response of the muscle.

3. Thirdly, a drug called carbachol (243) may be used.



(243)

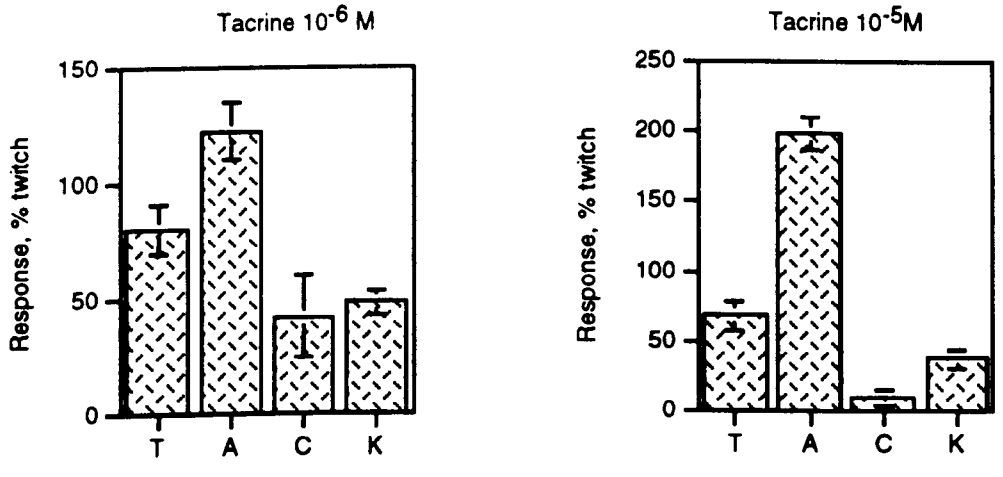
Carbachol is the amide analogue of ACh and possesses a similar potency but it is not readily hydrolysed by cholinesterase enzymes. The assay is used to test the ability of a compound to block ACh receptors. If a drug is found to have this action, carbachol would not produce its characteristic effect of stimulating the

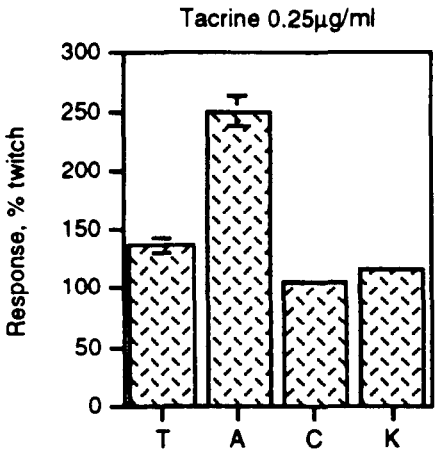
muscle. If the drug has no action in this area, the results would be no different from the control.

4. Fourthly, the potassium ion may also be used to produce a response by depolarising the synaptic membrane. However, a high concentration of potassium ions is used in this instance to remove the membrane potential and hence determine the effects of any new drug on muscle contractility by enhancing the neurotransmitter release in the manner of 4-aminoquinoline (8) (see *chapter 2*).

(ii) The Pharmacological Effects of Tacrine

Figure 6: Graphs demonstrating the effects of tacrine, at differing concentrations ($10^{-6}M$, $10^{-5}M$, 0.25μg/ml), on the chick biventer cervicis muscle.





[T = Twitch response - indirect stimulation
A = Acetylcholine (10^{-3} M)
C = Carbachol (2×10^{-5} M)
K = KCl (K^+ 4×10^{-2}M)]

The effect of tacrine is demonstrated by the graphs in *figure 6* above at different concentrations of the drug. It can be seen that the greatest effects were with the administration of ACh indicating a powerful anticholinesterase action. Augmentation of the twitch response is also seen but there is no effect on the carbachol or potassium ion assays indicating that tacrine, at least at these concentrations does not block nicotinic receptors nor enhance the release of ACh.

(iii) Using Rat Brain Tissue to Determine Effects on Brain Chemistry

Rat brain preparations were used in this series of pharmacological assays to determine four different actions - acetylcholinesterase and butyrylcholinesterase inhibition[181,182, 183] and the inhibition of 5-HT and noradrenaline uptake[184, 185].

This series of tests was used to define directly the anticholinesterase capabilities of each drug and also to investigate the possibility of alternative actions on brain tissue.

Again, taking tacrine results as illustrative of the kinds of values expected from its analogues, it can be seen that fairly low concentrations of tacrine are required for the inhibition of acetylcholinesterase and butyrylcholinesterase, both of the free amine and the maleate salt. However, fairly high molarities are required for tacrine to have an effects in the monoamine assays, although it will be seen that these are not the highest values that would be discovered. This indicates the monoamine uptake inhibition of tacrine in higher doses as discussed in *chapter 3*.

Table 1: Values obtained with tacrine in the rat brain assays.

	IC_{50} μM (± SEM, n = 5)			
	AChEase	**BuChEase**	**5-HT**	**NA**
Tacrine	0.078 (0.0078)	0.025 (0.0016)	7.6	6.7
Tacrine maleate	0.094 (0.0045)	0.028 (0.0038)	10.6	1.8

(b) Preliminary Pharmacological Findings

The following results were obtained for the compounds described in *chapter 5*. The majority were subjected to both the muscle response and the rat brain assays, although latterly only the rat brain assay was used.

Key to biological results. The figures comprise of the graphs from the chick muscle assays, the tables describe the figures obtained from the rat brain assays.

Figures 7, 8; table 2 - **Group 1** compounds are the compounds investigating the variation of ring size.

Figures 9, 10; table 3 - **Group 2** compounds are bridged and known compounds,

Figures 11, 12, 13 and 14 , table 4 - **Group 3** compounds investigate ring size with a fused benzene ring.

Table 5 - **Group 4** compounds investigate the presence of a heteroatom.

Figure 7 - **Group 1:** Effects of compounds (**10^{-5}M**) on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation (**T**), acetylcholine (**A, 10^{-3}M**), carbachol (**C, 2 x 10^{-5}M**), and KCl (**K, 4 x 10^{-2}M**).

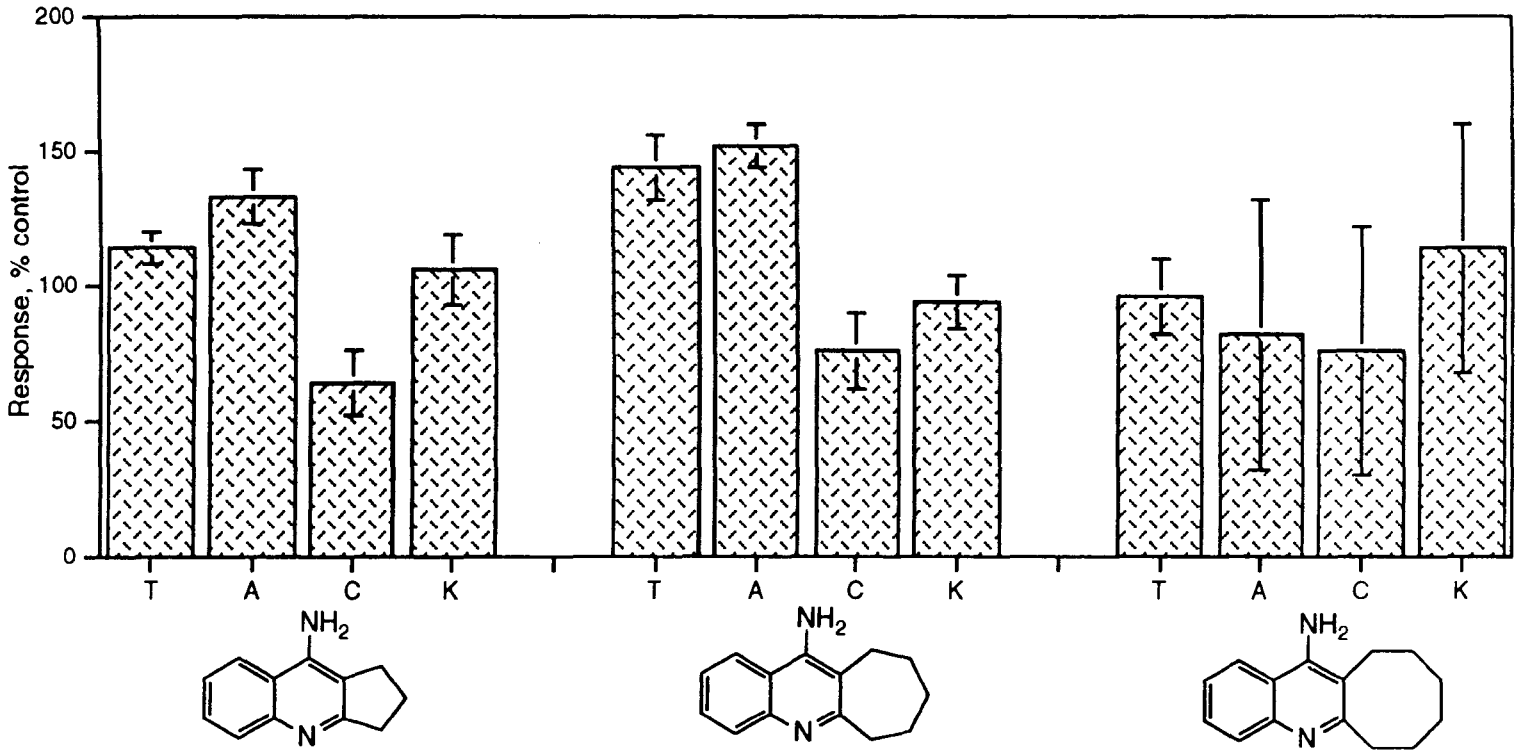


Figure 8 - **Group 1:** Effects of compounds **(10^{-6}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation **(T)**, acetylcholine **(A, 10^{-3}M)**, carbachol **(C, 2 x 10^{-5}M)**, and KCl **(K, 4 x 10^{-2}M)**.

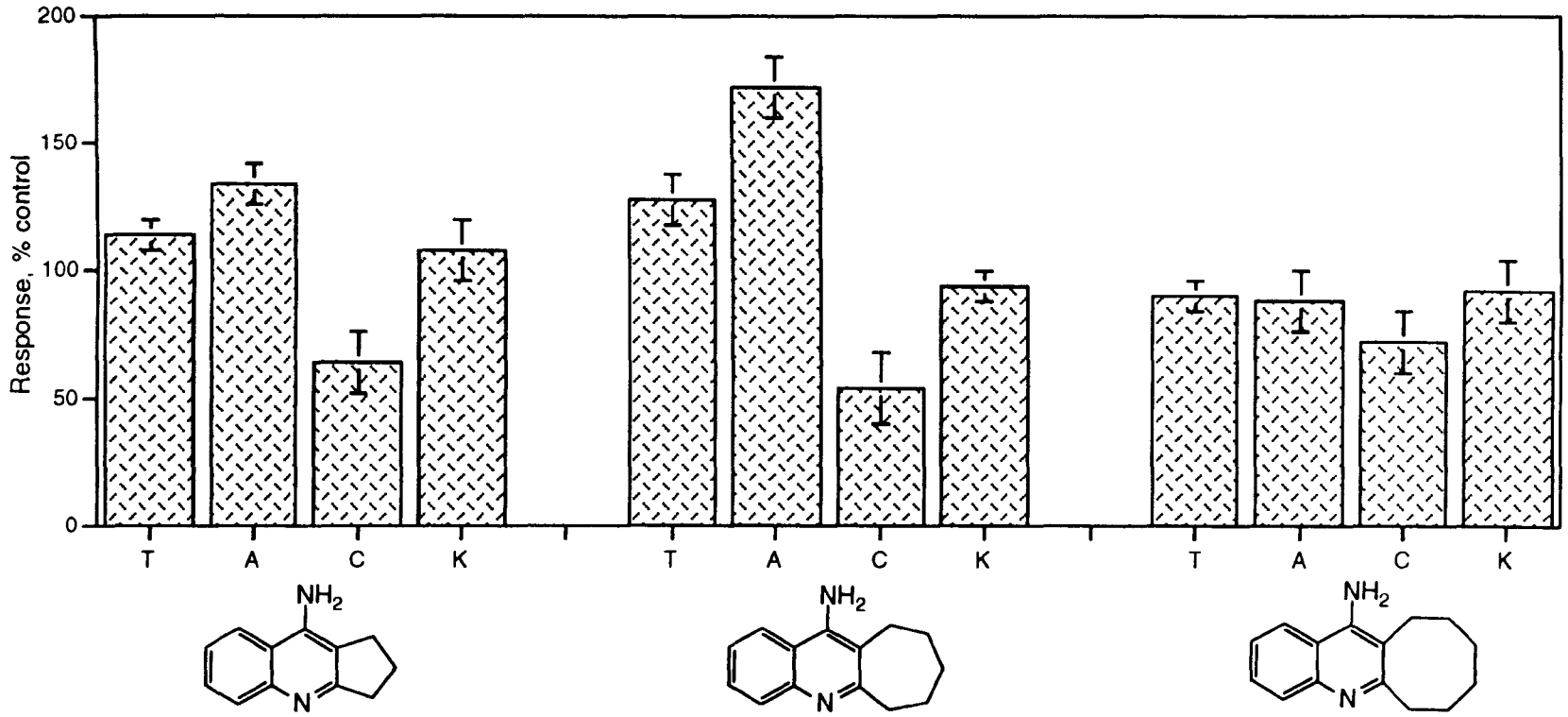


Figure 9 - **Group 2:** Effects of compounds **(10^{-5}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation **(T)**, acetylcholine **(A, 10^{-3}M)**, carbachol **(C, 2 x 10^{-5}M)**, and KCl **(K, 4 x 10^{-2}M)**.

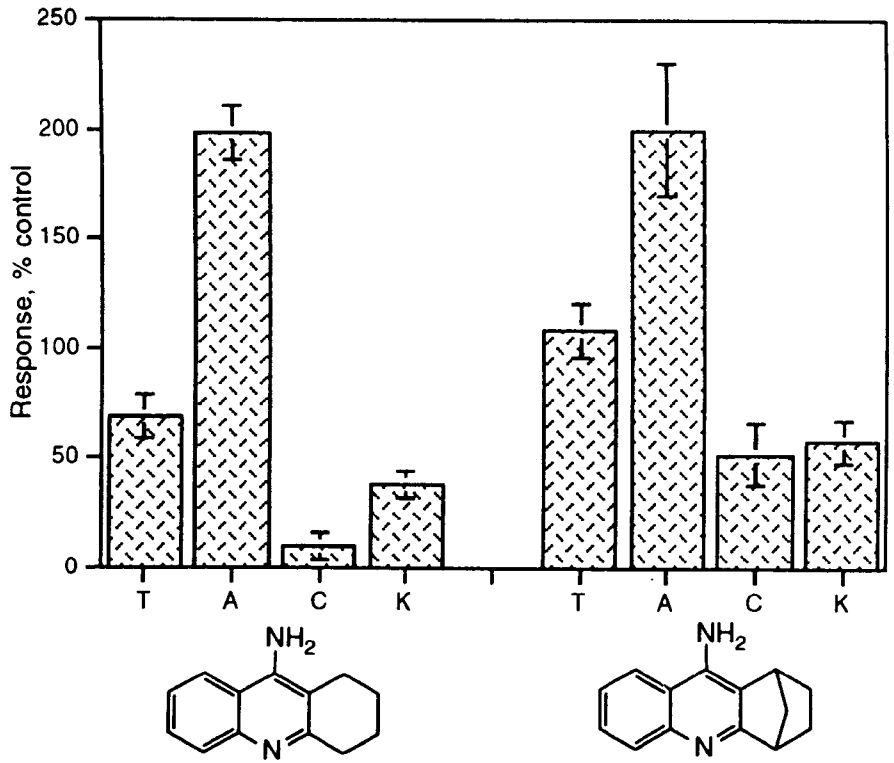


Figure 10 - **Group 2:** Effects of compounds **(10^{-6}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation **(T)**, acetylcholine **(A, 10^{-3}M)**, carbachol **(C, 2 x 10^{-5}M)**, and KCl **(K, 4 x 10^{-2}M)**.

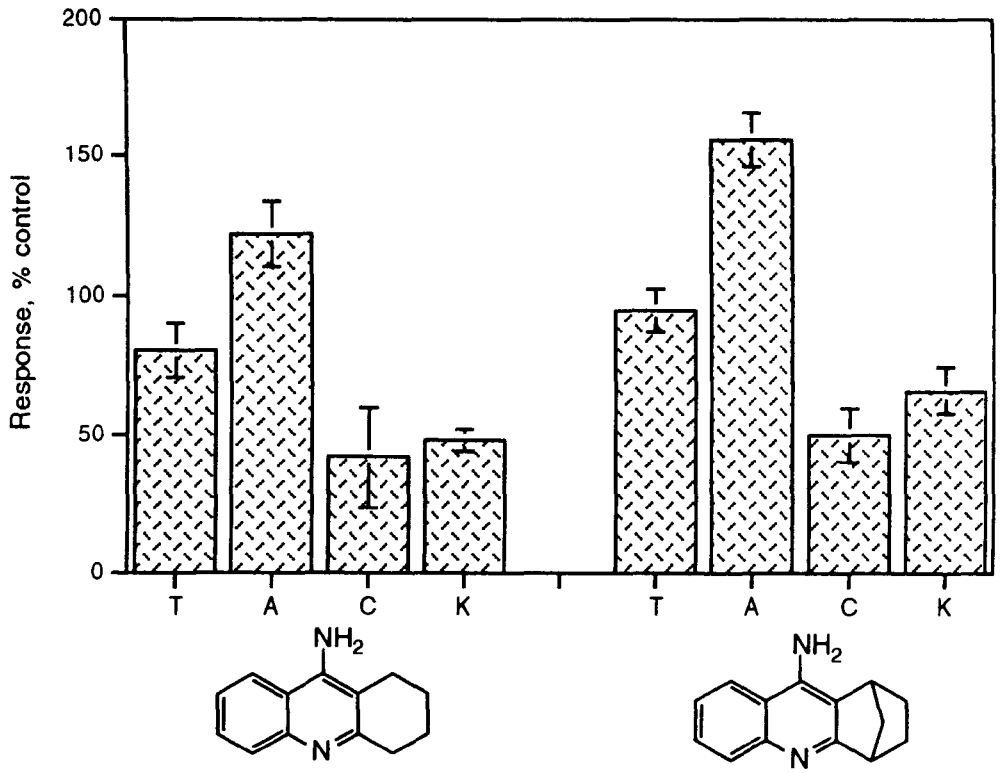


Figure 11 - **Group 3:** Effects of compounds **(2.5 x10^{-6}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation **(T)**, acetylcholine **(A, 10^{-3}M)**, carbachol **(C, 2 x 10^{-5}M)**, and KCl **(K, 4 x 10^{-2}M)**.

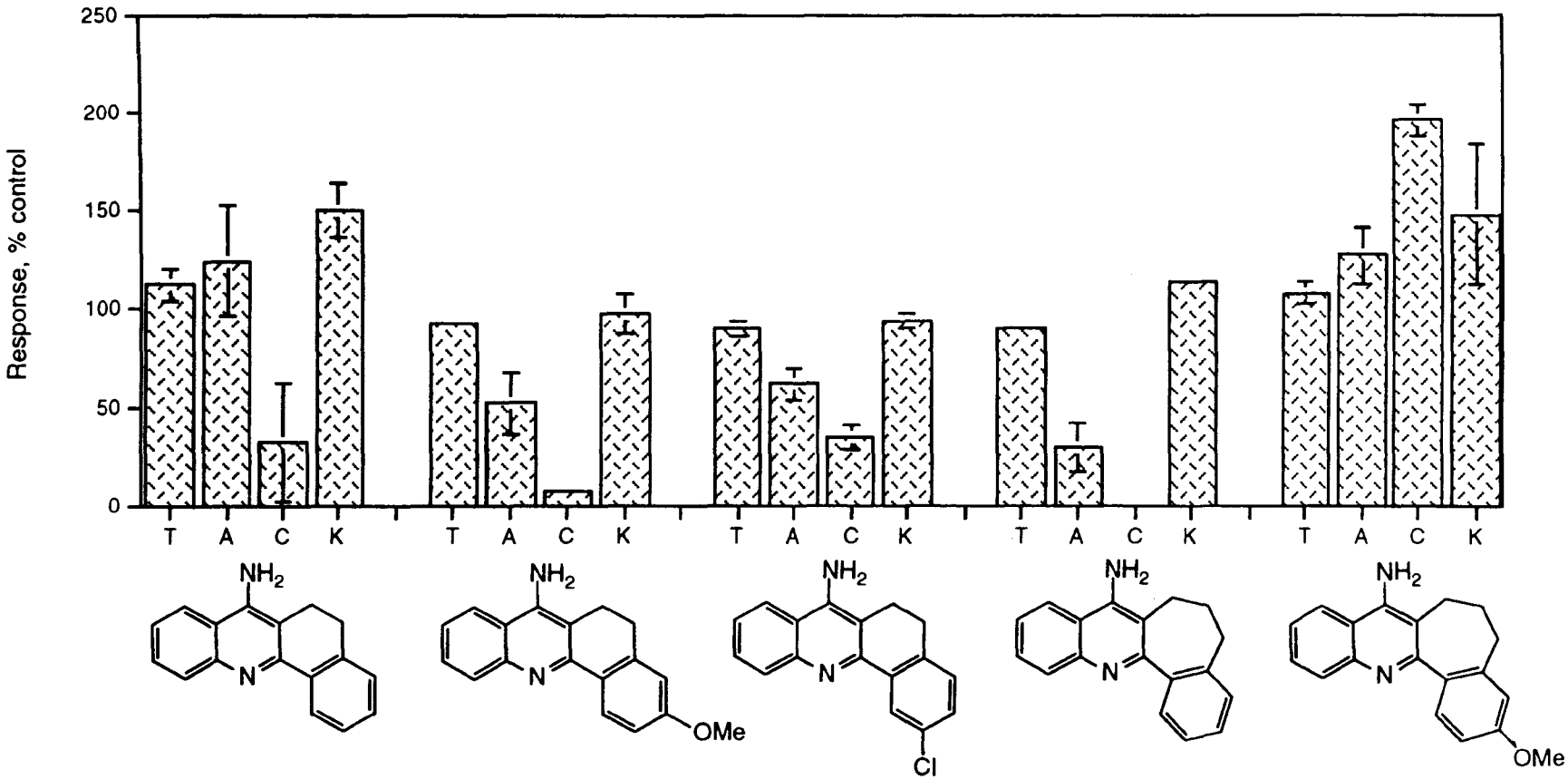


Figure 12 - **Group 3:** Effects of compounds **(0.25 x10^{-6}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation **(T)**, acetylcholine **(A, 10^{-3}M)**, carbachol **(C, 2 x 10^{-5}M)**, and KCl **(K, 4 x 10^{-2}M)**.

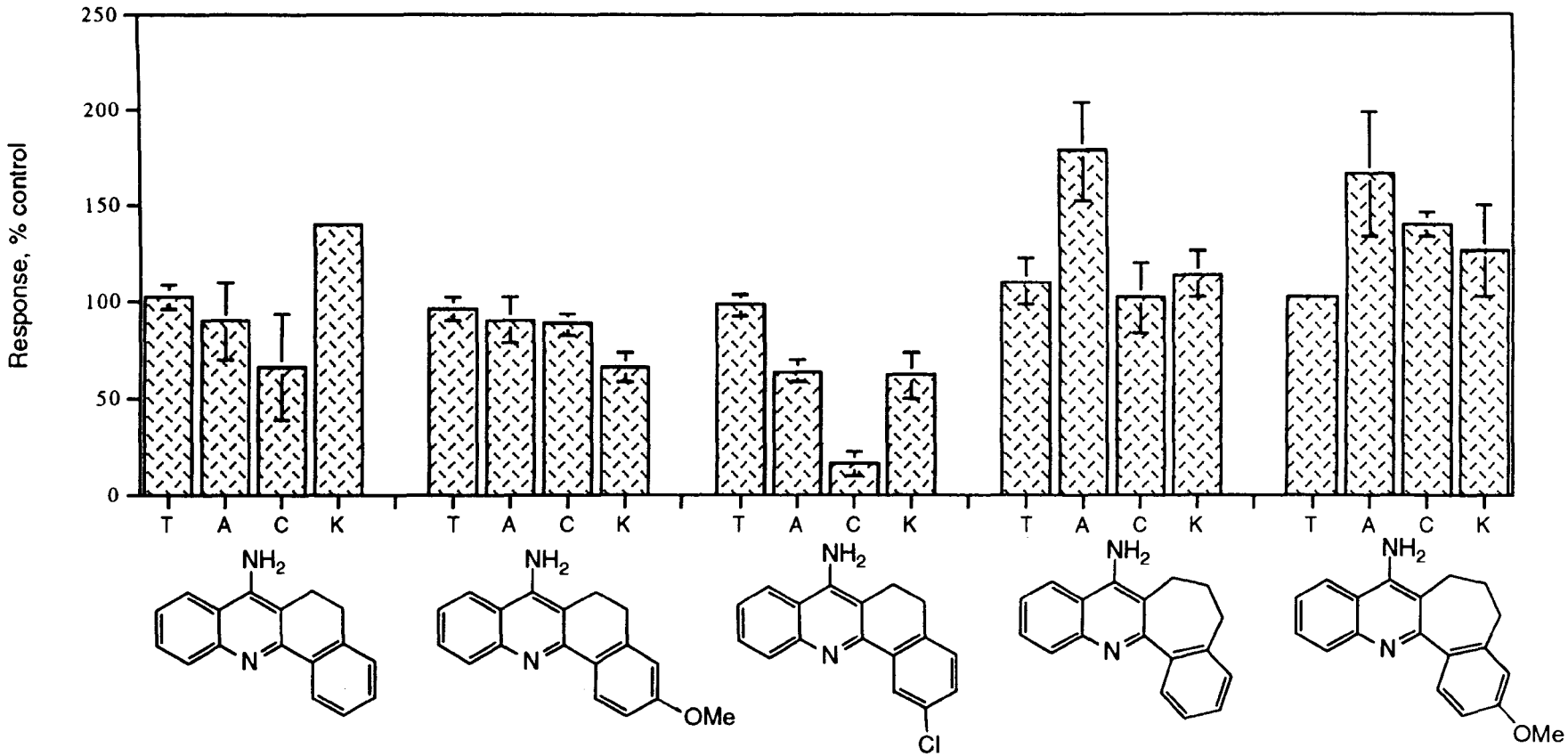


Figure 13 - **Group 3:** Effects of compounds as their **maleate salts (10^{-5}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation **(T)**, acetylcholine **(A, 10^{-3}M)**, carbachol **(C, 2 x 10^{-5}M)**, and KCl **(K, 4 x 10^{-2}M)**.

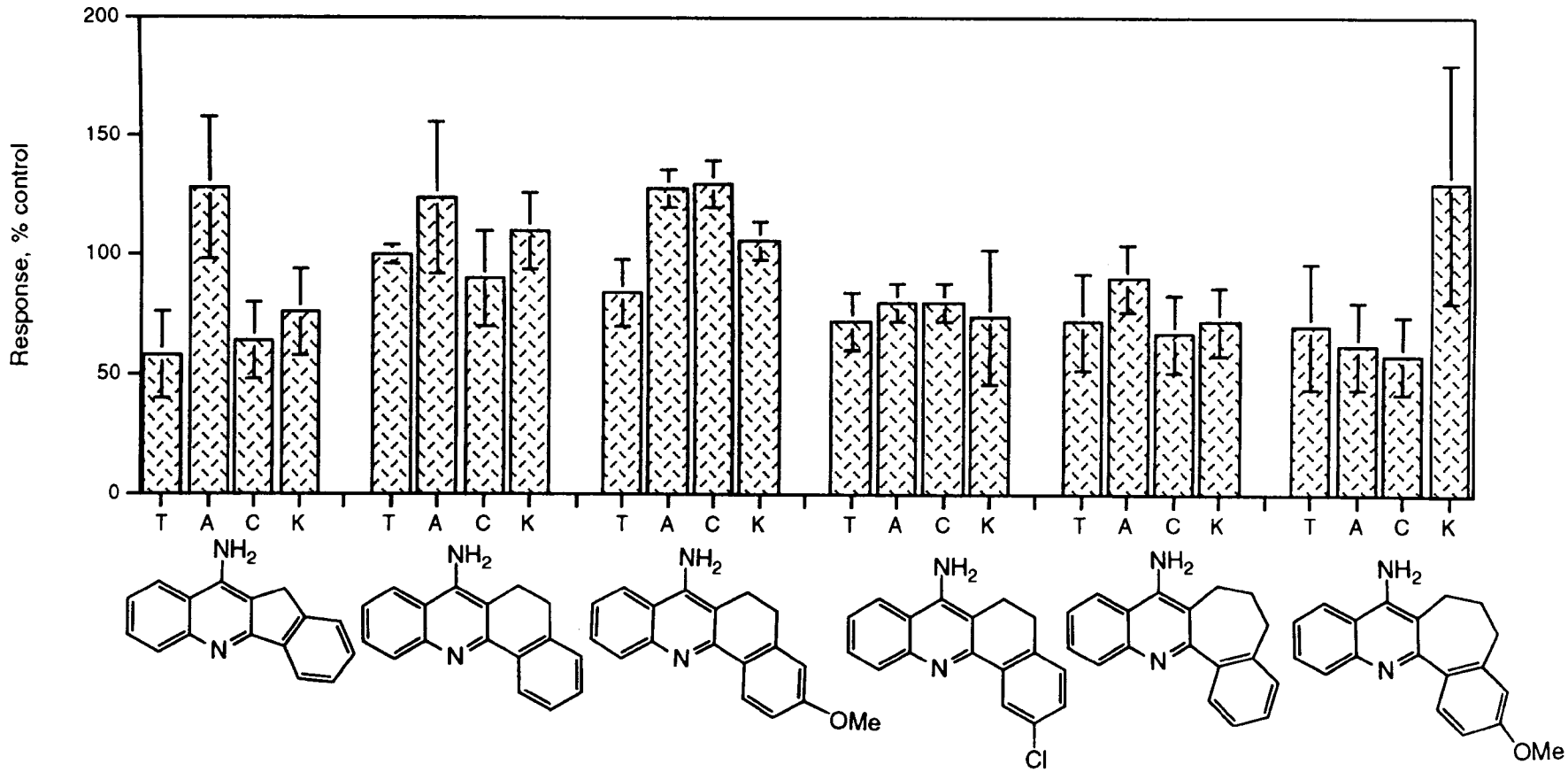


Figure 14 - **Group 3:** Effects of compounds as their **maleate salts (10^{-6}M)** on responses of chick biventer cervisis nerve muscle preparations to indirect stimulation (**T**), acetylcholine (**A**, 10^{-3}**M**), carbachol (**C**, **2 x** 10^{-5}**M**), and KCl (**K**, **4 x** 10^{-2}**M**).

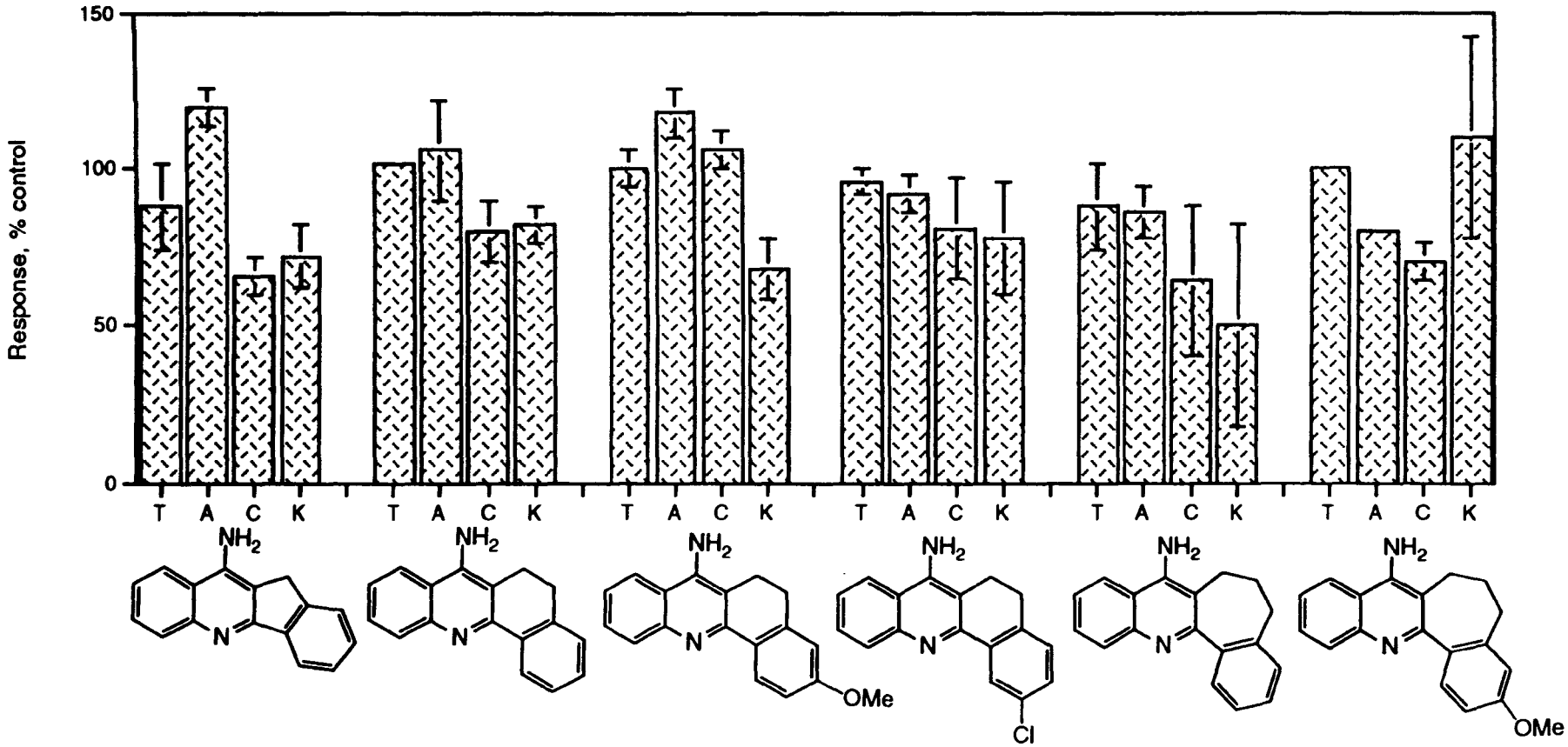


Table 2 - **Group 1**

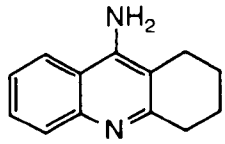
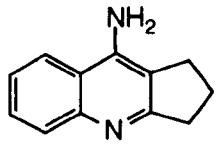
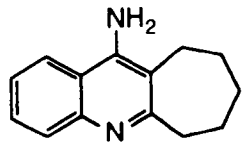
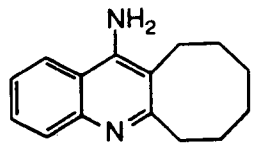
		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(36)		0.078 (0.0078)	0.025 (0.0016)	7.6	6.7
maleate salt		0.094 (0.0045)	0.028 (0.0038)	10.6	1.8
(162)		0.056 (0.0039)	0.052 (0.0082)	21	
maleate salt		0.069 (0.0066)	0.047 (0.0015)	19.2	
(164)	 maleate salt	0.039 (0.0083)	0.014 (0.0015)	3.6	6.9
(166)	 maleate salt	9.1 (0.60)	2.3 (0.079)	1.1	1.8

Table 3 - **Group 2**

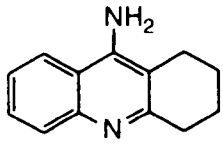
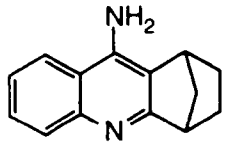
		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(36)		0.078 (0.0078)	0.025 (0.0016)	7.6	6.7
(211)		0.24 (0.032)	0.041 (0.0052)	30.2	6.1

Table 4 - **Group 3**

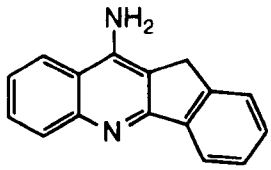
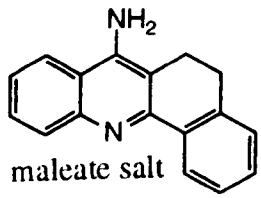
		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(168)	 maleate salt	0.35 (0.059)	3.1 (0.40)	2.2	2.3
(170)	 maleate salt	37.4 (2.9) 7.8 (0.6)	3.3 (0.056) 3.8 (0.60)	4.6 2.0	12.1 13.2

Table 4 continued - **Group 3**

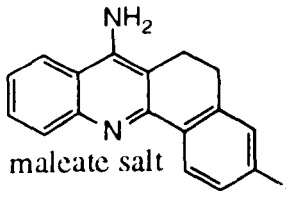
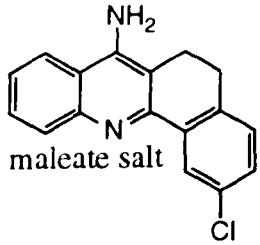
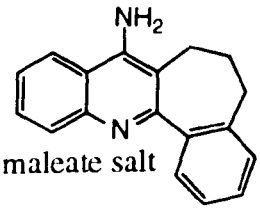
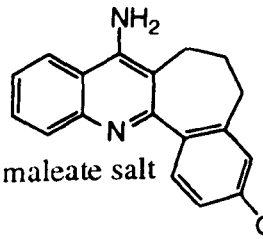
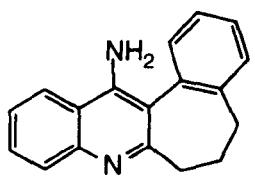
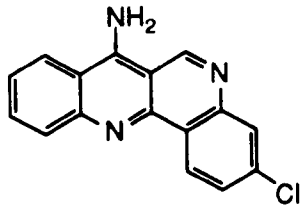
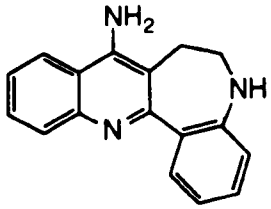
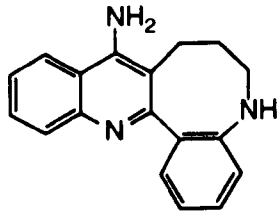
		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(172)		144.1 (7.3)	no block	14.3	41
	maleate salt	17.9 (1.4)	37.1 (8.2)	5.2	6.1
(174)		47.5 (2.9)	10.3 (0.70)	5.8	1.4
	maleate salt	17.1 (0.99)	1.9 (0.15)	2.1	12.2
(176)		166.1 (74.3)	36.0 (9.0)	4.7	
	maleate salt	20.6 (1.6)	4.0 (0.57)	1.0	1.9
(178)		68.8 (4.2)		0.7	3.6
	maleate salt	17.4 (0.094)	34.4 (6.7)	0.02	1.8
(183)		38.2 (8.1)	26.4 (0.44)	0.02	

Table 5 - **Group 4**

		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(220)		no block	no block	>95.2	0.54
(241)	 $2H_2O.2HCl$	69.4 (3.6)	18.9 (2.6)	4.2	1.8
(242)		391.1 (51.3)	6.7 (0.64)	6.9	2.5

(c) Conclusions

Examination of the pharmacological results gave indications of emerging patterns when the results of the tacrine analogues were compared, and hence it was possible to decide upon the types of structural features that were undesirable for biological activity. Below, each group of compounds will be discussed separately.

(i) Group 1

Firstly, studying the effects of changing the size of the saturated ring demonstrated that replacement of the cyclohexyl ring with a cyclopentyl or cycloheptyl ring did

not alter the anticholinesterase activity significantly. There was even an indication that the cycloheptyl analogue was possibly even more potent than tacrine in this respect. The eight-membered compound, however, showed a marked reduction in overall activity and this fact was deemed to be the first conclusion from the pharmacological results *i.e.* a ring of greater size than the cycloheptyl analogue decreased activity markedly. The reason for this was almost certainly due to the increased bulk and flexibility of the cyclooctyl ring, compared to the smaller rings. Conversely, this molecule (166) had the best profile of this group where amine uptake inhibition was concerned.

(ii) Group 2

The effect of inserting a methylene bridge into the tacrine nucleus was to decrease activity in most of the assays (the noradrenaline assay was comparable). The question that arose from this result concerned the attempted preparation of the camphor derived molecule. Would the dimethyl-methano bridge, with its greater bulk decrease the activity even more? If this was found to be the case then a more definite conclusion could be drawn about bridging in that particular area of the molecule in relation to activity. Also, if the two enantiomers of camphor could be used successfully then perhaps an insight might be achieved into the direction (if any) from which this hindrance arises. If activity decreased, bridging would perhaps constitute an overall undesirable feature.

(iii) Group 3

The next group of results concerned the "fusing" of a benzene ring to each of the cyclopentyl, cyclohexyl and cycloheptyl compounds. Some concern was felt over the increased size of the molcules, but the increased lipophilicity and the substitution of the compounds could potentially indicate some areas of interest.

Overall, anticholinesterase activity was reduced in these molecules, with the maleate salt exhibiting a greater activity. This was probably due to the very insoluble nature of the free amines, with the salt being more soluble in aqueous solution, delivering the drug more efficiently to the tissues. However, the results were not encouraging. The tetralone-derived analogues showed an order of anticholinesterase activity: the unsubstituted analogue > 3-methoxy- > 4-chloro-derivative. The benzosuberone derived compounds showed comparable results (to each other) and are intermediate in activity between the tetralones. The indanone compound is the most interesting compound in this series. A much smaller reduction on anticholinesterase activity is balanced with a greater inhibition of amine uptake behaviour than tacrine. This was a significant discovery and raised questions as to how manipulation of the molecule could increase the anticholinesterase activity while still retaining the good 5-HT uptake inhibition. Surveying the amine-uptake inhibition results all the compounds in this group there is a good activity, higher than tacrine. The 13-amino-3,4-benzo-5-methoxy-2,3,4,5-[*1H*]-cyclohepta-[*b*]-quinoline compound (178) shows an excellent activity and was equivalent in this context to the 13-amino-3,4-benzo-2,5-dihydro-[*1H*]-cyclohepta-[*b*]-quinoline (176), its unsubstituted analogue.

This group has indicated that fusion of a benzene group does not enhance anticholinesterase activity, but instead has a much greater effect on 5-HT uptake inhibition.

(iv) Group 4

Insertion of a nitrogen atom into the structure as with the compounds in this group demonstrated a marked reduction in anticholinesterase activity. 5-HT and noradrenaline uptake inhibition results were comparable to the control values.

To summarise:

1. The cholinesterase assays indicated that eight-membered rings, bridging, fused benzene rings and nitrogen atom insertion in this manner were all undesirable.

2. The benzene fused analogues showed very interesting results concerning the 5-HT uptake inhibition assays and this has potential for further investigation.

3. The indanone-derived molecule 6-amino-[*5H*]-[2,3]-benzocyclopenta-[*b*]-quinoline (168) displayed an interesting balance of dual activities in acetylcholinesterase inhibition and monoamine uptake inhibition.

4. The main conclusion was that the compounds most closely related to tacrine *i.e.* the cyclopentyl and cycloheptyl analogues were the most active.

(d) An In Vivo *Study of Tacrine and its Cycloheptyl Analogue*

In vivo studies were carried out to discern the effects of tacrine and 9-amino-2,3,4,5-[*1H*]-cyclohepta-[*b*]-quinoline (164) on learning in mice[186]. In this test each mouse must find its way from a white area of a box to a dark area. The control experiment indicated that on each day for five days the mouse would take less time to locate the dark area as the days progressed. Administration of scopolamine (a muscarinic antagonist used to induce memory loss) on the sixth day meant that the mouse reverted to its original, unlearned time. On the seventh day, without the effects of the scopolamine, the mouse easily found the dark area again.

The results of the control experiment in bar graph indicating the rate of learning by the mouse are shown in *figure 15.*

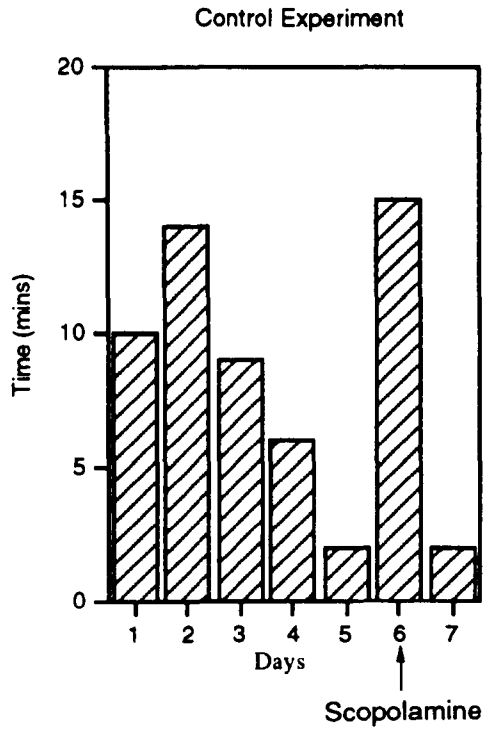


Figure 15: Results of the control experiment for the rate of learning of the mouse.

The investigation focussed on whether or not administration of tacrine and 9-amino-2,3,4,5-[*1H*]-cyclohepta-[*b*]-quinoline (164) would enhance learning in the mouse. From the pharmacological experiments carried out at Strathclyde for this project, it was already known that the 9-amino-2,3,4,5-[*1H*]-cyclohepta-[*b*]-quinoline compound was just as effective an anticholinesterase as tacrine, if not more so. It was therefore expected that the two compounds would have comparable effects.

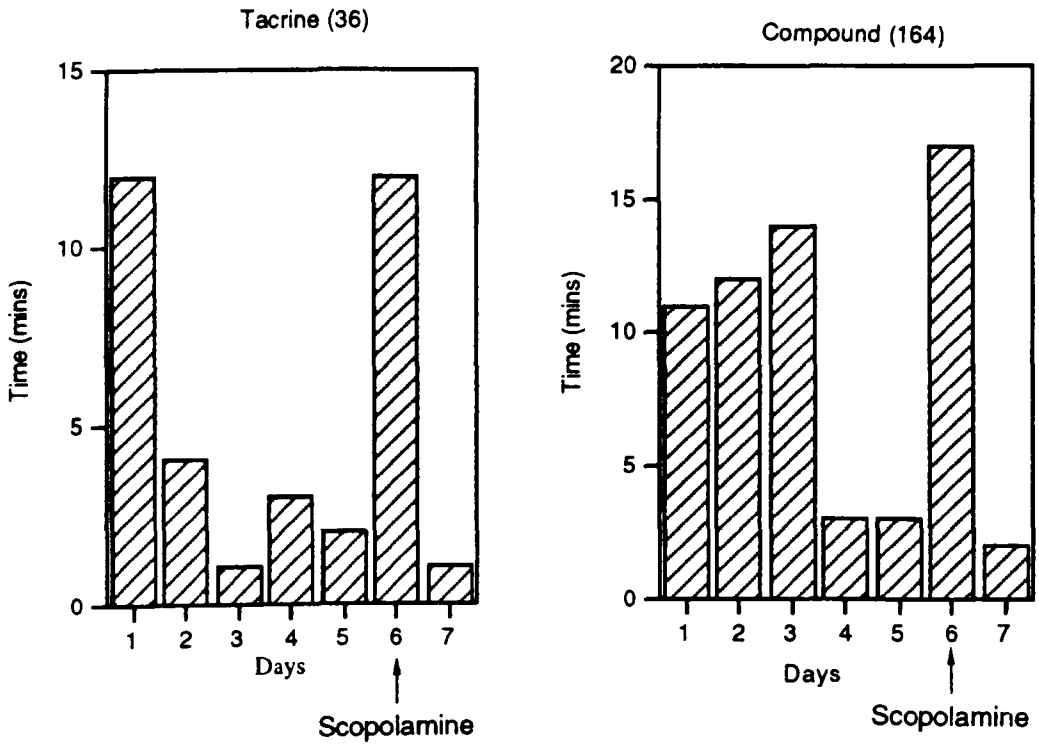


Figure 16: Results of mouse learning expereiment showing the effects of tacrine (36) and 9-amino-2,3,4,5-tetrahydro-[1H]-cyclohepta-[b]-quinoline (164).

However, it can be seen from the results above that tacrine did have a marked effect on the learning ability of the mouse. The location of the dark area was more effectively remembered than in the control experiment until the scopolamine was administered, giving complete reversal of learning. The seventh day saw a return to the learned state.

Surprisingly, this pattern of behaviour was not reproduced with the cycloheptyl derivative. If anything, the learning seemed retarded. This result perhaps gives more evidence towards the growing body of data concerning tacrine and its "extra" effects other than simply acting as an anticholinesterase. The most interesting question surrounds the particular activity of tacrine that exhibits these differences in the *in vivo* experiments which were not apparent in the *in vitro* tests. Perhaps elucidation of the full pharmacological profile of the cycloheptyl analogue

will reveal the pharmacological action in which it is deficient compared to tacrine and, if answers could be obtained, this could have significance in the study of the memory processes and Alzheimer's disease.

Chapter 7

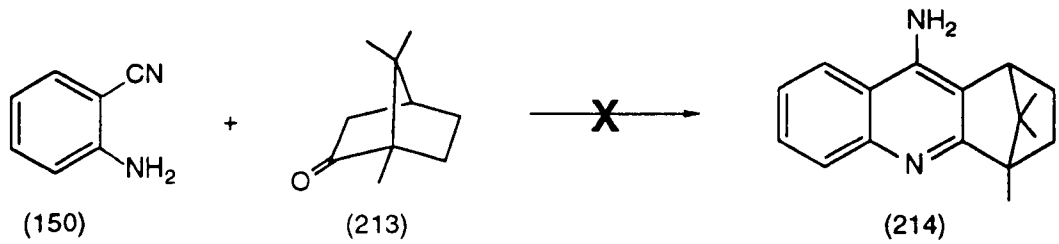
SYNTHESIS OF TACRINE ANALOGUES VIA IMINES AND ENAMINES

(a) Further Design

To design compounds for the latter half of the project, it was now the time to address certain questions that had been raised by examination of the pharmacological results from the ranges of analogues synthesised so far. Firstly, synthesis of camphor analogues would give a greater indication to the desirability of bridging as a structural feature for an anticholinesterase function. Secondly, in order to provide inhibitors of acetylcholinesterase with a potential dual activity in 5-HT uptake inhibition, indanone-derived compounds were designed to investigate this possibility. Thirdly, it was obvious that the most potent anticholinesterase was still tacrine itself or its close analogues, so the use of cyclic ketones was envisaged that would produce molecules of a structure very similar to tacrine but with added functionalisation. Manipulation of this functionalisation was then anticipated to yield more information into the binding of tacrine with the enzyme, for example, with the introduction of a potential hydrogen bond donors or acceptors (*e.g.* amino, carbonyl or hydroxyl groups).

(b) Synthesis of Bridged Analogues

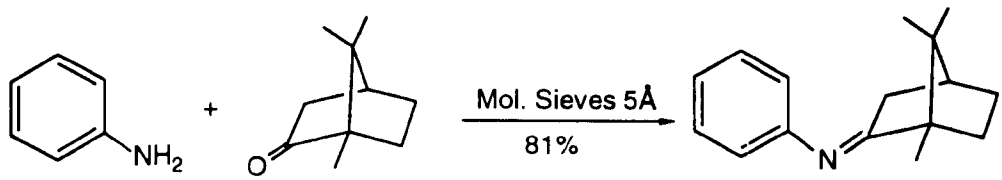
Synthesis of 9-amino-1,4-methano-1,2,3,4-tetrahydro-4,11,11-trimethyl-acridine (214) had been attempted using the standardised method of cyclodehydration between R(+)-camphor (213) and anthranilonitrile (150) (*Chapter 5*), but this had not been successful in any instance *(Scheme 43)*.



(150) (213) (214)

Scheme 43

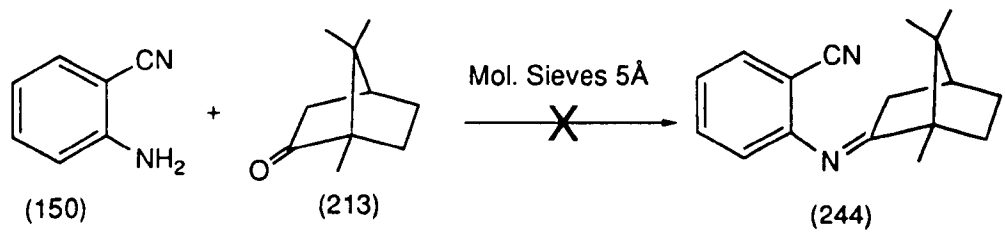
It was then decided that synthesis *via* the intermediate imine would constitute an alternative pathway as it was known that a camphor-derived imine (243) could be produced in good yield from aniline (100) and R(+)-camphor (213) using a very mild method of dehydration, molecular sieves[187] *(Scheme 53)*.



(100) (213) (243)

Scheme 53

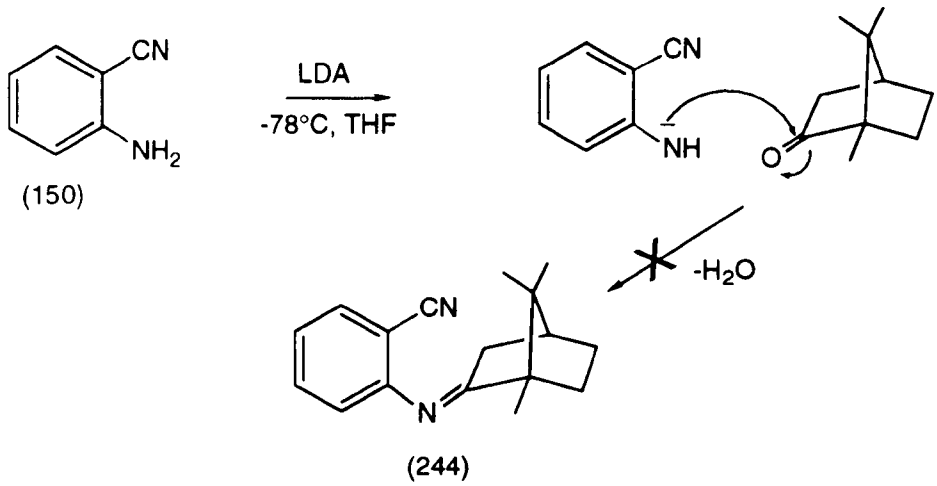
However, substituting the aniline (100) with anthranilonitrile (150) did not produce the desired product (244) and this was attributed to the influence of the nitrile and its electron-withdrawing properties causing a decrease in the nucleophilicity of the amino group to such an extent that no imine could be isolated *(Scheme 54)*. Attempting this procedure with titanium tetrachloride in the manner described by White *et al.*[151] was also unsuccessful.



(150) (213) (244)

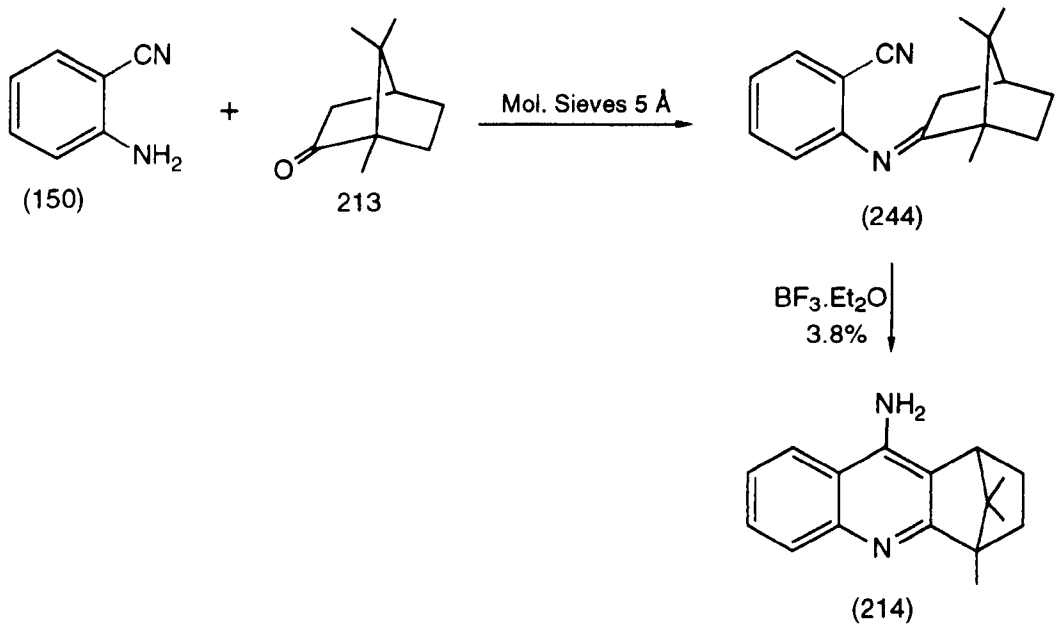
Scheme 54

The next strategy was to utilise a strong, non-nucleophilic base to deprotonate the amino group of the anthranilonitrile (150), turning it into a strong nucleophile, and then adding the R(+)-camphor (213). Lithium diisopropylamide was synthesised in the usual manner[188] by adding *n*-butyllithium to diisopropylamine at -78°C and this base added *via* syringe to the reactants. The reaction was warmed to room temperature and stirred over two days during which time aliquots were removed, quenched with water and analysed by tlc. This showed only the presence of starting materials *(Scheme 55)*.



Scheme 55

One of the problems with the analysis of a camphor imine was the fact that its NMR spectrum was very similar to a mixture of the starting materials and would potentially be identical if contaminated by them. Also, the nature of imines being such that they would be hydrolysed in an acidic medium such as the silica gel used in thin layer column chromatography. Analysis was therefore attempted with alumina tlc plates but indicated only starting materials, which would have masked the presence of the imine anyway. A small sample was analysed by mass spectrometry in an attempt to detect a mass ion that corresponded to the imine. The resultant spectrum returned did indeed have a peak possibly corresponding to the mass of the imine although it also indicated the starting materials present. Being the only tangible evidence for the presence of a small percentage of imine an attempt was made to synthesise the product by removing the molecular sieves after a specific period of time (*i.e.* 1 hour) and, instead of isolating the imine, adding the boron trifluoride diethyl etherate in order to cyclise any imine present. This strategem was successful, and although very low yielding, produced enough of the bridged tacrine analogue for pharmacological testing *(Scheme 56).*



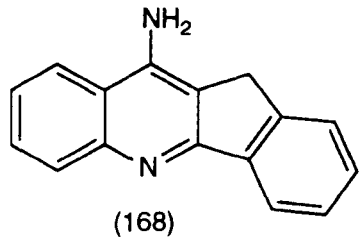
Scheme 56

It should be noted that success was only achieved when the reactants were heated at reflux in the presence of molecular sieves for around one hour. Any longer (or shorter) period of time ensured that the reaction was completely unsuccessful.

R(+)-Camphor (213) was introduced to the project in an attempt to produce information on binding due to its homochiral nature. It was felt that subsequent use of its enantiomer, S(-)-camphor, would further enhance this investigation into the nature of the binding of tacrine-type molecules, altering the position of the methano bridge to the opposite face of the flat 4-aminoquinoline moiety. However, after many attempts it was concluded that this reaction was completely unsuccessful when attempted with S(-)-camphor. No product could be detected, even with the use of a kugelrohr distillation apparatus.

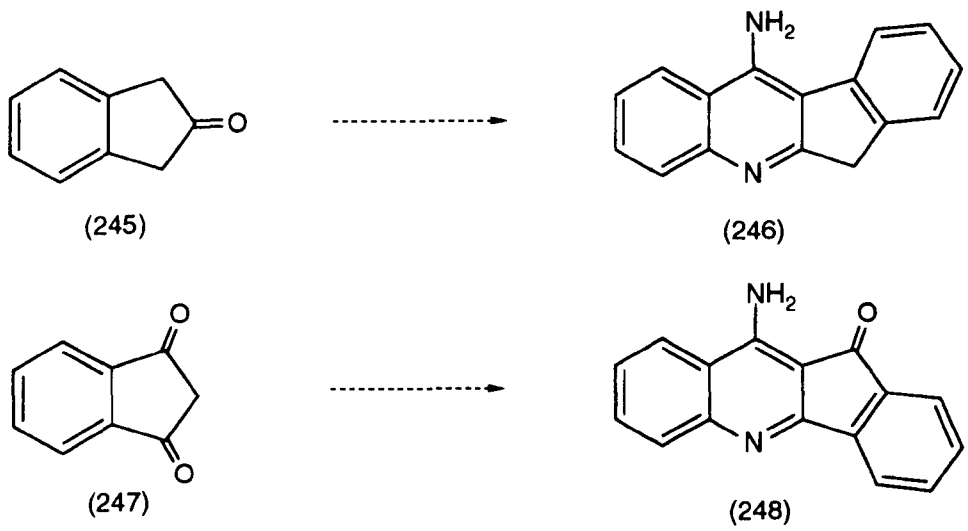
(c) Use of Indanones

With the discovery that the tacrine analogue derived from 1-indanone (168) showed a dual activity in the inhibition of both acetylcholinesterase **and** 5-HT uptake, the next step in this particular area was to consider the possibilities of variation on the basic structure (168).



(168)

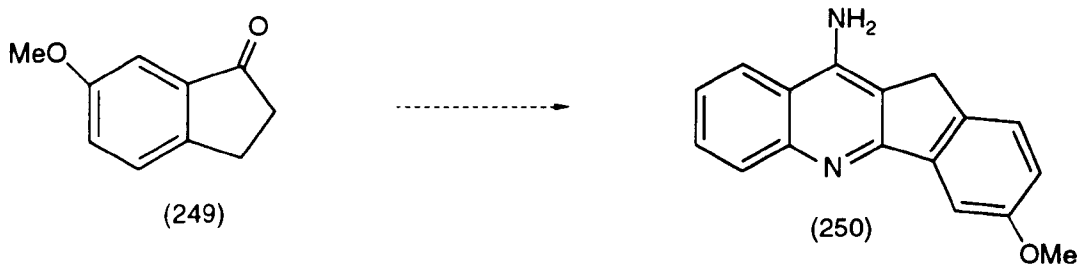
Commercially available indanones were proposed for the preliminary investigations, compounds such as indan-2-one (245), indan-1,3-dione (247) and 6-methoxyindan-1-one (249) shown here with their target analogues *(Scheme 57).*



(245) (246)

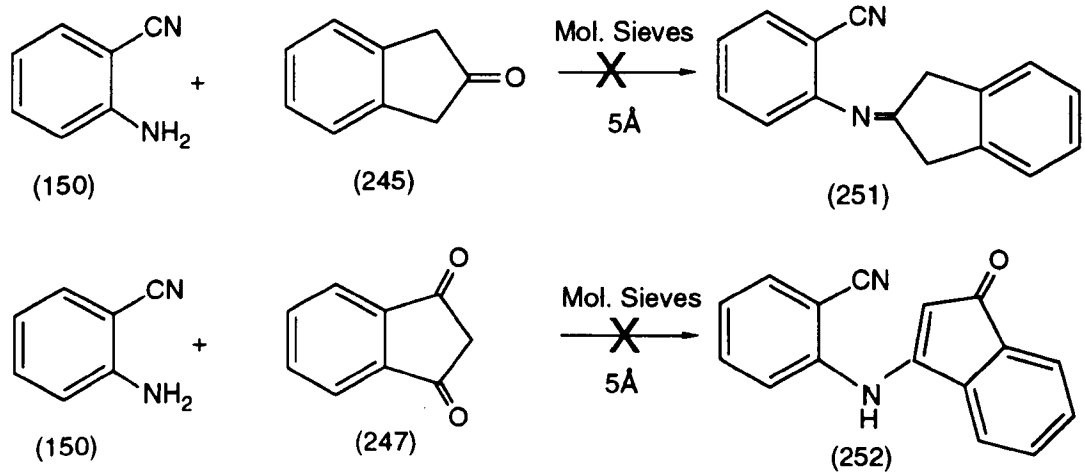
(247) (248)

Scheme 57



Scheme 57 continued

On subjecting the indanone starting materials to the cyclodehydration reaction with anthranilontrile (150), attempts to isolate these products failed. The reactions yielded only decomposition products. Assuming that the indanones required more sensitive handling, particularly in view of the poor yield attained with 1-indanone as described in *Chapter 5*, it was decided that success may be better achieved by again proceeding *via* the imine or enamine intermediates. The mildest method, that of molecular sieve dehydration [187], was attempted with both indan-2-one (245) and indan-1,3-dione (247) but in each case again produced decomposition products *(Scheme 58)*.

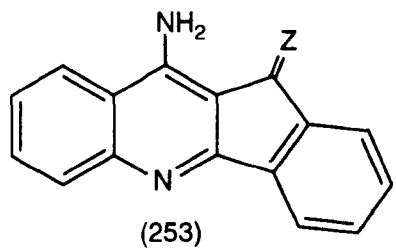


Scheme 58

Attempts to secure the imine from indan-1,3-dione (247) by dehydration was also attempted with the conventional method of acid catalysis *i.e.* *p*-toluenesulphonic acid with the use of a Dean and Stark water separator. Again, the reaction failed and produced only decomposition products.

6-Methoxyindan-1-one (249) was subject to the cyclodehydration procedure in the hope that the electron donating properties of the methoxy group would improve the yield of reaction over the simple indan-1-one. In this instance, using the boron trifluoride method no product could be isolated. It is proposed that the ring activating properties of the substituent were negated by its position on the aromatic ring because no resonance would be achieved which would contribute to the reaction.

Due to time restraints it was decided to abandon the use of indanones themselves and approach the construction of these types of tacrine analogues from another angle. The wisdom of this decision was confirmed by the discovery of some Polish work which contained similar indan-1,3-dione-derived products (253)[189].



(253)

e.g. R = $MeNH_2$, Z = O

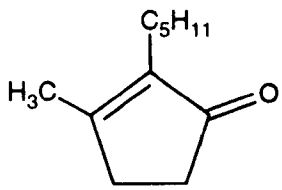
(d) Use of Cyclotene

With the stated aim of designing compounds very closely related to the tacrine nucleus but with additional functionalisation attention turned towards a cyclopentenone derivative 2-hydroxy-3-methyl-cyclopent-2-enone (254a) or, envisaged as the minor tautomer, 3-methylcyclopentan-1,2-dione (253b). The trivial name for this compound is cyclotene and it exists 100% in the enolic form[190] *(Scheme 59).*

(254a) (254b)

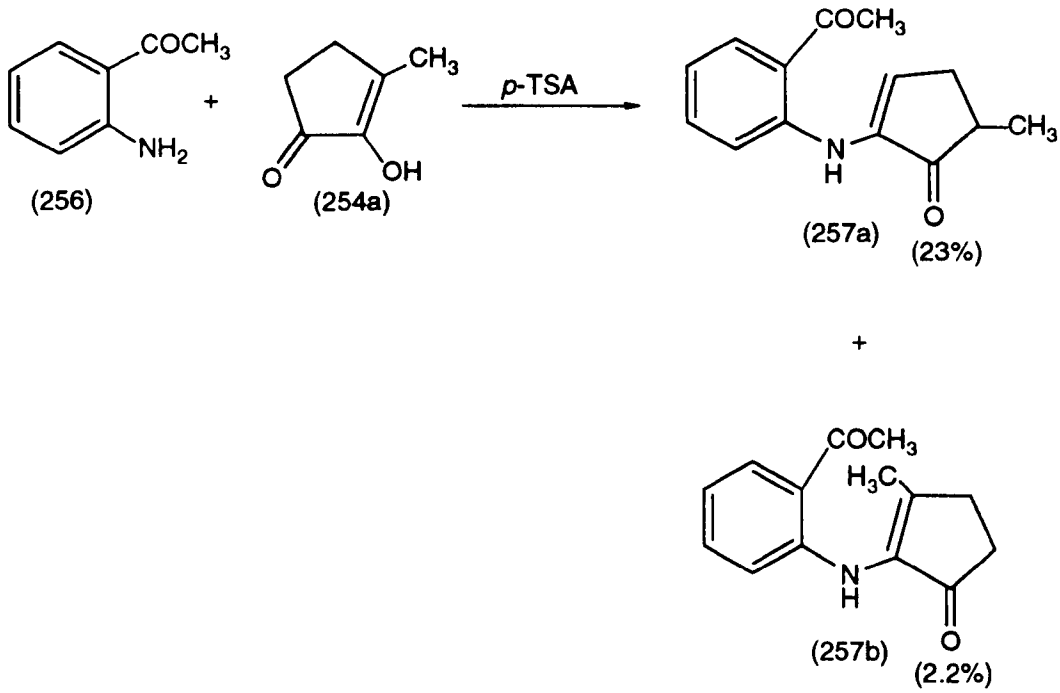
Scheme 59

Cyclotene is found in several natural sources[191,192,193,194] and was used as a starting material in the synthesis of dihydrojasmone (255) in the 1960's[195,196].



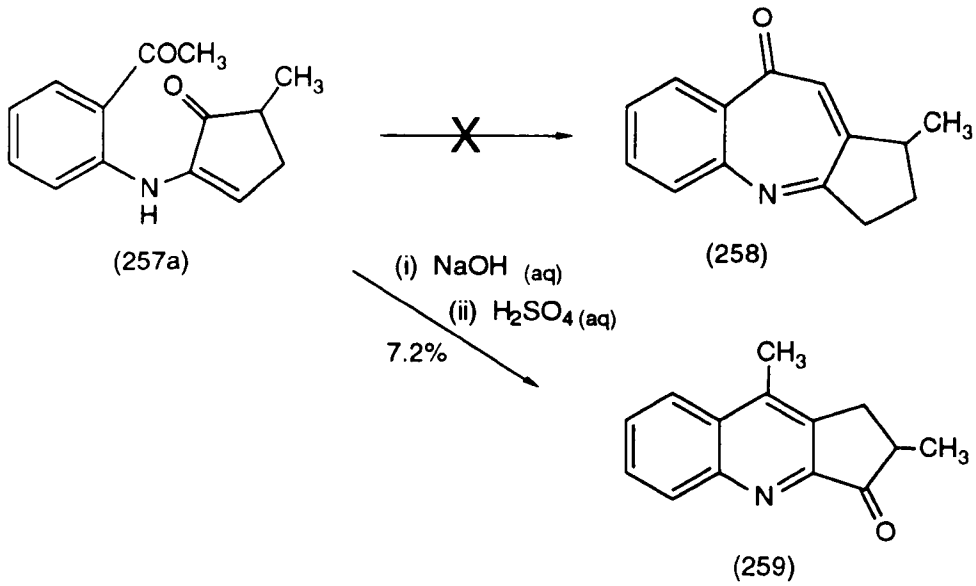
(255)

In 1970, Proctor *et al.* utilised cyclotene as a starting material in the attempted syntheses of an azatropone (258) *via* an enamine intermediate (257)[197] *(Scheme 60).*



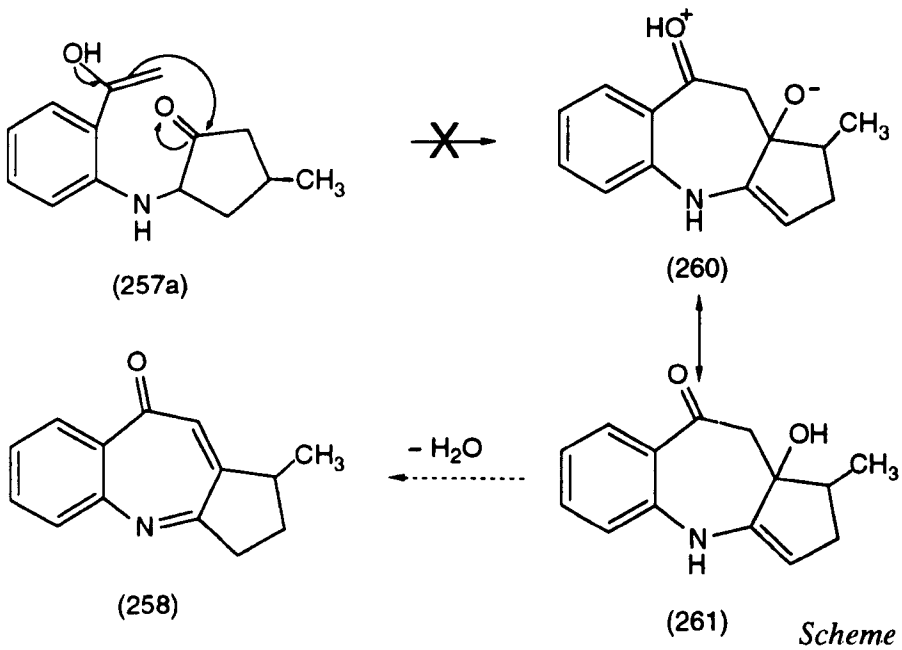
Scheme 60

The enamine was prepared from the reaction of cyclotene (254a) and *ortho*-amino-acetophenone (256) in an acid-catalysed dehydration reaction. Proctor *et al.* had hoped that an intramolecular aldol condensation would occur with the enamine to give the desired azatropone (258) but the product that was obtained from the reaction did not correlate with the expected spectroscopic data. Further analysis indicated that a different cyclisation had taken place *(Scheme 61).*

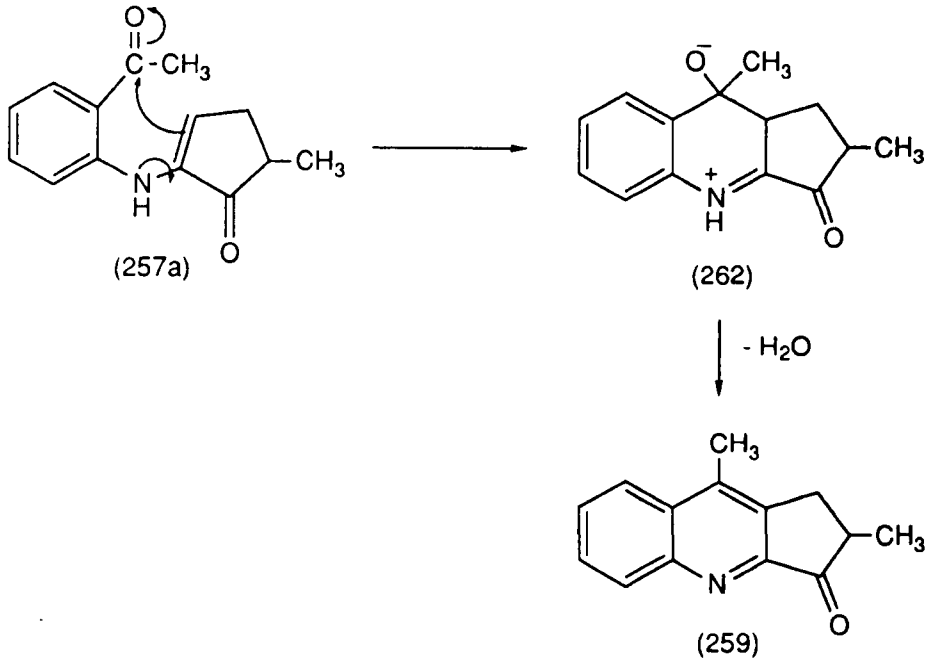


Scheme 61

Presumably, instead of the aldol condensation occurring between the acetyl group and the carbonyl of the cyclotene moiety *(Scheme 62)* , the double bond contained within the cyclotene moiety had attacked the acetyl carbonyl group instead, subsequent dehydration producing the fully aromatic quinoline (259) *(Scheme 63)*.



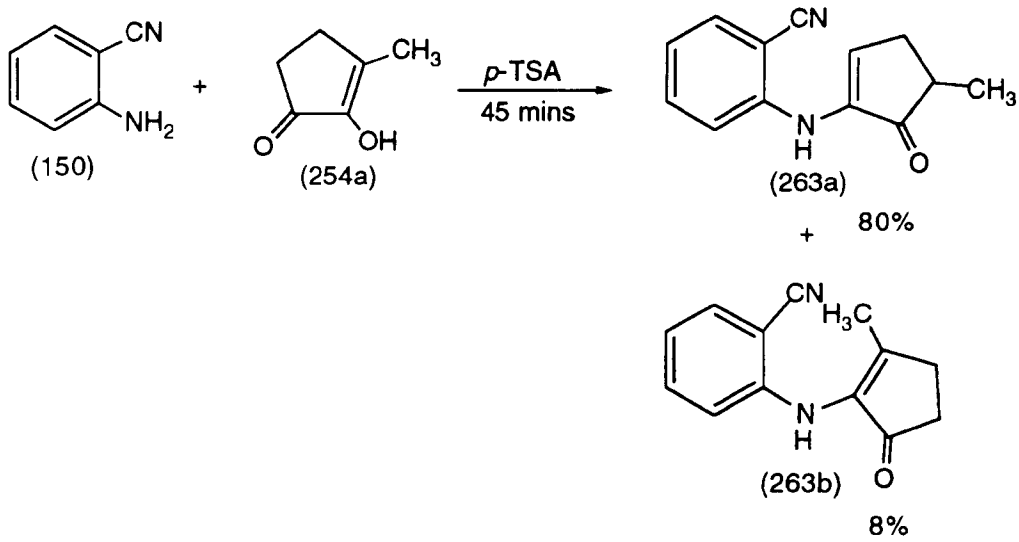
Scheme 62



Scheme 63

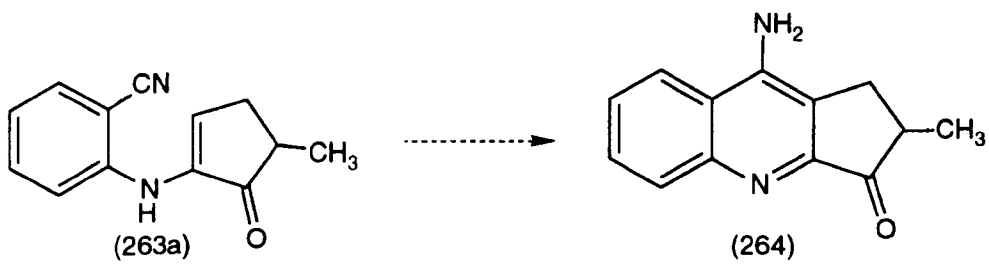
It can be seen that compound (259) is similar to the type of molecule that was required for this project. Visualising the replacement of the methyl group with an amino group there would exist a tacrine isomer with the additional functionalisation of the carbonyl group which could be subjected to extensive manipulation in the search for potent anticholinesterases.

The first step, then, was to attempt the preliminary dehydration reaction substituting the amino-acetophenone (256) with anthranilonitrile (150) *(Scheme 64)*. This was achieved by heating the reactants at reflux for 45 minutes in the presence of catalytic *p*-toluenesulphonic acid with a Dean and Stark water separator.



Scheme 64

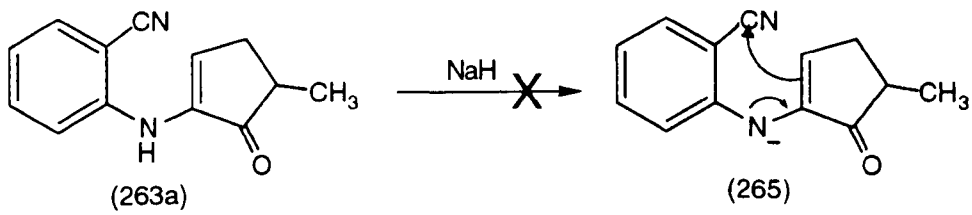
The resultant dark red solution yielded a dark brown oil on evaporation of the solvent and tlc analysis indicated the presence of two products. After purification by column chromatography the desired enamine intermediate (263a) was isolated in an excellent 80% yield, the other isomer (263b) present in an 8% yield. What was now required was the cyclisation of this enamine to produce a compound that could be further modified *i.e.* the cyclopenta-[*b*]-quinoline (264) *(Scheme 65).*



Scheme 65

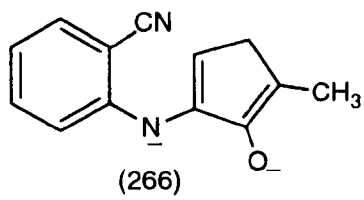
The further transformations that were envisaged for this cyclised product would only be worthwhile if it was possible to produce reasonable quantities of the enamine initially, and so began the search for the appropriate reagent to effect cyclisation.

Using the boron trifluoride method, and the zinc chloride diethyl etherate were both unsuccessful, but allowed recovery of the enamine due to its very stable nature. Sodium hydride was then utilised in an attempt to promote cyclisation but this failed, even after the addition of a further equivalent of the reagent *(Scheme 66).*



Scheme 66

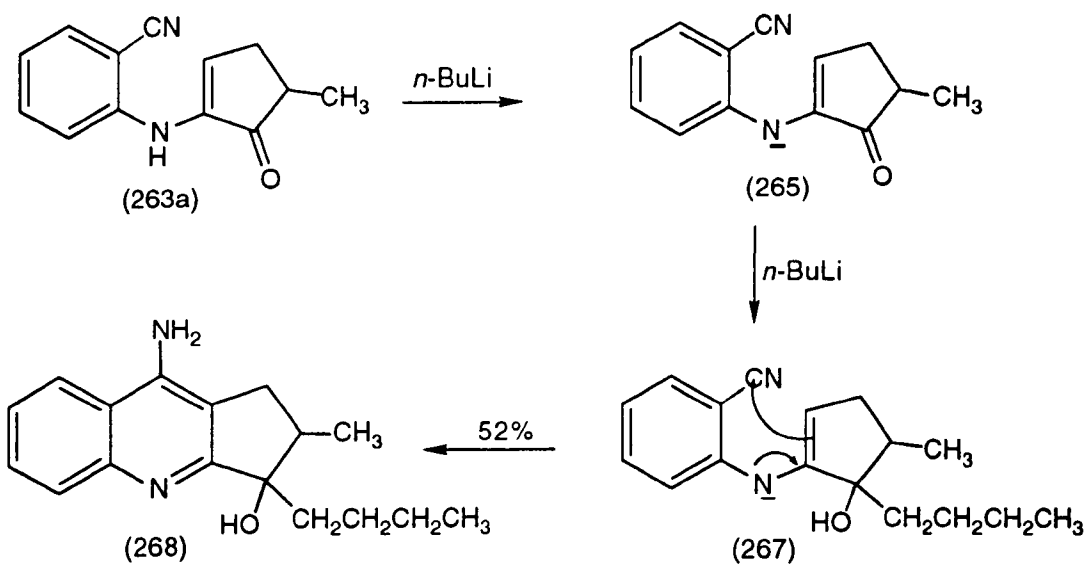
The reason for the failure of sodium hydride to have any effect upon the enamine was ascribed to the fact that the first equivalent of the base would remove the amino proton to give the stable anion (265) and the second equivalent would remove the proton α- to the carbonyl resulting in a stable cyclopentadiene moiety (266) *(Scheme 67).*



Scheme 67

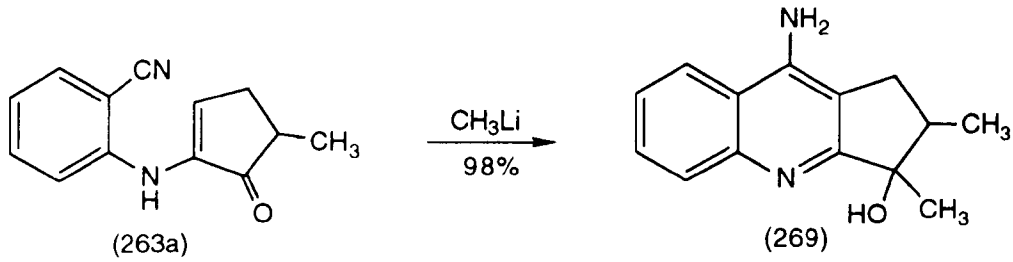
After this failure to react, the enamine was treated with a nucleophilic base in the shape of *n*-butyllithium. As before, one equivalent of the base did not

produce a reaction and the starting material was isolated after quenching the reaction. The second attempt of this reaction used two equivalents of the base and after quenching, tlc analysis indicated that a reaction had taken place as the R_f value of the new product corresponded to a cyclised compound NMR spectroscopy revealed that the addition of a second equivalent of the *n*-butyllithium produced a nucleophilic attack on the carbonyl group, ridding the enamine of its stabilising influence and thus enhancing the nucleophilicity of the alkene, permitting cyclisation *(Scheme 68).* The yield of the cyclised product (268) was optimised at 52% when the solvent was changed from toluene to anhydrous THF.



Scheme 68

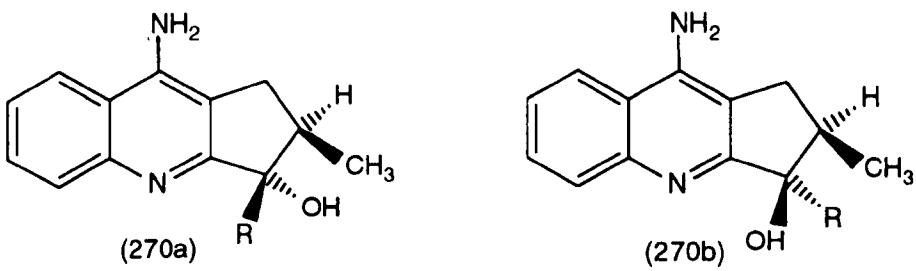
The reaction was repeated with the replacement of the *n*-butyllithium with methyllithium and it was again successful giving an almost quantitative yield of the cyclised product *(Scheme 69).*



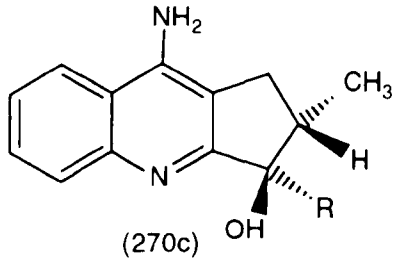
Scheme 69

Unfortunately, the range of this reaction could not be extended as it was found that *t*-butyllithium did not take part in the reaction probably due to steric hindrance. Furthermore, the use of phenyllithium was unsuccessful when used in this manner. However, the cyclisation itself gave a useful insight into the process required to obtain a cyclised product from the cyclotene enamine and it prompted much thought into how the reaction could be further developed.

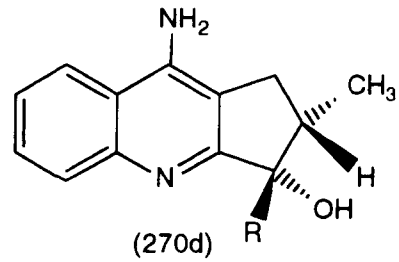
Firstly though, it was realised that the tertiary alcohols so produced by this reaction would not exist in diastereomerically pure form, but presumably as a mixture of all four possible isomers (270a,b,c,d)*(Scheme 70)*.



Scheme 70



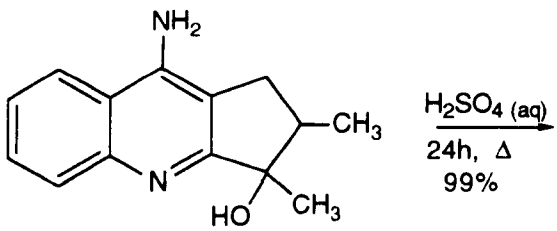
(270c)



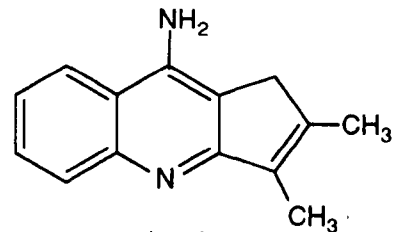
(270d)

Scheme 70 continued

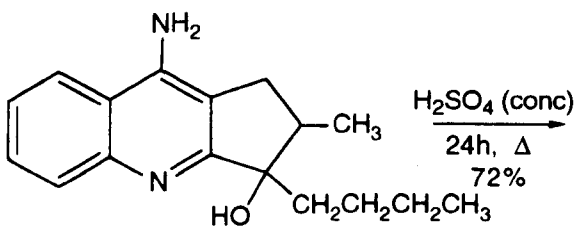
Before commencing the design of synthetic strategies towards any or all of these isomers it is suggested that they be subject to molecular modelling studies with the acetylcholinesterase enzyme to determine if further effort would be worthwhile. But, initially, it was simple enough to remove the stereochemical complexity by dehydration of the alcohols to their corresponding alkenes (271) and (272) *(Scheme 71)*.



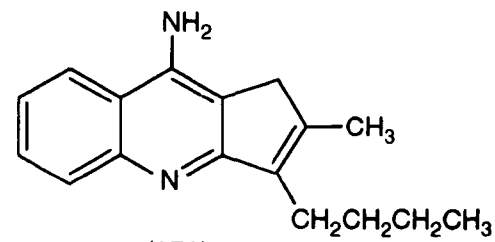
(267)



(271)



(268)



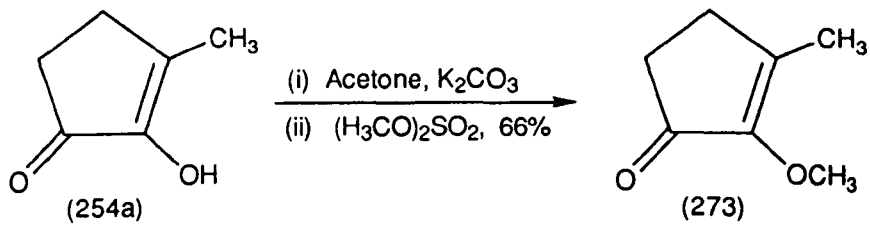
(272)

Scheme 71

(e) Further Attempts at Cyclisation of the Cyclotene Enamine

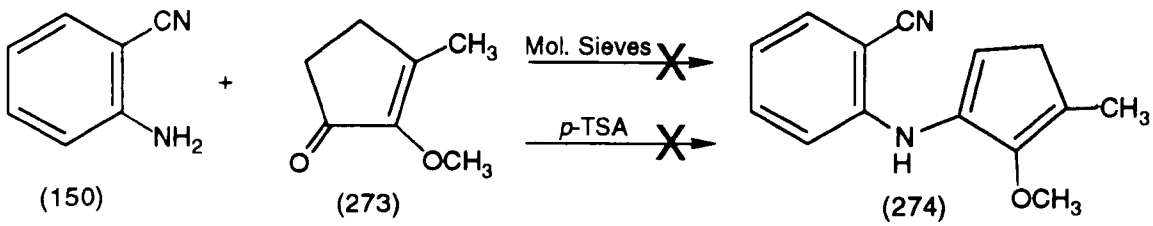
Noticing that the cyclotene enamine was a highly functionalised but particularly stable molecule, steps were taken to remove the stabilising influence of the carbonyl group that the enamine possessed. It was expected that this could be achieved by alteration of cyclotene prior to formation of the enamine, or by some modification of the enamine itself.

A simple preparation was discovered which allowed the facile preparation of the methyl ether derivative of cyclotene by stirring the enone in acetone with potassium carbonate and adding dimethyl sulphate dropwise[198]. After a period of reflux, filtration and purification yielded the required derivative (273) *(Scheme 72).*



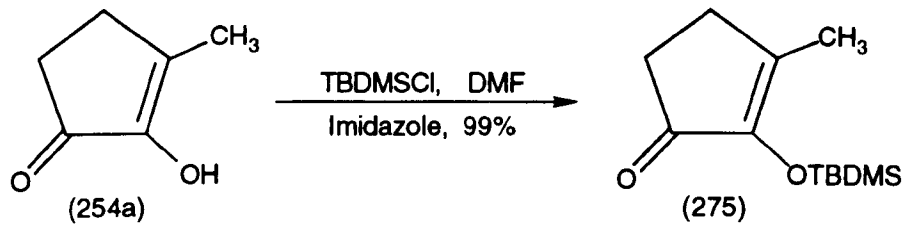
Scheme 72

Unfortunately, this derivative was quite reactive and gave only decomposition products when reacted with anthranilonitrile (150) using, firstly, molecular sieves and, secondly, *p*-toluenesulphonic acid *(Scheme 73).* This was ascribed to the fact that by making the methyl ether derivative, the hydrogen bond stabilisation that cyclotene has was removed, leaving the methyl derivative free to enolise to the cyclopentadiene which would be expected to dimerise under the reaction conditions.



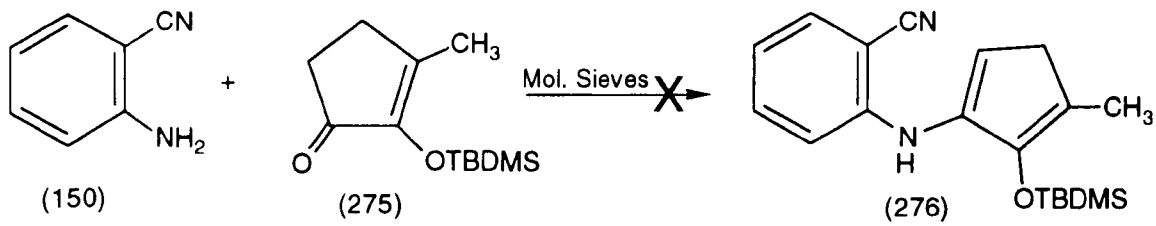
Scheme 73

Utilising the method of Corey *et al.*[199], an alternative protecting group was tried. Cyclotene (254a) was placed in DMF with *tert*-butyldimethylsilyl chloride and two equivalents of imidazole and stirred at 36°C for 24 hours *(Scheme 74).*



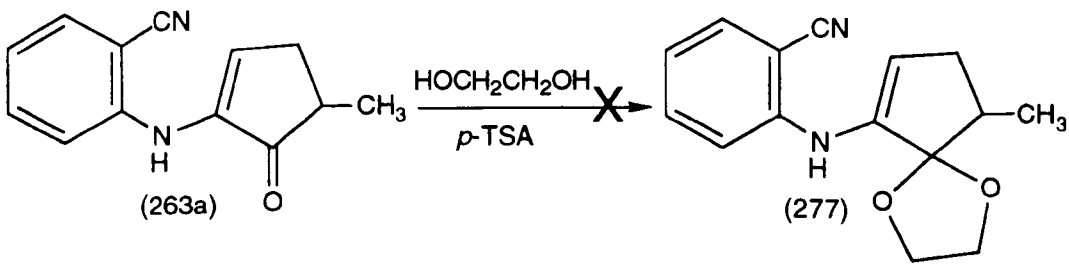
Scheme 74

In a reversal of the previous result, when the silylated cyclotene (275) was subjected to reaction with anthranilonitrile (150) and molecular sieves and heated to reflux, no reaction could be observed. Tlc analysis indicated merely the presence of starting materials *(Scheme 75).*



Scheme 75

It was clear that attempting the synthesis of enamines from these cyclotene derivatives was impossible and an alternative strategy had to be investigated. It was subsequently decided to attempt modification of the cyclotene enamine itself with the conventional use of ethan-1,2-diol in an effort to produce the acetal from the carbonyl group[200](*Scheme 76*).



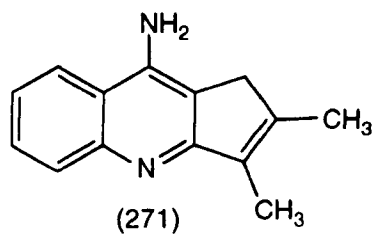
Scheme 76

Again, this strategy was unsuccessful, producing only decomposition products. It was reasoned that removing the stabilising influence of the carbonyl group rendered the enamine sensitive to hydrolysis under the reaction conditions and it was decided to abandon this pathway.

(f) Further Development with Organolithium Reagents

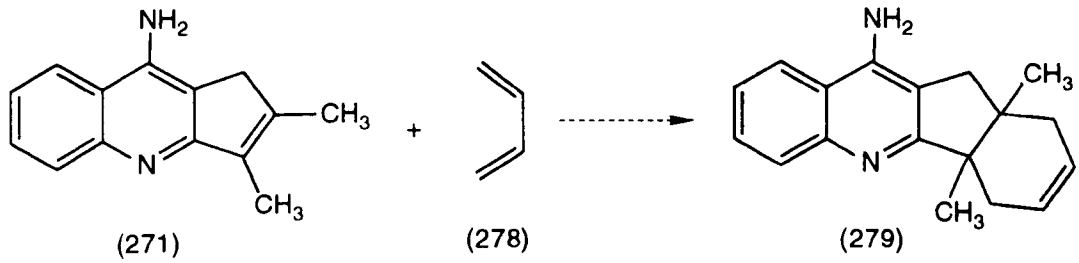
Further use of simple alkyllithiums in the manner previously described was expected to produce more tacrine analogues, but at this point in the project it was felt that development of the chemistry was becoming the more dominant requirement. Increasing knowledge of the types of molecules that were exhibiting activity made it possible to list several design criteria *i.e.* those discussed in the conclusions of *Chapter 6*, but when these were acted upon, say, with the use of camphor or indanones, the chemistry was synthetically unsuccessful. It was thought that the products could have been synthesised by alternative methods but these would have taken time to elucidate, and it was already known at this point that the camphor-derived analogue possessed no particular activity. It was felt that chemistry routes had to be developed that would produce compounds of choice and would allow extensive variation of the starting materials. To this end, it was felt that the alkyllithium cyclisation of the cyclotene enamine was a useful discovery and rather than produce more analogues, which could be done at any stage, further development was considered.

Consider the dehydrated product as exemplified by the 9-amino-2,3-dimethyl-[*1H*]-cyclopent-[*b*]-quinoline compound (271), readily available in almost quantitative yields from cheap starting materials.



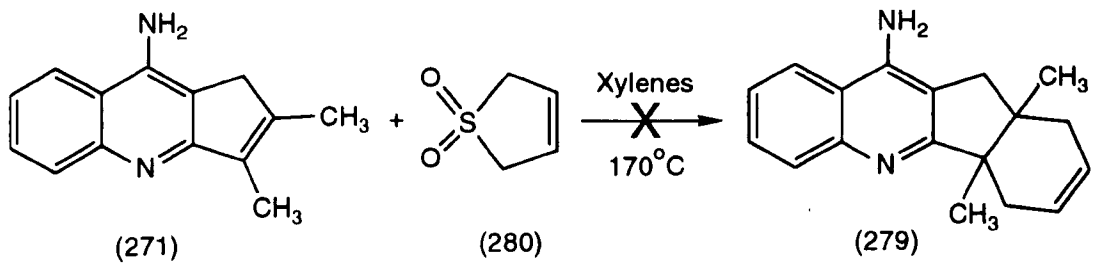
(271)

It was visualised that the double bond of the cyclopentyl ring could possibly take part in a Diels-Alder cycloaddition with 1,3-butadiene (278). This would have given the potential product (279) of which it can be seen has an obvious resemblance to the indanone-derived products described in *section (c)*, to which general synthetic routes failed *(Scheme 77)*.



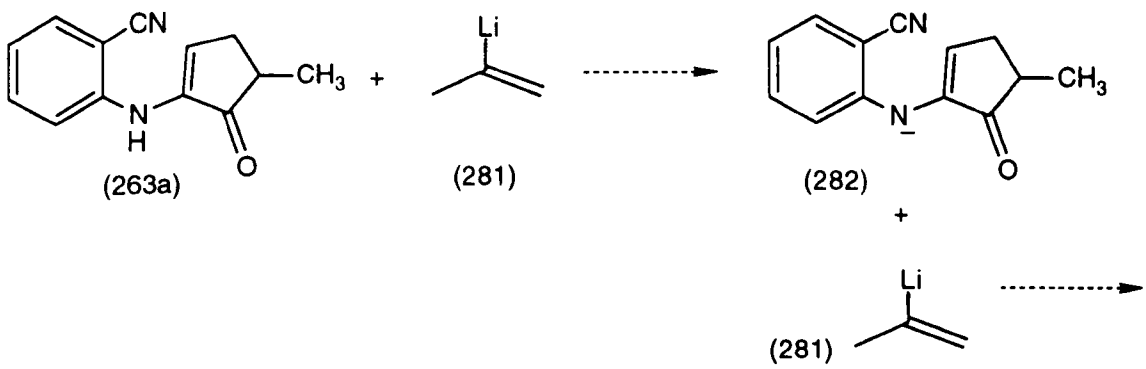
Scheme 77

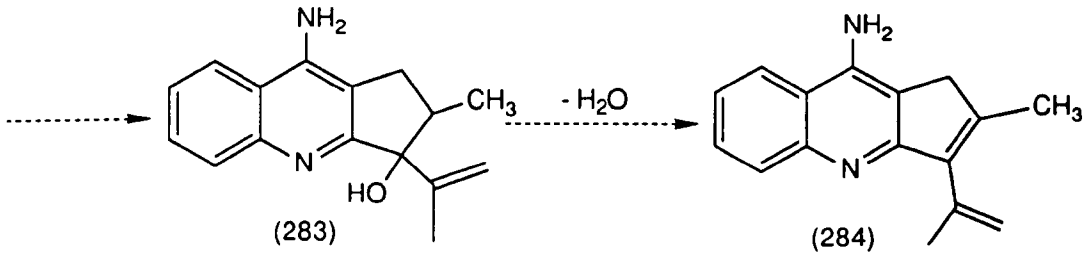
Diels-Alder reactions are very versatile[201] and a successful reaction with compound (271) would therefore have further potential. However, it was found that the reaction was unsuccessful when compound (271) was placed in xylenes with 3-sulpholene (280) and heated overnight, the 3-sulpholene decomposing to 1,3-butadiene (278) *in situ (Scheme 78)*.



Scheme 78

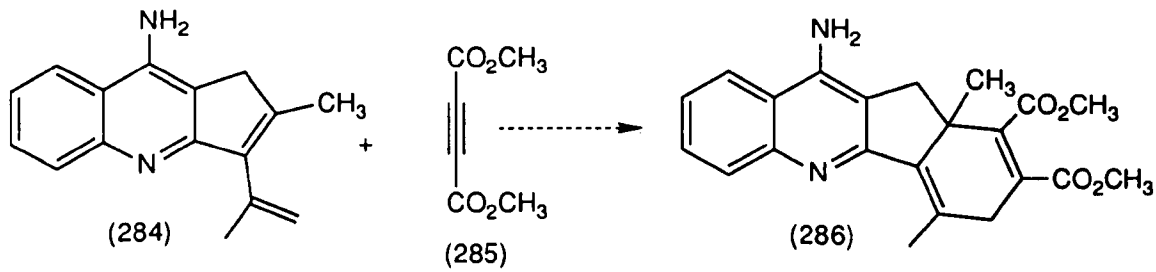
It is perhaps not surprising that this Diels-Alder reaction failed to proceed since such transformations are known to occur most favourably between alkenes possessing electron-withdrawing substituents and dienes with electron-donating substituents. In the case of the above reaction, the alkene is a simple one with an electron-donating methyl substituent, and is hence a poor dienophile. Furthermore, the diene is unsubstituted. A reverse electron demand Diels-Alder could have been investigated where a diene with electron-withdrawing substituents may have been more suitable for the system represented by compound (271). However, another strategy was considered which seemed to have greater accessibility and took advantage of the fact that the cyclotene enamine was cyclised by alkyllithiums. More specifically, use of vinyllithium reagents (281) were proposed to replace the previously used alkyllithiums. Using two equivalents of the vinyllithium (281), the tertiary alcohol (283) so produced could then be dehydrated, thereby producing a diene (284) which could potentially be of greater reactivity in a Diels-Alder reaction *(Scheme 79).*





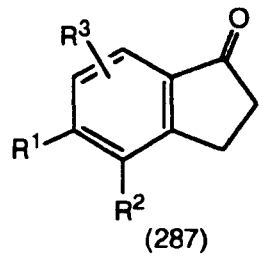
Scheme 79

Subsequent use of strong dienophiles such as dimethyl acetylene dicarboxylate (DMAD) (285) might then produce compounds with the required indanone-type carbon skeleton and with the additional advantage of further functionalisation *(Scheme 80).*



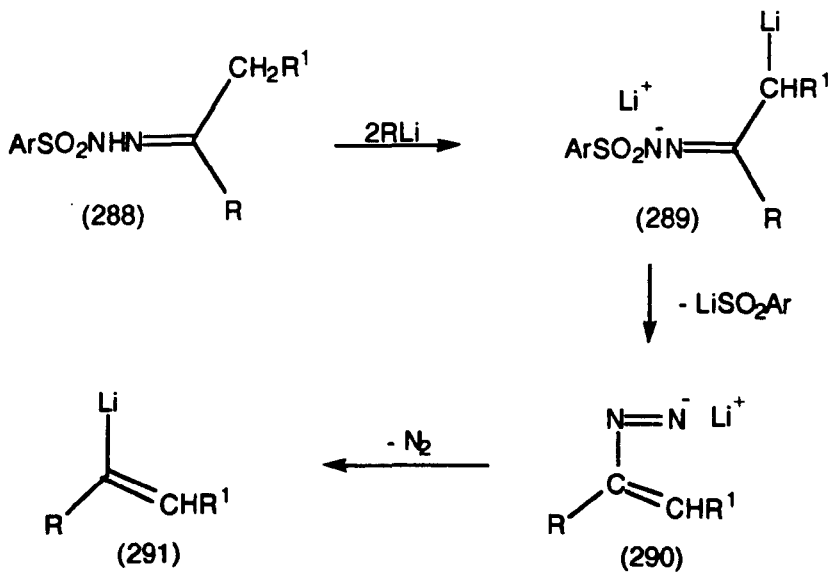
Scheme 80

This new strategy seemed an excellent alternative to the direct use of functionalised indanones. These types molecules would only otherwise be accessible by the individual synthesis of each indanone (287) as required. This route, with the use of vinyllithiums, has much scope for development. Used in conjunction with the versatile Diels-Alder reaction, the route described above has much greater potential for development.



(287)

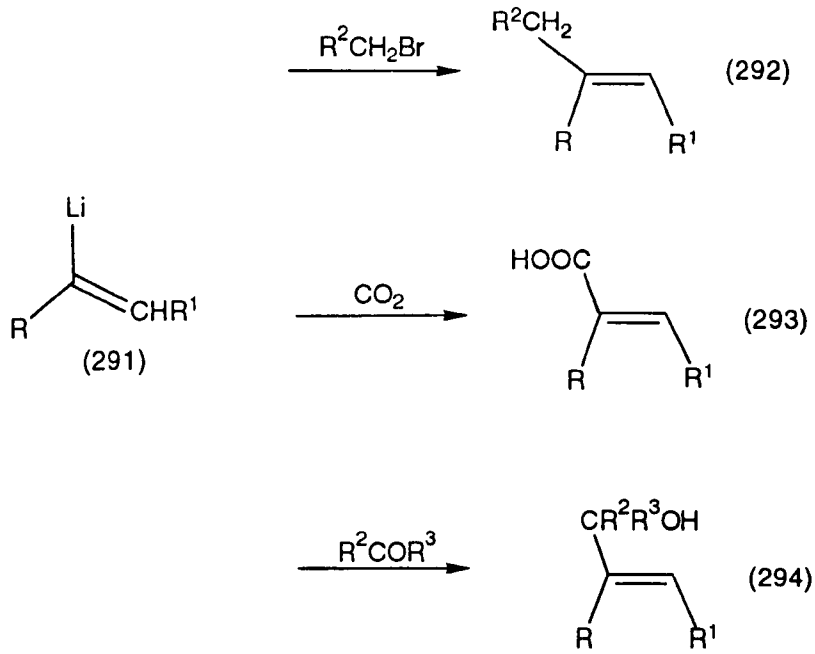
Firstly though, the synthesis of the vinyllithiums themselves had to be investigated. This was conveniently achieved by a modification[202] of the Shapiro alkene synthesis[203], a reaction which utilises trialkylbenzenesulphonylhydrazones in the conversion of ketones to alkenes *(Scheme 81)*.



Scheme 81

Typically, the arylhydrazone (288) is treated with two equivalents of an alkyllithium, removing two protons and losing the arylsulphinate to give the vinyl

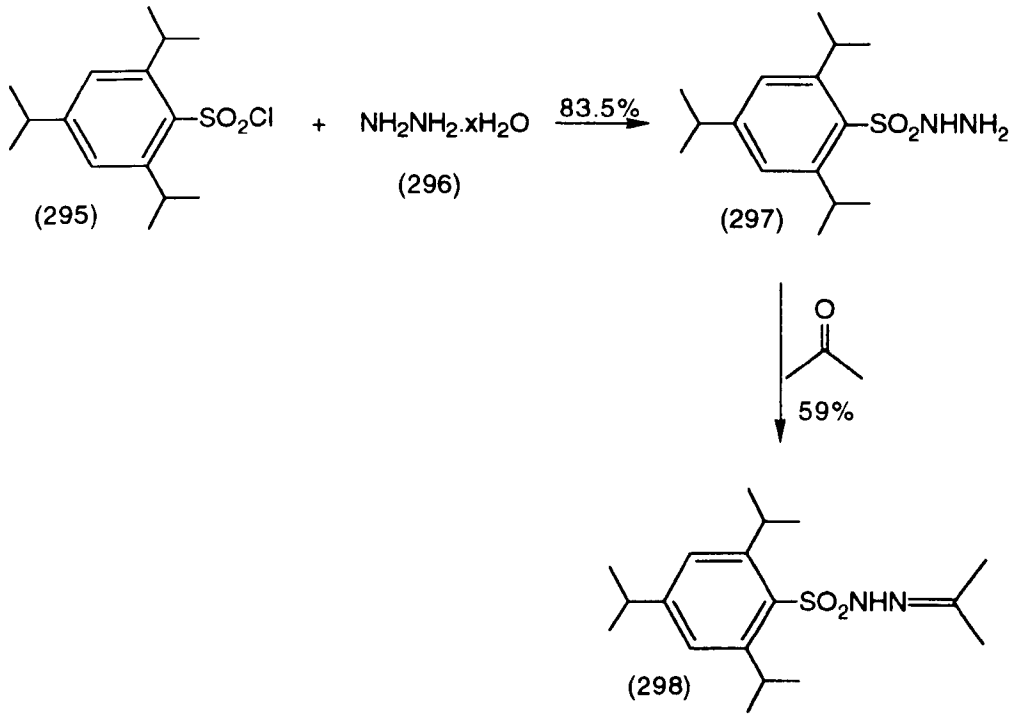
diimide anion (290). Contact with a proton source then gives the alkene after loss of molecular nitrogen. However, the vinyllithium intermediate may also be trapped by electrophiles such as alkyl halides[204], carbon dioxide[205], and ketones[204] to give substituted alkenes (292), α,β-unsaturated acids (293) and alcohols respectively (294) *(Scheme 82).*



Scheme 82

It was anticipated that the vinyllithium would react with the carbonyl group of the cyclotene enamine and then act as a base to cyclise to the cyclopenta-[*b*]-quinoline as described in *Scheme 79.* The range of this reaction was expected to be extended by using different ketones for the synthesis of the vinyllithium reagent, but to begin with acetone was to be used in the preliminary investigation. 2,4,6-Tri-isopropylbenzenesulphonyl chloride (295) was stirred in THF at -10°C and hydrazine hydrate (296) was added dropwise with further stirring. This produced the hydrazide (297) which was isolated and purified. To a suspension of the

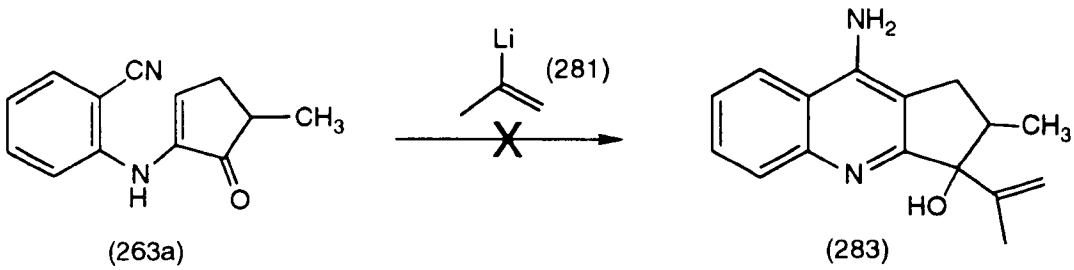
hydrazide (297) in methanol was added acetone, dropwise, and this produced, in minutes, a precipitation of the hydrazone (298) *(Scheme 83).*



Scheme 83

After synthesising the hydrazone (297) in good yield, a quantity was placed in a solution of tetramethylenediamine (TMEDA) in *n*-hexane, cooled to -78°C, and *n*-butyllithium was added dropwise. As the temperature was permitted to warm to 0°C, nitrogen was evolved. On the cessation of gas evolution, a solution of cyclotene enamine in THF was added and stirred overnight. When quenched with water and extracted with diethyl ether, a brown oil was isolated. This seemed promising by tlc analysis, but NMR spectroscopy showed an unidentifiable mixture of compounds which did not contain the expected signals. The tertiary alcohol

that was to result from this reaction was not produced despite several attempts *(Scheme 84).*



Scheme 84

It was proposed that polymerisation and other side reactions were responsible for the failure of this reaction. Perhaps utilisation of the cerium chloride modification with the lithium compounds[163] might provide the desired nucleophilic attack and subsequent cyclisation of the cyclotene enamine.

Chapter 8

FURTHER PHARMACOLOGICAL RESULTS AND CONCLUSIONS

(a) Further Pharmacological Findings

The following data were obtained from two groups of compounds. Firstly, the compounds derived from norcamphor and camphor will demonstrate the effects on activity of a methylene and substituted methano bridge on the tacrine nucleus (**group 5**). Secondly, the group of compounds derived from cyclotene are examined (**group 6**).

The rat brain assays were carried out using the same procedure as for the results obtained in chapter 6.

*Table 5 - **Group 5***

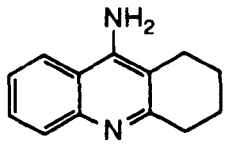
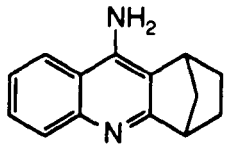
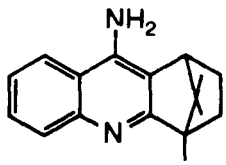
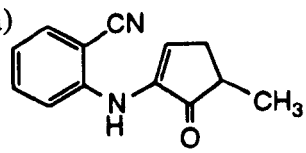
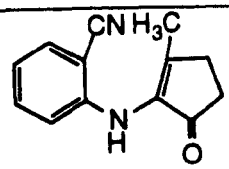
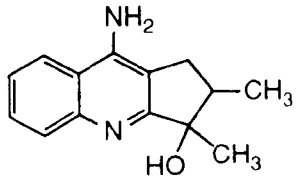
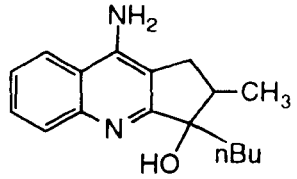
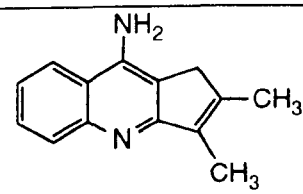
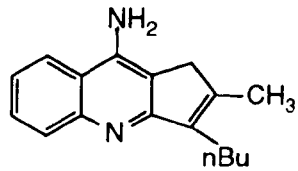
		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(36)		0.078 (0.0078)	0.025 (0.0016)	7.6	6.7
(211)		0.24 (0.032)	0.041 (00052)	30.2	6.1
(213)		110 (5.3)	88 (10)	13	

*Table 6 - **Group 6***

		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(263a)		84.6% at 100μM	88.8% at 100μM	77% at 100μM	
(263b)		79.8% at 100μM	92.4% at 100μM	23.7 at 100μM	

		IC_{50} μM (± SEM, n = 5)			
		AChEase	**BuChEase**	**5-HT**	**NA**
(269)		134 (0.45)	12.2 (0.55)	4.4	
(268)		91 (26)	81 (3.8)	15.9	19
(271)		3.5 (0.42)	5.9 (0.51)	5.1	
(272)		25 (1.3)	42 (2.8)	20	

(b) Conclusions

The conclusions concerning the group 5 molecules and the effects of bridging are that activity is reduced on the introduction of a methylene bridge and markedly reduced when this bridge is substituted. This suggests that further manipulation of this type of bridging could prove fruitless as the reason for this reduction in activity must be steric factors. This also tends to suggest that the bridged compound produced by the Japanese, 9-amino-8-fluoro-1,2,3,4-tetrahydro-2,4-methanoacridine (209)[170], derived its enhanced activity from the presence of the fluoro substituent, although of course this is not conclusive. Other work in this laboratory, however, suggests that enhancement of activity due to the presence of a

fluoro substituent in this particular position[206] and this should be considered for the future design of tacrine analogues.

With **group 6** the biological results were interesting though disappointing. Synthesis of the tertiary alcohols (268) and (269) conferred upon the molecule another hydrogen bond donor - the hydroxyl group - which might have had potential for binding to the enzyme. However, it became apparent that the hydroxyl group does not enhance binding at all, and its removal by dehydration did increase activities in each case although not to any useful extent.

The reason for these compounds not having much activity when their bulk does not seem to be an adverse factor seems to be the presence of the hydroxyl group in this position. It should be pointed out, however, that these compounds exist as diastereoisomers (270a, b, c, d) (see *Chapter 7*). If separated, or synthesised separately, each compound would not be expected to exhibit great activity, but it is suggested is that these isomers are subjected to a molecular modelling study with the acetylcholinesterase enzyme to deduce if any areas of interaction between the molecules and enzyme occur.

Dehydration removed the question of stereochemistry and produced flat molecules with a double bond conjugated with the aromatic system. Having removed the polar hydroxyl group it was hoped that there would be increased π-electron interactions with the aromatic amino acids residues lining the enzyme's active gorge, but this also did not appear to be the case.

The monoamine assay results for **groups 5** and **6** were unremarkable.

Chapter 9

SUMMARY AND FUTURE WORK

(a) Summary

It is now possible to summarise the achievements of this project. Systematic investigations of tacrine analogues were carried out which, after biological testing, allowed several conclusions to be drawn about the structural features that would or would not be required in a compound with potential use as a centrally active anticholinesterase drug.

Rings of greater size than seven-membered saturated rings lowered activity, as did bridging of the saturated rings. Fusing a benzene ring to the acridine nuclei did not enhance activity, nor with benzene rings fused to the cyclopentyl and cycloheptyl analogues.

Biological results showed that tacrine, when tested *in vivo,* gave increased learning while the cycloheptyl analogue, 9-amino-2,3,4,5-tetrahydro-[*1H*]-cyclohepta-[*b*]-quinoline (164) (which is almost equipotent to tacrine in anticholinesterase activity) did not. This result served to underline the conclusions drawn by other research groups which indicated that the other pharmacological actions of tacrine seem to bring about the required overall enhancement of learning and memory, whereas other centrally active drugs active solely as an anticholinesterase could not achieve this. It is therefore suggested

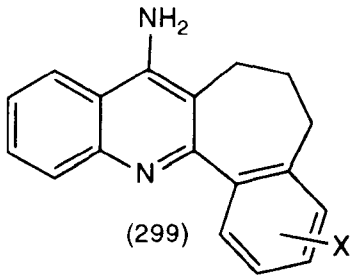
that 9-amino-2,3,4,5-tetrahydro-[*1H*]-cyclohepta-[*b*]-quinoline (164) be tested for alternative pharmacological activities for a comparison with the range of actions of tacrine. This might indicate a particular pharmacological effect which could then be investigated for its part in the learning and memory processes.

The most successful part of the project pertained to the discovery that certain derivatives that had been synthesised exhibited remarkable activity in the 5-HT uptake inhibition assays. Since such compounds were obtained in a straightforward manner, it is now envisaged that an investigation of these amine uptake inhibitors (and their potential antidepressant effects) could readily be accessed.

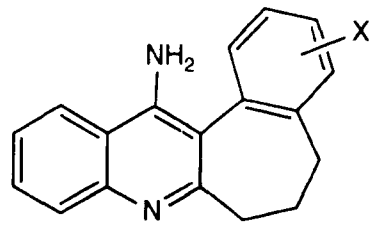
Concerning the synthesis of alternatives to tacrine for use against AD, it has been realised that there are no simple answers. No single drug was found to have a superior pharmacological profile to tacrine and this result seems to be mirrored by other researchers in the area. However, this project has attempted to open into a new area of synthesis which is envisaged to give novel ranges of tacrine analogues suitably functionalised for further manipulations.

(b) Further Synthesis of 5-HT Uptake Inhibitors

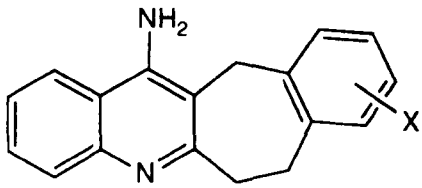
The following targets have been envisaged for an investigation into the structure-activity relationships of the benzocyclohepta-[*b*]-quinoline molecules. These ranges involve shifting the position of the fused benzene ring around the cycloheptyl moiety.



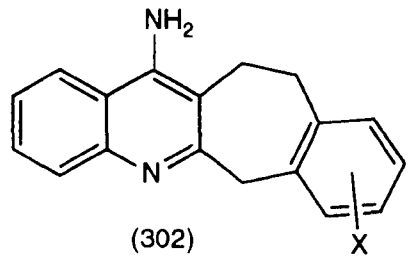
(299)



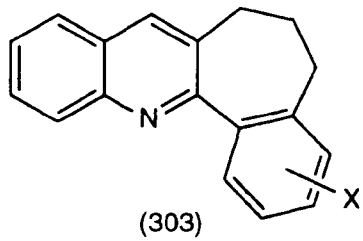
(300)



(301)

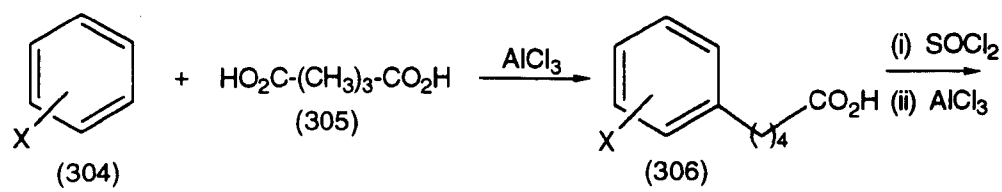


(302)

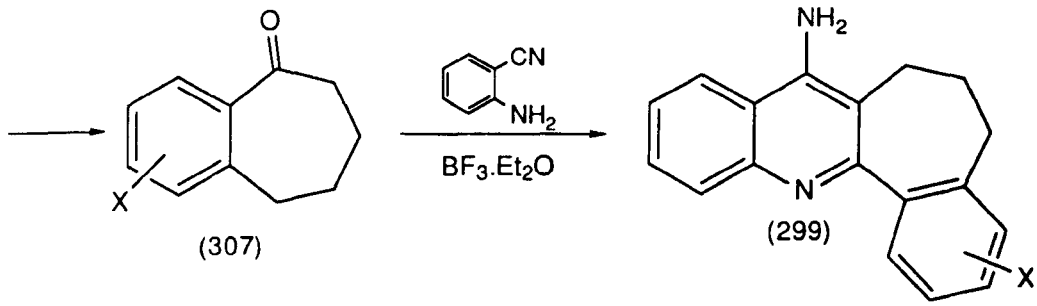


(303)

Compounds derived from (299) are expected to be obtained in the following manner. Acylation of a substituted benzene (304) followed by an intramolecular acylation, probably *via* the acid chloride, to give the required benzosuberone (307) for incorporation into the cyclodehydration reaction with anthranilonitrile (150) would give the target molecule (299) *(Scheme 85).*

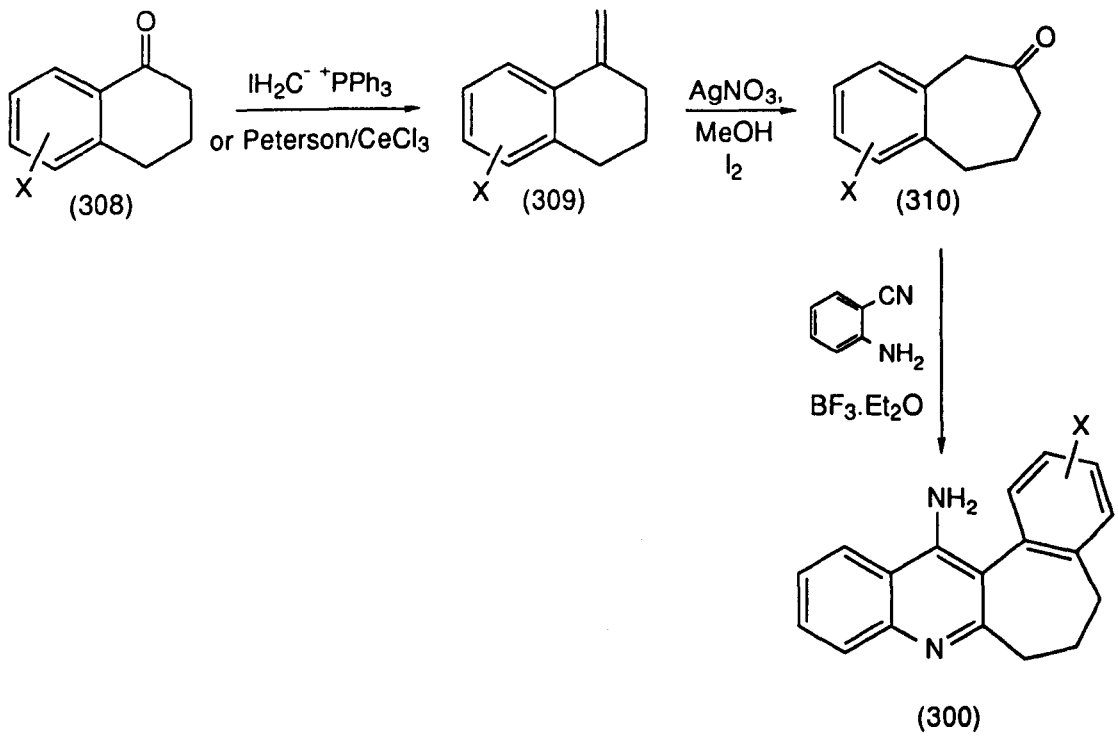


(304) (305) (306)



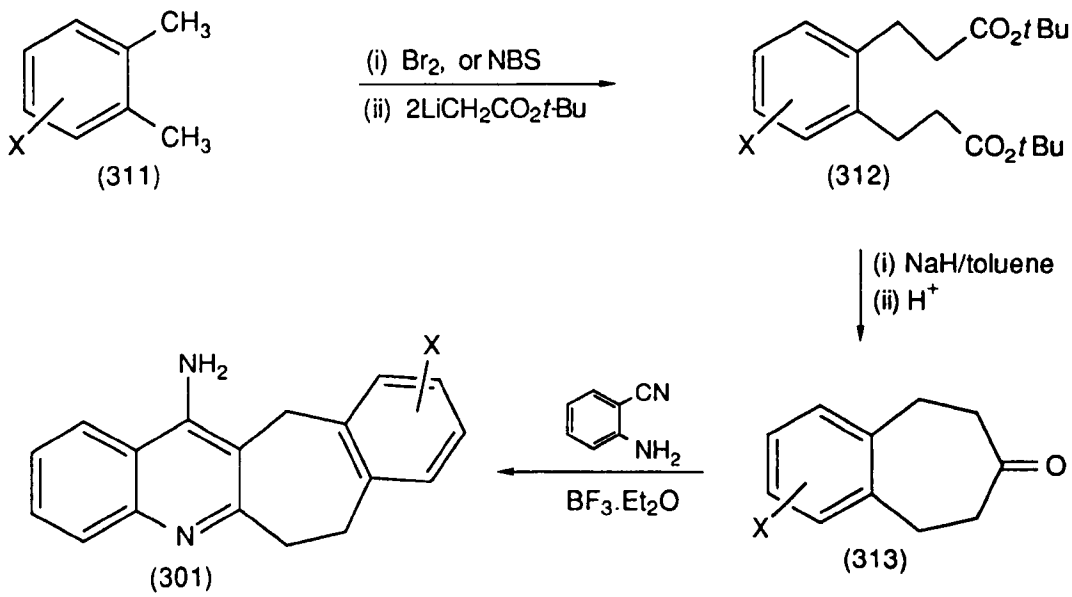
Scheme 85

Utilising methodology already discussed in *Chapter 5*, compounds derived from the target (300) could be made using the ring expansion reaction. Beginning with variously substituted 1-tetralones (308), methylenation to the exocyclic alkene (309) is envisaged with subsequent ring expansion. Incorporation of the product (310) into the cyclodehydration reaction with anthranilonitrile (150) would then give substituted target molecules derived from the target structure (300) *(Scheme 86).*



Scheme 86

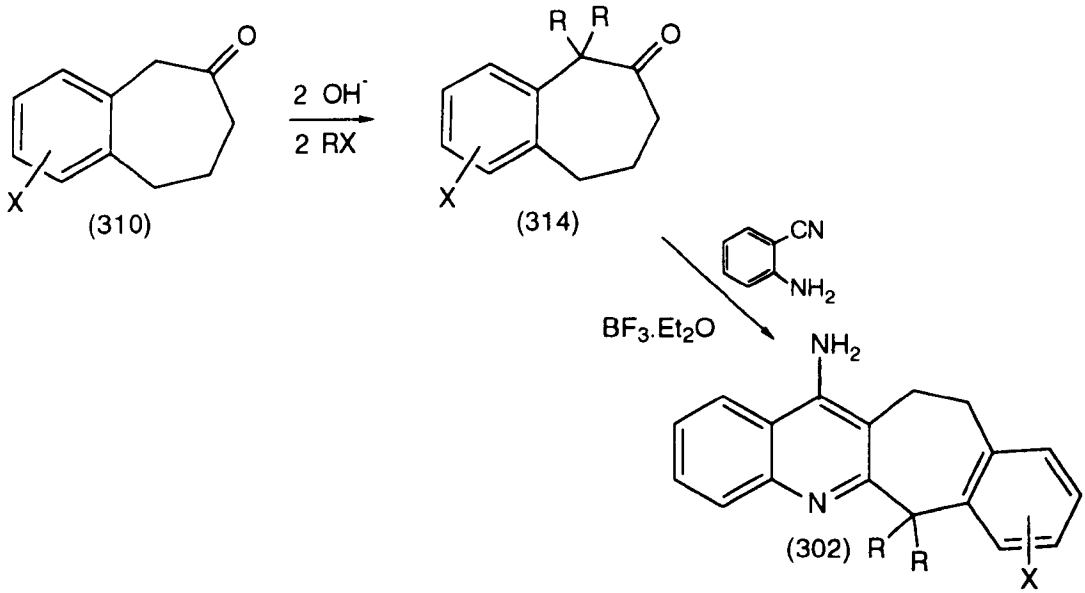
In order to obtain compounds derived from target (301), synthesis of 3-benzosuberones (313) must first be achieved. This is conventionally done by reaction between lithium *tert*-butylacetate and *ortho*-xylylene dibromide, the dibromide itself synthesised from *ortho*-xylene and reaction with molecular bromine or *N*-bromosuccinimide (NBS). A Dieckmann condensation reaction and subsequent decarboxylation then leads to the desired 3-benzosuberone (313) and its incorporation into the cyclodehydration reaction is envisaged to give target molecules derived from (301) *(Scheme 87)*.



Scheme 87

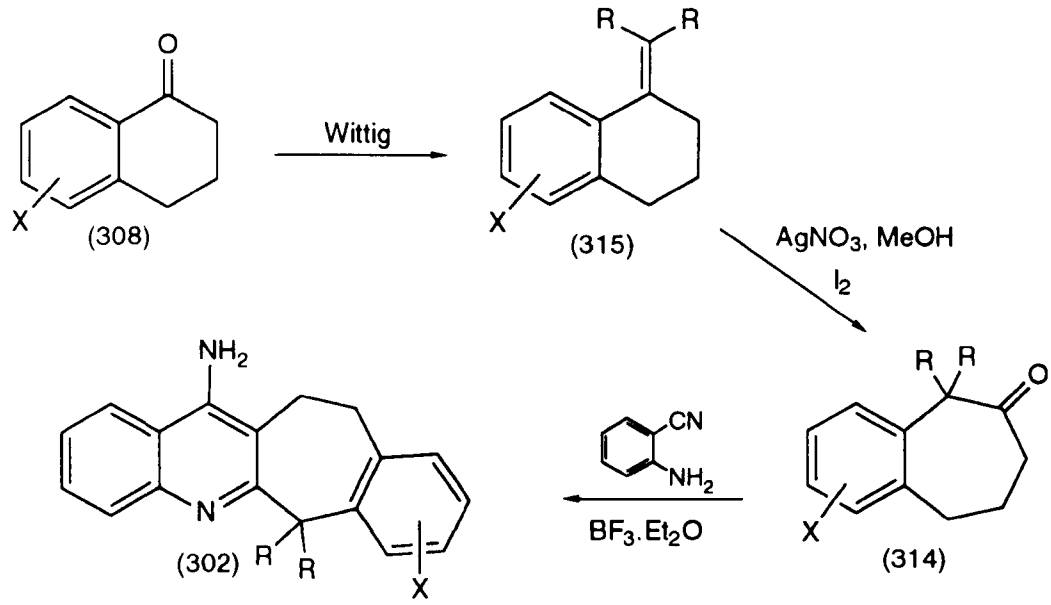
To shift the fused benzene to the final position on the cycloheptyl ring reveals a small problem. The benzosuberones required for this are the 2-benzosuberones (310) whose synthesis were described in *Chapter 5* and in *Scheme 86* above. The problem concerns the fact that the α-methylene group that attacks the nitrile of the anthranilonitrile (150) will always, in this case, be the benzylic

methylene due to the stabilising conjugation with the aromatic ring. This means that this methylene must be disubstituted to prevent reaction at this position. The alternative methylene will therefore have to react during the cyclodehydration reaction with anthranilonitrile (150) and the orientation of the benzene group will be subsequently altered to the required position. Consider the use of two equivalents of a base to deprotonate the α-methylene of 2-benzosuberone and two equivalents of an alkyl halide to provide the substitution *(Scheme 88).*



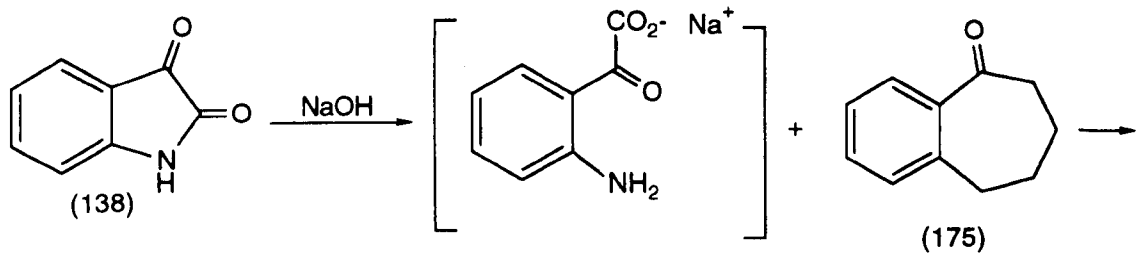
Scheme 88

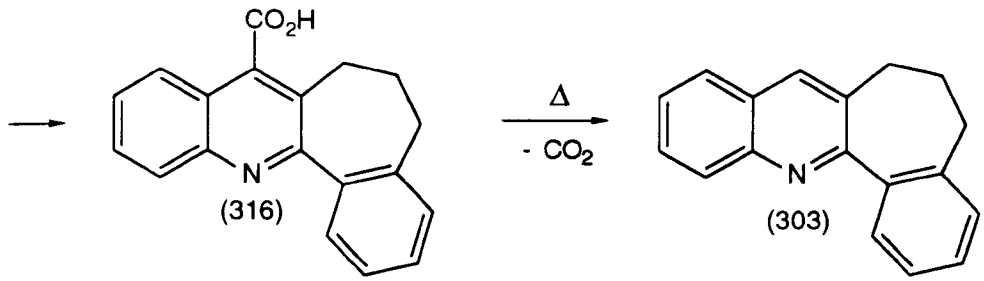
Alternatively, olefination of 1-tetralones (308) to give disubstituted exocyclic alkenes (315), followed by ring expansion to the disubstituted 2-benzosuberone (314) would furnish the required molecule (314) for use in the cyclodehydration reaction *(Scheme 89).*



Scheme 89

Finally, to investigate the effects of the amino group itself on biological activity, the following method is proposed to produce the target compound (303). Previously, in *Chapter 4*, the use of isatin (138) was described. Subjection of this molecule to a base, then condensation with, in this case, 1-benzosuberone (175) would give the 8-carboxylic acid derivative (316). Decarboxylation on heating would then remove this group to give the target (303) *(Scheme 90)*. If, on biological testing this compound had an enhanced activity then substitution of anthranilonitrile (150) with isatin (138) in the above reactions could be envisaged for further investigations.

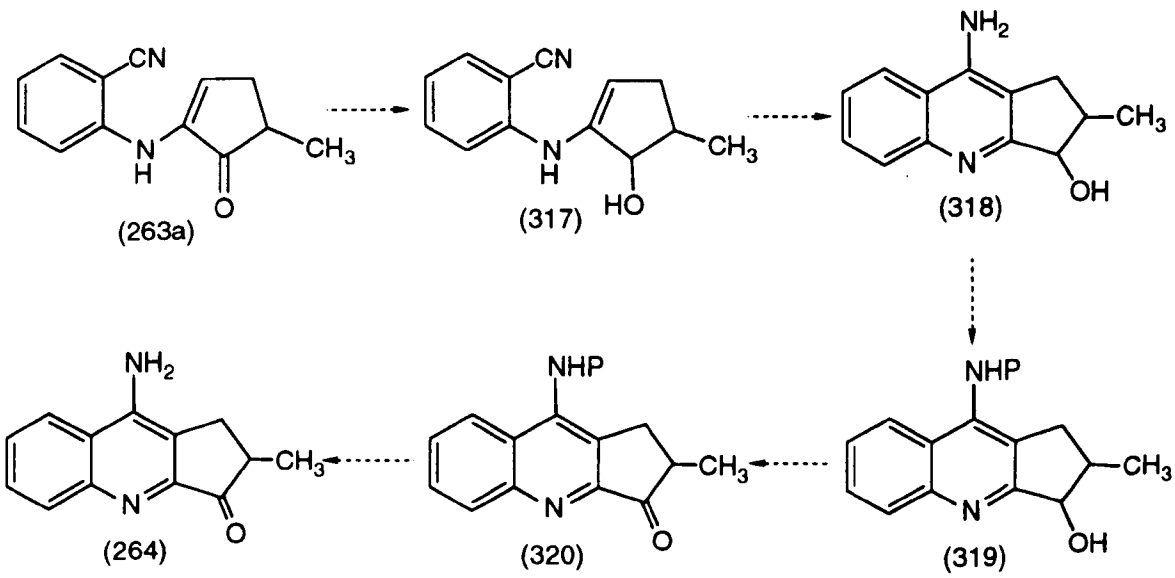




Scheme 90

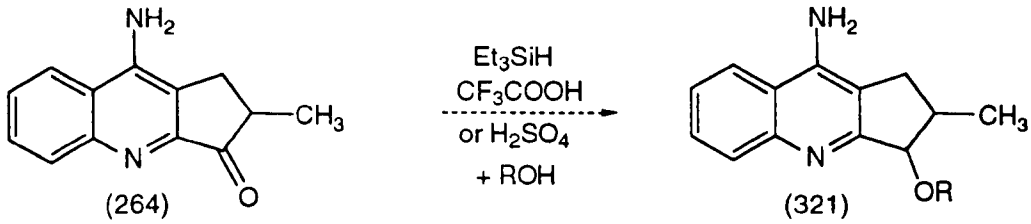
(c) Proposed Novel Tacrine Analogues

Continuing the work with cyclotene, it is envisaged that the fully cyclised product - 9-amino-1,2-dihydro -2-methyl-3-oxo-cyclopenta-[*b*]-quinoline (264) may be obtained in the following manner. Reduction of the carbonyl group of the cyclotene enamine with a mild reducing agent such a sodium borohydride would give the alcohol (317). Cyclisation with a mild base *e.g.* potassium carbonate would give the secondary alcohol (318). In order to introduce a carbonyl group at this point, protection of the amino group, followed by oxidation then deprotection of the amino group might potentially give the required ketone (264) *(Scheme 91).*

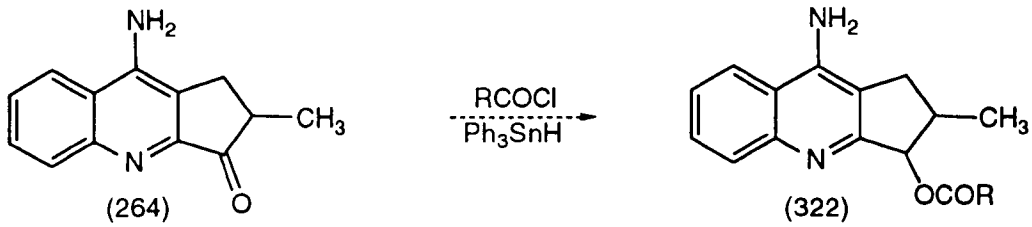


Scheme 91

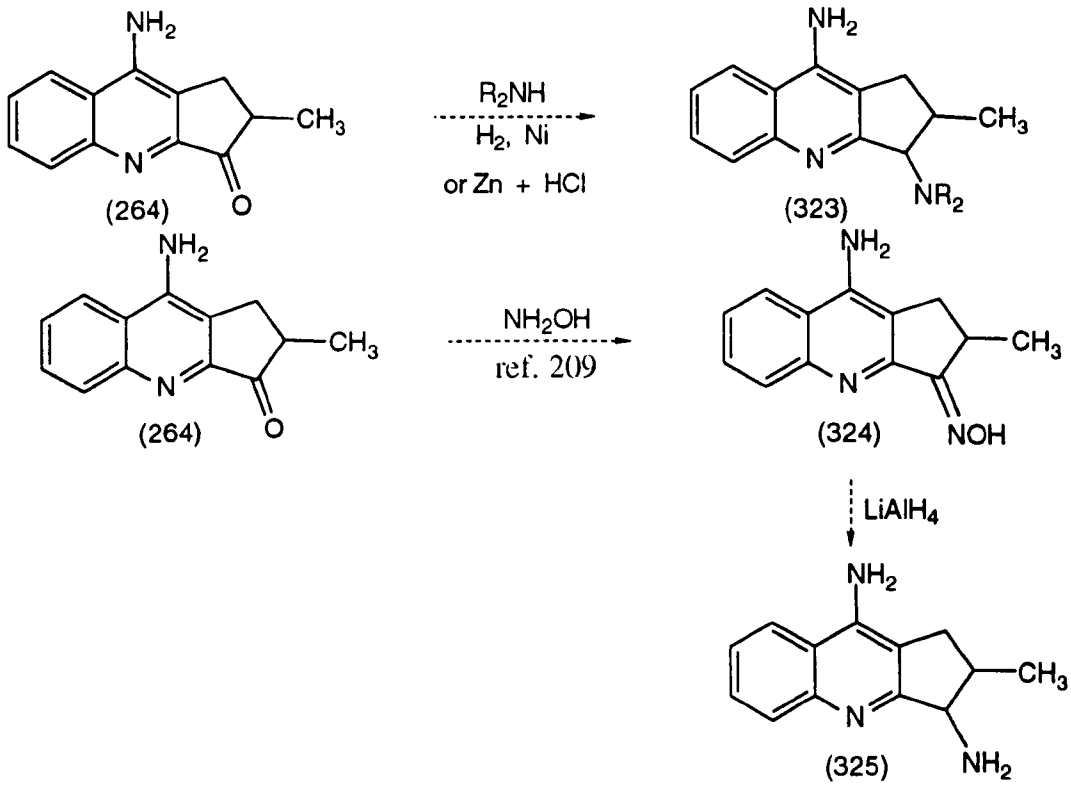
If this strategy was successful and high yielding, it would be worth pursuing the manipulation of the keto group with what is today an extensive arsenal of organic reagents for this purpose. Consider the following examples that introduce alterntive functionalisation to replace the carbonyl group on the compound (264) that we desire to synthesis *(Scheme 92)*.

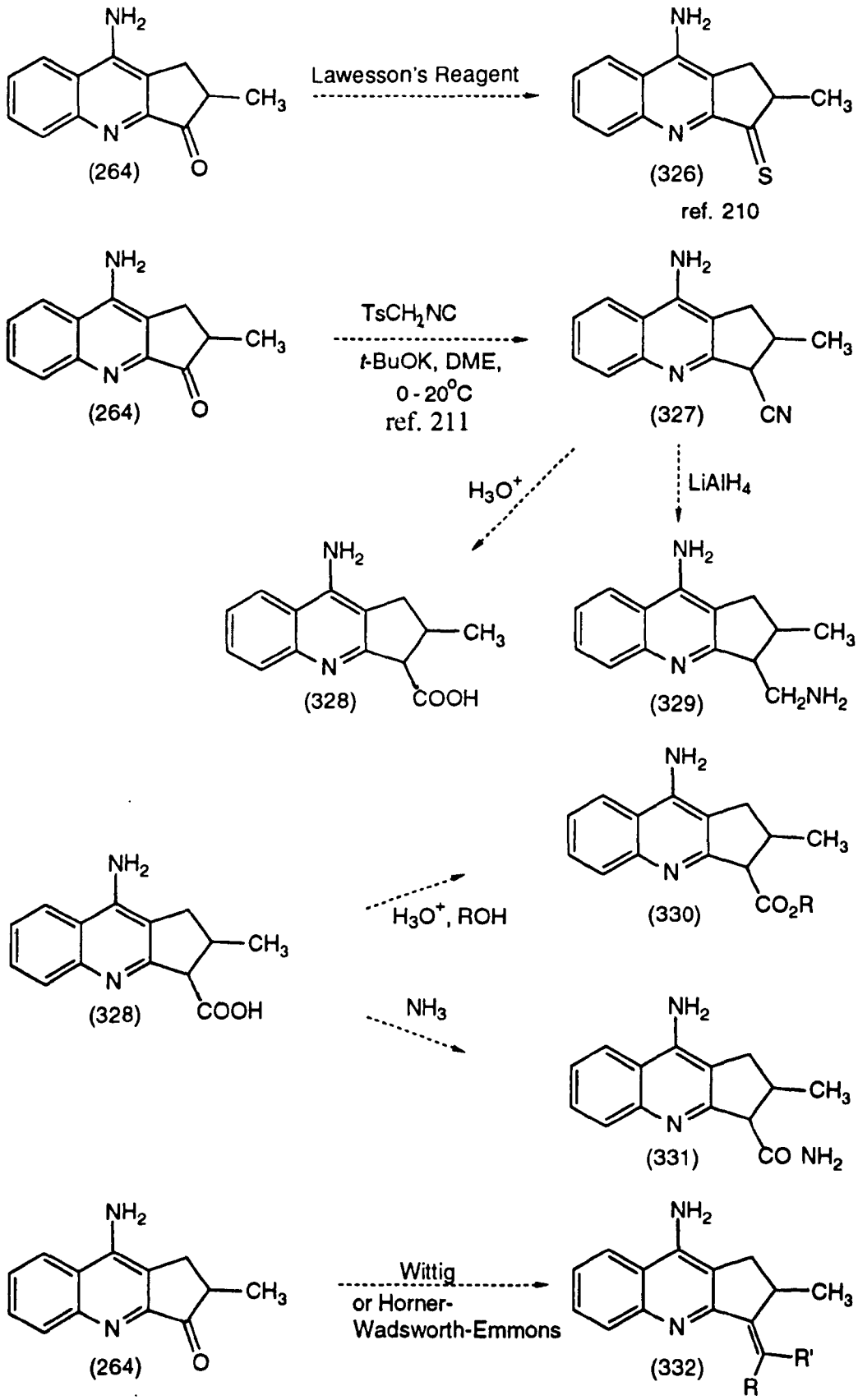


ref. 207



ref. 208



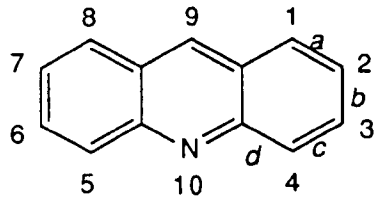

NH2
CH3
N
O
(264)
Lawesson's Reagent
S
(326)
ref. 210
TsCH2NC
t-BuOK, DME,
0 - 20°C
ref. 211
CN
(327)
H3O+
LiAlH4
COOH
(328)
CH2NH2
(329)
H3O+, ROH
CO2R
(330)
NH3
CO NH2
(331)
Wittig
or Horner-
Wadsworth-Emmons
R'
R
(332)

Scheme 92

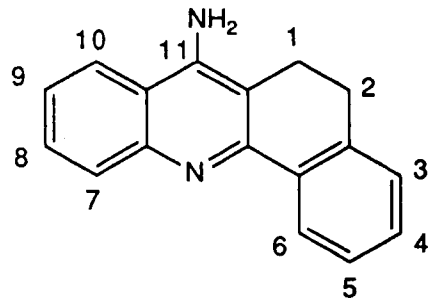
Reductive transformations are proposed to give ethers (321) , esters (322) and amines (323). Synthesis of the oxime (324) can lead to further functionalisation to the primary amine by conventional reduction with lithium aluminium hydride. Lawson's reagent could potentially provide the thione (326) and use of tosyl methyl isocyanide with potassium *tert*-butoxide could give the nitrile (327) which leads to further possibilities such as the carboxylic acid (328) and the acid derivatives (330) and (331) as well as reduction to the amine (329). Alkenations using the Wittig and Horner-Wadsworth-Emmons reactions could be utilised to produce novel analogues,

In the future, it could be imagined that should the above strategies for cholinesterase inhibition show promise, the routes used for the preparation of cyclotene-derived analogues could be extended to six- and seven-membered analogues. Shifting the position of the carbonyl group could also be considered.

Finally, it is also recommended that the work encompassing the use of vinyllithium reagents be investigated, perhaps with the use of the cerium chloride modification, as suggested in *Chapter 7*, as the versatility of this work would be undeniable once the initial chemistry concerning the cyclisation, dehydration and Diels-Alder reactions was mastered.

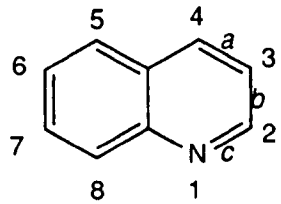


acridine

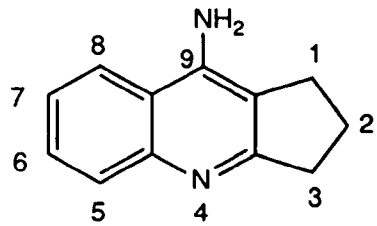


11-amino-1,2-dihydro-benz-[*c*]-acridine

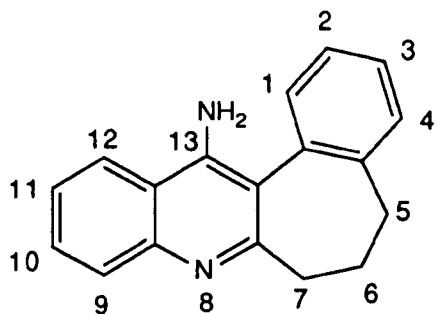
Acridines are an exception to IUPAC rules for nomenclature.



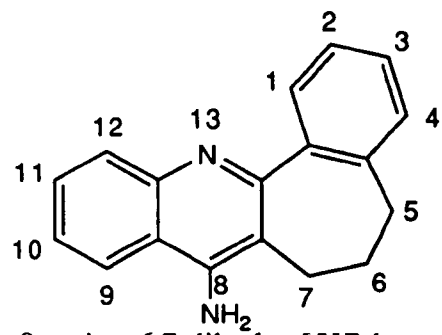
quinoline



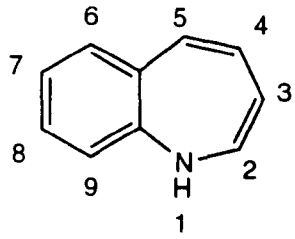
9-amino-2,3-dihydro-[*1H*]-cyclopenta-[1,2-*b*]-quinoline



13-amino-6,7-dihydro-[*5H*]-benzo-[3,4]-cyclohepta-[1,2-*b*]-quinoline

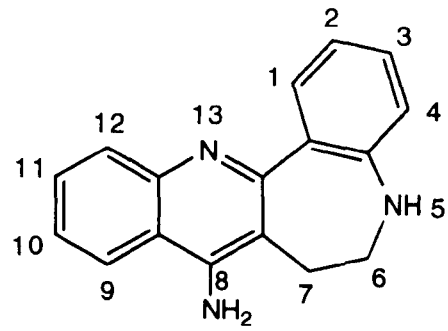


8-amino-6,7-dihydro-[*5H*]-benzo-[6,7]-cyclohepta-[1,2-*b*]-quinoline

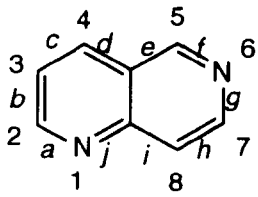


[*1H*]-1-benzazepine

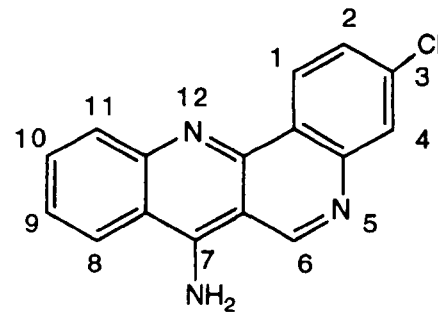
(Benzazocine similar)



8-amino-6,7-dihydro-[3,2-*b*]-quinolino-[4,5-*d*]-[*5H*]-1-benzazepine

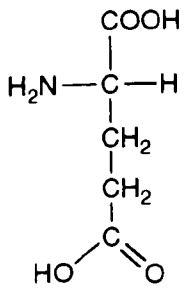


1,6-naphthyridine

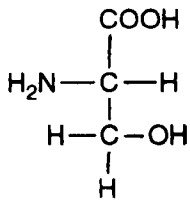


7-amino-3-chloro-dibenzo-[*b,h*][1,6]-naphthyridine

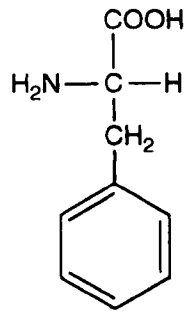
Appendix 2 - AMINO ACID STRUCTURES



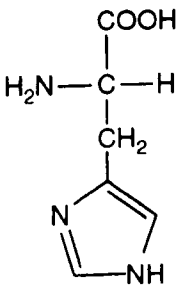
Glu - Glutamate



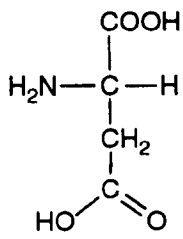
Ser - Serine



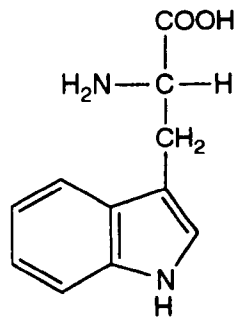
Phe - Phenylalanine



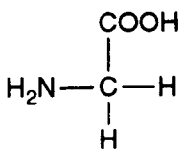
His - Histidine



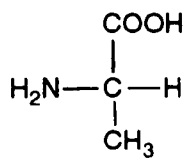
Asp - Aspartate



Trp - Tryptophan



Gly - Glycine



Ala - Alanine

EXPERIMENTAL CHAPTER

- Melting points were obtained on a Gallenkamp melting point apparatus in open capillaries and are uncorrected.

- ^{1}H NMR spectra were recorded on a Perkin-Elmer R32 spectrometer operating at 90MHz, on a Bruker WM250 spectrometer operating at 250.13MHz and on a Bruker AMX400 spectrometer operating at 400.13MHz, with the WM250 and the AMX400 in Fourier Transform mode. *J* values are given in Hertz. ^{13}C spectra were recorded on a Bruker AMX400 operating at 100.62MHz in Fourier Transform mode.

- Infrared spectra were recorded on a Unicam Mattson 1000 series FTIR spectrometer as thin films, Nujol mulls or in solution cells.

- Flash column chromatography was carried out using CAMLAB Art. Nr 81538 MN Kieselgel 60 (0.040 - 0.063mm) and Merck 7735 silica gel type 60 (0.125 - 0.250mm). Samples were applied in solution or adsorbed onto silica.

- Mass spectra were obtained on a AEIMST double-focusing mass spectrometer, modified with a solid state console using a GEC 905 computer based data system.

- Carbon, hydrogen and nitrogen analysis were determined on a Carlo Erba 1106 analyser using a technique based on the classical Pregl Dumas method.

Halogens were determined by combusting the sample in an oxygen flask containing hydrogen peroxide and potassium hydroxide and titrating an alcoholic solution of the products with mercuric nitrate using diphenylcarbazone as indicator (Mercurimetric method).

Sulphur was determined by combusting the sample in an oxygen flask containing hydrogen peroxide and water and titrating an alcoholic solution of the products with barium perchlorate using the mixed indicator THORON and methylene blue.

- Toluene was dried over sodium; THF was pre-dried with anhydrous sodium sulphate and distilled over calcium hydride or sodium benzophenone ketyl.

- All solutions were dried over anhydrous sodium sulphate or anhydrous magnesium sulphate and filtered.

- Thin layer chromatography was carried out on plastic sheets pre-coated with 0.25mm silica gel containing fluorescent indicator UV_{254}, or with 0.2mm aluminium oxide also with fluorescent indicator UV_{254}, both supplied by CAMLAB.

9-Amino-1,2,3,4-tetrahydroacridine (36)

(a) Using anhydrous zinc chloride[149]

Anthranilonitrile (150) (10.0g, 84.7mmol) and cyclohexanone (116) (10.0g, 102.0mmol) were placed in toluene (100ml) in a three-necked round-bottomed flask fitted with an overhead stirrer and reflux condenser. Anhydrous zinc chloride (23.0g, 169mmol) was then added and the mixture stirred and heated at reflux for 10min. After cooling, the toluene was decanted off and the remaining solids were washed with cold toluene. The solids were then heated at reflux with sodium hydroxide (2M, 120ml) for 1h, cooled and organic components were extracted with chloroform. After drying, the solvent was evaporated *in vacuo* to give the title compound (36) (13.6g, 81% crude).

(b) Using zinc chloride diethyl etherate

Anthranilonitrile (150) (5.0g, 42.3mmol) and cyclohexanone (116) (5.0g, 51.0mmol) were suspended in toluene (120ml) in a three-necked round-bottomed flask fitted with an overhead stirrer and reflux condenser. Zinc chloride diethyl etherate (1M, 51ml, 51.0mmols) was added *via* syringe and the reaction heated at reflux for 24h. The toluene was decanted off and the remaining solids were heated at reflux with sodium hydroxide (2M, 120ml) for 24h. After cooling the organic components were extracted with chloroform, the solution dried and the solvent evaporated *in vacuo* to give the title compound (36) (4.3g, 52% crude).

(c) Using tin(iv) chloride

Anthranilonitrile (2.0g, 16.9mmol) and cyclohexanone (1.8g, 18.6mmol) were placed in toluene (120ml) in a three-necked round-bottomed flask fitted with a reflux condenser. Under a nitrogen atmosphere tin(iv) chloride (2.2ml, 18.6mmol) was added*via* syringe and the stirred reaction was heated at reflux for 24h. The toluene was decanted off and the remaining solids heated at reflux with sodium hydroxide (2M, 120ml) for 5h. The organic components were extracted with chloroform and the organic layers were combined, dried and the solvent evaporated *in vacuo* to give the title compound(36) (1.3g,39% crude).

(d) Using titanium(IV) chloride

Anthranilonitrile (150) (2.0g, 16.9mmol) and cyclohexanone (116) (1.8g, 18.6mmol) were placed in a three-necked round-bottomed flask fitted with a reflux condenser. Toluene (85ml) was added and, under a nitrogen atmosphere, titanium(IV) chloride (2.1ml, 18.6mmol). The reaction became viscous and a yellow solid precipitated out which turned brown after heating at reflux for 2h. The toluene was decanted off and the remaining solids were heated at reflux with sodium hydroxide (2M, 90ml) for 2h. The organic components were extracted with chloroform and the organic layers were combined, dried and the solvent evaporated *in vacuo* to give the title compound (36) (1.81g, 54% crude).

(e) Using polyphosphoric acid (PPA)

In a three-necked round-bottomed flask fitted with an overhead stirrer and drying tube, phosphorus pentoxide (101.1g, 1.39mmol) was added to orthophosphoric acid [H_3PO_4, (d = 1.69), 62.4ml, 0.9mol] and stirred vigorously. The reaction became very hot and within 30 min clear, viscous PPA was formed. When the reagent was cool, anthranilonitrile (150) (5.0g, 42.3mmol) and cyclohexanone (116) (5.0g, 51.0mmol) were added and the mixture stirred at RT. At this temperature a yellowish colour formed, but when a sample was removed and analysed by TLC chromatography it was found to consist of starting materials. The reaction temperature was then raised to 100°C and stirred for 24h. Again, a sample was taken for analysis and showed a single fluorescent spot on TLC analysis. The reaction was terminated and the mixture poured onto iced water and stirred for 1h. The resulting precipitate was filtered to give a greenish solid. On recrystallisation from ethyl acetate/ethanol (1:1) this gave a yellow solid 4.1g, 50%, (m.p. 215 - 217°C). The product did not correspond to the title compound (36) on analysis.

(f) Using boron trifluoride diethyl etherate

Anthranilonitrile (150) (5.0g, 42.3mmol) and cyclohexanone (116) (5.0g, 51.0mmol) were placed in a three-necked round-bottomed flask and toluene (100ml) was added. Boron trifluoride diethyl etherate (1M, 5ml, 40mmol) was added and the mixture heated at reflux for 5h. The toluene was decanted off and the remaining solids were stirred and heated at reflux with sodium hydroxide (2M, 100ml) for about 1h. The alkaline solution was repeatedly extracted with diethyl ether. The ethereal layers were combined, dried and the solvent evaporated *in vacuo* to give the title compound (36) (7.4g, 89% crude).

Characterisation of 9-amino-1,2,3,4-tetrahydroacridine

Recrystallised: m.p. 178 - 180°C (aq. EtOH) [lit.[212] 178 - 179°C]

Found: C, 78.3; H, 7.5; N, 13.9; M^+, 198.1144. Calculated for $C_{13}H_{14}N_2$: C, 78.75; H, 7.1; N, 14.1%; M^+, 198.1157.

δ_H(400MHz, $CDCl_3$) 1.86 - 1.95 (4H, m, 2C*H*$_2$), 2.57 (2H, t, J = 6.2, C*H*$_2$), 3.01 (2H, t, J = 5.5, C*H*$_2$), 4.68 (2H, br s, exchanges with D_2O, NH_2), 7.33 (1H, td, J = 8.2, 1.2, aryl-*H*), 7.54 (1H, td, J = 6.8, 1.3, aryl-*H*), 7.67 (1H, dd, J = 8.4, 0.8, aryl-*H*), 7.88 (1H, d, J = 8.4, aryl-*H*).

Maleate salt

M.p. 50 - 55°C

Found: C, 64.45; H, 5.8; N, 8.7. Calculated for $C_{13}H_{14}N_2.C_4H_4O_4$

C, 64.95; H, 5.8; N, 8.9%.

δ_H(250MHz, CD_3OD) 1.97 (4H, m, CH_2), 2.60 (2H, m, CH_2), 2.98 (2H, m, CH_2), 6.23 (2H, s, maleate), 7.59 (1H, m, aryl-*H*), 7.71 (1H, m, aryl-*H*), 7.84 (1H, m, aryl-*H*).

Hydrochloride salt

M.p. (dec) 252 - 256°C

Found: C, 58.0; H, 7.2; N, 10.5; Cl, 14.05. Calculated for $C_{13}H_{14}N_2$.HCl. $2H_2O$: C, 57.65; H, 7.1; N, 10.35; Cl, 13.1%.

GENERAL PROCEDURE FOR THE CYCLODEHYDRATION REACTION

Anthranilonitrile (150) , ketone (1.1 equivalents) and sodium-dried toluene (120ml) were placed in a three-necked round-bottomed flask fitted with an overhead stirrer. Boron trifluoride diethyl etherate (1.1 equivalents) was added slowly *via* syringe and the reaction mixture heated at reflux for 24h. On cooling, the toluene was decanted and, to liberate the product, the remaining solids were treated with sodium hydroxide (2M, 120ml) and heated at reflux for 24h. After cooling, the organic components were extracted with chloroform and the organic layers were combined, dried and the solvent evaporated *in vacuo* to give the desired product.

In each case, the compounds made were easily identifiable by tlc analysis where they showed as a characteristic baseline spot (solvent: ethyl acetate).

In each case, a sample was recrystallised from the stated solvent for analysis and pharmacological testing.

9-Amino-2,3-dihydro-[1H]-cyclopenta-[1,2-b]-quinoline (162)

Anthranilonitrile (150) (5.0g, 42.3mmol), cyclopentanone (161) (4.1g, 46.7mmol), boron trifluoride diethyl etherate (1M, 10ml, 81.8mmol) were treated according to the general procedure (pg. 183) to give the title compound (162) (3.2g, 41% crude).

Recrystallised: m.p. 180 - 182°C (aq. EtOH)

Found: C, 78.15; H, 6.5; N, 14.95. Calculated for $C_{12}H_{12}N_2$: C, 78.35; H, 6.55; N, 15.2%.

δ_H(250MHz, $CDCl_3$) 2.19 (2H, quintet, J = 7.6, CH_2), 2.88 (2H, t, J = 7.5, CH_2), 3.10 (2H, t, J =7.8, CH_2), 4.65 (2H br s, exchanges with D_2O, NH_2), 7.33 (1H, t, J = 8.05, aryl-*H*), 7.57 (1H, t, J = 8.11, aryl-*H*), 7.71 (1H, d, J = 8.3, aryl-*H*), 7.89 (1H, d, J = 8.4, aryl-*H*).

Maleate salt

M.p. 100 - 105°C.

Found: C, 63.9; H, 5.3; N, 9.3. Calculated for $C_{12}H_{12}N_2.C_4H_4O_4$: C, 64.0; H, 5.4; N, 9.3%.

δ_H(250MHz, CD_3OD) 2.34 (2H, quintet, J = 7.8, CH_2), 2.97 (2H, t, J = 7.1, CH_2), 3.23 (2H, t, J =7.8, CH_2), 6.23 (2H, s, maleate), 7.61 (1H, td, J = 6.9, 1.3, aryl-*H*), 7.35 (1H, d, J = 8.5, aryl-*H*), 7.86 (1H, td, J = 6.9, 1.2, aryl-*H*), 8.23 (1H, dd, J = 8.5, 0.7, aryl-*H*).

11-Amino-2,3,4,5-tetrahydro-[1H]-cyclohepta-[1,2-b]-quinoline (164)

Anthranilonitrile (150) (10.0g, 84.7mmol), cycloheptanone (163) (11.4g, 101.8mmol) and boron trifluoride diethyl etherate (1M, 12.5ml, 102.3mmol) were treated according to the general procedure (pg. 183) to give the title compound (164) (10.9g, 61% crude).

Recrystallised: m.p. 167 - 171°C (EtOH) (lit.[108] 178°C).

Found: C, 78.9; H, 7.55; N, 13.05. Calculated for $C_{14}H_{16}N_2$: C, 79.2; H, 7.6; N, 13.2%.

δ_H(250MHz, $CDCl_3$) 1.64 - 1.92 (6H, m, 3C*H*$_2$), 2.74 (2H, m, C*H*$_2$), 3.13 (2H, m, C*H*$_2$), 4.68 (2H, br s, exchanges with D_2O, N*H*$_2$), 7.39 (1H, td, *J* = 7.00, 1.22, aryl-*H*), 7.56 (1H, td, *J* = 6.89, 1.31, aryl-*H*), 7.68 (1H, dd, *J* =8.31, 0.74, aryl-*H*), 7.91 (1H, d, *J* = 8.41, 0.61, aryl-*H*).

Maleate salt

M.p. 161 - 163°C

Found: C, 65.5; H, 6.15; N, 8.4. Calculated for $C_{14}H_{16}N_2.C_4H_4O_4$: C, 65.8; H, 6.1; N, 8.5%.

δ_H(250MHz, CD_3OD) 1.65 - 1.96 (6H, m, 3C*H*$_2$), 2.91 (2H, m, C*H*$_2$), 3.13 (2H, m, C*H*$_2$), 6.25 (2H, s, maleate), 7.61(1H, td, *J* = 6.8, 1.4, aryl-*H*), 7.75 (1H, d, *J* =8.4, 0.5, aryl-*H*), 7.85 (1H, td, *J* = 6.7, 1.2, aryl-*H*), 8.28 (1H, dd, *J* = 8.5, 0.5, aryl-*H*).

12-Amino-1,2,3,4,5,6-hexahydrocycloocta-[1,2-b]-quinoline (166)

Anthranilonitrile (150) (5.0g, 42.3mmol), cyclooctanone (165) (6.1ml, 46.7mmol) and boron trifluoride diethyl etherate (1M, 5.6ml, 46.7mmol) were treated according to the general procedure (pg. 183) to give the title compound (166) (7.4g, 78% crude).

Recrystallised: m.p. 198 - 200°C (EtOH), (lit.[112] 198 - 200°C).

Found: C, 79.7; H, 8.25; N, 12.2. Calculated for $C_{15}H_{18}N_2$: C, 79.7; H, 8.0; N, 12.4%.

δ_H(250MHz, $CDCl_3$) 1.20 - 1.55 (4H, m, 2CH_2), 1.60 - 1.95 (4H, m, 2CH_2), 2.87 (2H, m, CH_2), 3.10 (2H, m, CH_2), 4.70 (2H, br s, exchanges with D_2O, NH_2), 7.39 (1H, td, J = 6.8, 1.3, aryl-*H*), 7.57 (1H, td, J = 8.3, 1.4, aryl-*H*), 7.70 (1H, dd, J = 8.3, 0.7, aryl-*H*), 7.95 (1H, dd, J = 8.4, 0.6, aryl-*H*).

Maleate salt

M.p. 168 - 170°C

Found: C, 66.4; H, 6.4; N, 8.0. Calculated for $C_{15}H_{18}N_2.C_4H_4O_4$: C, 66.65; H, 6.5; N, 8.2%.

δ_H(250MHz, CD_3OD) 1.35 - 1.56 (4H, m, 2CH_2), 1.57 - 1.95 (4H, m, 2CH_2), 2.9 - 3.0 (2H, m, CH_2), 3.05 - 3.15 (2H, m, 2CH_2), 6.24 (2H, s, maleate), 7.62 (1H, t, J = 8.1, aryl-*H*), 7.76 (1H, d, J = 7.8, aryl-*H*), 7.83 (1H, t, J = 6.7, aryl-*H*), 8.31 (1H, d, J = 8.4, aryl-*H*).

6-Amino-4,5-benzo-[5H]-cyclopenta-[1,2-b]-quinoline (168)

Anthranilonitrile (150) (4.6g, 38.9mmol), indan-1-one (167) (5.6g, 42.4mmol) and boron trifluoride diethyl etherate (1M, 5.3ml, 43.3mmol) were treated according to the general procedure (pg. 183) to give the title compound (168) (0.25g, 2.6% crude).

Recrystallised: m.p. (dec) 239 - 240°C (EtOH).

Found: C, 82.50; H, 5.2; N, 12.0; M^+, 232.1006. $C_{16}H_{12}N_2$ requires C, 82.7; H, 5.2; N, 12.1%; M^+, 232.1000.

δ_H(250MHz, CD_3OD) 3.78 (2H, s, C*H*$_2$), 7.20 - 7.69 (5H, m, aryl-*H*), 7.94 (1H, d, J = 8.5, aryl-*H*), 8.03 (1H, d, J = 8.4, aryl-*H*), 8.10 - 8.25 (1H, m, aryl-*H*).

Maleate salt

M.p. 212 - 214°C.

Found: C, 68.6; H, 4.5; N, 7.9. $C_{16}H_{12}N_2.C_4H_4O_4$ requires C 68.95; H, 4.6; N, 8.05%.

δ_H(250MHz, DMSO-*d*6) 3.91 (2H, s, C*H*$_2$), 2.60 - 4.70 (2H, br s, exchanges with D_2O, N*H*$_2$), 6.06 (2H, s, maleate), 7.50 - 8.00 (6H, m, aryl-*H*), 8.18 (1H, d, J = 7.2, aryl-*H*), 8.43 (1H, d, J = 8.4, aryl-*H*), 8.71 (2H, br s, exchanges with D_2O, maleate).

11-Amino-1,2-dihydro-benz-[c]-acridine (170)

Anthranilonitrile (150) (2.0g, 16.9mmol), 1-tetralone (3,4-dihydro-[*2H*]-naphthalen-1-one) (169) (2.7g, 18.5mmol) and boron trifluoride diethyl etherate (1M, 2.3ml, 18.8mmol) were treated according to the general procedure (pg. 183) to give the title compound (170) (3.75g, 90% crude).

Recrystallised: m.p. 138 - 140°C (EtOH).

Found: C, 83.1; H, 5.8; N, 11.3; M^+, 246.1159. $C_{17}H_{14}N_2$ requires C, 82.9; H, 5.7; N, 11.3%; M^+, 246.1157.

δ_H(90MHz, $CDCl_3$) 2.6 - 3.1 (4H, m, $2CH_2$), 4.55 (2H, br s, exchanges with D_2O, NH_2), 7.10 - 7.80 (6H, m, aryl-*H*), 8.00 - 8.20 (1H, m, aryl-*H*), 8.5 - 8.7 (1H, m, aryl-*H*).

Maleate salt

M.p. 207 - 209°C.

Found: C, 69.90; H, 4.8; N, 7.6; $C_{17}H_{14}N_2.C_4H_4O_4$ requires C, 69.6; H, 5.0; N, 7.7%.

δ_H(250MHz, CD_3OD) 2.7 - 3.2 (4H, m, CH_2), 6.22 (2H, s, maleate), 7.3 - 7.7 (4H, m, aryl-*H*), 7.87 (1H, t, J = 7.3, aryl-*H*), 7.95 - 8.2 (2H, m, aryl-*H*), 8.35 (1H, d, J = 7.6, aryl-*H*).

11-Amino-1,2-dihydro-3-methoxybenz-[c]-acridine (172)

Anthranilonitrile (150) (5.0g, 42.3mmol), 5-methoxy-1-tetralone (171) (8.1g, 46.0mmol) and boron trifluoride diethyl etherate (1M, 5.7ml, 46.6mmol) were treated according to general procedure (pg. 183) to give the title compound (172) (5.5g, 47% crude).

Recrystallised: m.p. 126 - 129°C ($CHCl_3$).

Found: C, 78.3; H, 5.65; N, 10.0; M^+, 276.1265. $C_{18}H_{16}N_2O$ requires C, 78.2; H, 5.8; N, 10.1%; M^+, 276.1263.

δ_H(90MHz, $CDCl_3$) 2.5 - 3.2 (4H, m, 2C*H*$_2$), 3.9 (3H, s, C*H*$_3$), 4.6 (2H, br s, exchanges with D_2O, N*H*$_2$), 6.8 - 7.0 (1H, d, J = 9, aryl-*H*), 7.2 - 7.8 (2H, m, aryl-*H*), 8.0 - 8.35 (1H, m, aryl-*H*).

Maleate salt

M.p. (dec.) 210 - 216°C.

Found: C, 67.3; H, 5.0; N, 7.1. $C_{18}H_{16}N_2O.C_4H_4O_4$ requires C, 67.35; H, 5.1; N, 7.1%.

δ_H(250MHz, DMSO-*d6*) 2.70 - 3.05 (4H, m, C*H*$_2$), 3.89 (3H, s, C*H*$_2$), 6.03 (2H, s, maleate), 7.29 (1H, d, J = 8.1, aryl-*H*), 7.52 (1H, t, J = 8.0, aryl-*H*), 7.64 (1H, t, J = 7.3, aryl-*H*), 7.81 (1H, d, J = 7.8, aryl-*H*), 7.90 (1H, t, J = 7.4, aryl-*H*), 7.13 (1H, d, J = 8.3, aryl-*H*), 8.45 (1H, d, J = 8.3, aryl-*H*), 8.57 (2H, br s, maleate).

11-Amino-1,2-dihydro-4-chlorobenz-[c]-acridine (174)

Anthranilonitrile (150) (5.0g, 42.3mmol), 6-chloro-1-tetralone (173) (8.8g, 48.7mmol) and boron trifluoride diethyl etherate (1M, 6.4ml, 52.4mmol) were treated according to the general procedure (pg. 183) to give the title compound (174) 3.3g, 28% (crude).

Recrystallised: m.p. 205 - 208°C (EtOH).

Found: C, 73.0; H, 4.6; N, 10.3; M^+, 280.0769. $C_{17}H_{15}N_2Cl$ requires C, 72.7; H, 4.7; N, 10.0%; M^+, 280.0767.

δ_H(90MHz, $CDCl_3$) 2.7 - 3.1 (4H, m, 2CH_2), 4.6 (2H, br s, exchanges with D_2O, NH_2), 7.1 - 8.2 (6H, m, aryl-*H*), 8.5 (1H, d, J = 8.1, aryl-*H*).

Maleate salt

M.p. (dec.) 220 - 221°C.

Found: C, 63.7; H, 4.05; N, 6.9; Cl, 9.1. $C_{17}H_{15}N_2Cl.C_4H_4O_4$ requires C, 63.6; H, 4.3; N, 7.05; Cl, 8.9%.

δ_H(250MHz, DMSO-d_6) 2.30 - 3.06 (4H, m, 2CH_2), 3.34 (2H, br s, NH_2), 6.05 (2H, s, maleate), 7.55 - 7.70 (3H, m, aryl-*H*), 7.88 (1H, t, J = 7.9, aryl-*H*), 8.07 (1H, d, J = 8.5, aryl-*H*), 8.19 (1H, d, J = 8.1, aryl-*H*), 8.43 (1H, d, J = 8.3, aryl-*H*), 8.51 (2H, br s, maleate).

8-Amino-6,7-dihydro-[5H]-benzo-[6,7]-cycloheptа-[1,2-b]-quinoline (176)

Anthranilonitrile (150) (2.0g, 16.9mmol), 1-benzosuberone (6,7,8,9-tetrahydrobenzocyclohepten-5-one) (179) (3.0g, 18.7mmol) and boron trifluoride diethyl etherate (1M, 2.6ml, 18.7mmol) were treated according to the general procedure (pg 183) to give the title compound (176) (3.5g, 80% crude).

Recrystallised: m.p. 190 - 192°C (EtOH).

Found: C, 83.5; H, 6.1; N, 10.6; M^+, 260.1304. $C_{18}H_{16}N_2$ requires C, 83.15; H, 6.2; N, 10.8%; M^+, 260.1314.

δ_H(90MHz, DMSO-d_6): 1.9 - 2.8 (6H, m, 3CH_3), 6.55 (2H, br s, exchanges with D_2O, NH_2), 7.1 - 7.95 (7H, m, aryl-*H*), 8.2 - 8.35 (1H, m, aryl-*H*).

Maleate salt

M.p. 186 - 188°C.

Found: C, 70.2; H, 5.4; N, 7.4. $C_{18}H_{16}N_2.C_4H_4O_4$ requires C, 70.2; H, 5.35; N, 7.4%.

δ_H(250MHz, CD_3OD) 2.25 (2H, br s, CH_2), 2.63 (2H, br s, CH_2), 5.07 (2H, br s, CH_2), 6.19 (2H, s, maleate), 7.35 - 8.05 (7H, m, aryl-*H*), 8.35 (1H, d, J = 8.4, aryl-*H*).

8-Amino-6,7-dihydro-3-methoxy-[5H]-benzo-[6,7]-cyclohepta-[1,2-b]-quinoline (178)

Anthranilonitrile (150) (5.0g, 42.3mmol), 7-methoxy-1-benzosuberone (177) (8.7g, 45.8mmol) and boron trifluoride diethyl etherate (1M, 6.2ml, 50.7mmol) were treated according to the general procedure (pg. 183) to give the title compound (178) (9.1g, 75% crude).

Recrystallised: m.p. 206 - 210°C (EtOH).

Found: C, 78.9; H, 6.4; N, 9.6; M^+, 290.1419. $C_{19}H_{18}N_2O$ requires C, 78.60 H, 6.2; N, 9.6%, M^+, 290.1419.

δ_H(90MHz, DMSO-d_6) 1.8 - 2.8 (6H, m, 3CH_2), 3.85 (3H, s, CH_3), 6.5 (2H, br s, exchanges with D_2O, NH_2), 6.75 - 8.4 (7H, m, aryl-*H*).

Maleate salt

M.p. 216 - 218°C.

Found: C, 67.6; H, 5.2; N, 6.6. $C_{19}H_{18}N_2O.C_4H_4O_4$ requires C, 68.0; H, 5.45; N, 6.9%.

δ_H(250MHz, CD_3OD) 2.20 - 2.75 (6H, m, 3CH_3), 3.89 (3H, s, CH_3), 6.21 (2H, s, maleate), 6.95 - 7.10 (2H, m, aryl-*H*), 7.60 - 7.72 (2H, m, aryl-*H*), 7.85 - 7.95 (2H, m, aryl-*H*), 8.35 (1H, d, J = 8.7, aryl-*H*).

Attempted preparation of 11-amino-3,4-dihydro-benz-[a]-acridine (180)

Anthranilonitrile (150) (5.0g, 42.3mmol), freshly distilled 2-tetralone (3,4-dihydro-[*1H*]-naphthalen-2-one) (179) (6.2ml, 46.7mmol) and boron trifluoride diethyl etherate (1M, 5.7ml, 46.7mmol) were treated according to the general procedure (pg. 183) to give an insoluble dark brown solid.
IR analysis showed no discernable features and the solids were insoluble in acid.

Methyl triphenyl phosphonium iodide (184)

To a cooled, stirred solution of triphenylphosphine (0.2g, 0.09mol) in anhydrous toluene (100ml) was added methyl iodide (6.7ml, 0.108mol) in toluene (100ml) dropwise over 1.5h. The cloudy solution was then stirred at RT for 24h. The resulting white precipitate was then filtered and washed thoroughly with toluene then washed with anhydrous diethyl ether to give the title compound (184) (35.9g, 98.8%).

1,2,3,4-Tetrahydro-1-methylene-naphthalene (185)

The above phosphonium salt $^{+}PPh_3CH_2\ I^{-}$ (184) (10g, 24.8mmol) was suspended in anhydrous THF (75 ml) in a round-bottomed flask fitted with a magnetic stirrer. The reaction was cooled to 0°C and *n*-butyllithium (1.5M solution in hexanes, 15.5ml, 24.8mmol) was added dropwise *via* syringe, giving a yellow solution which was stirred at RT for 45min. 1-Tetralone (169) (3.3ml, 24.8mmol) was added dropwise at RT and then stirred for 2h. The reaction was then heated at reflux for 45min and then stirred overnight at RT. The THF was evaporated *in vacuo* and the residue taken up in chloroform, washed with water, dried, and the solvent

evaporated *in vacuo* to give a purple oil (14.0g). This was purified by Kugelröhr distillation (0.2mmHg, 65°C) to give the title compound (185) as a clear oil (1.9g, 54%).

δ_H(250MHz, $CDCl_3$): 1.9 (m, 2H, CH_2), 2.5 (t, 2H, CH_2), 2.8 (t, 2H, CH_2), 4.95 (s, 1H, C*H*), 5.45 (s, 1H, C*H*), 7.0 - 7.6 (m, 4H, aryl-*H*).

2,3,4,5-Tetrahydrobenzocyclohepten-[1H]-2-one (192)

Silver nitrate (17.9g, 92mmol) was stirred vigorously in methanol (50ml) and water (50ml) until it had dissolved. A solution of 1,2,3,4-tetrahydro-1-methylene-naphthalene (185) (6.6g, 45.8mmol) in THF (100 ml) was added, followed immediately by iodine (12.7g, 50mmol). The mixture was stirred at RT for 30min, filtered through a pad of kieselguhr and the filtrate concentrated by the removal of the solvent *in vacuo*. The concentrate was dissolved in diethyl ether, washed first with water, then saturated sodium hydrogen carbonate solution, and finally with water. The ethereal solution was then dried and the solvent evaporated *in vacuo* to give the title compound (192) as a clear oil (3.9g, 53% crude).

δ_H(90MHz, $CDCl_3$) 2.0 (2H, m, CH_2), 2.55 (2H, t, CH_2), 3.0 (2H, m, CH_2), 3.8 (2H, s, CH_2), 7.2 (4H, s, aryl-*H*).

ν_{max}/cm^{-1} 1705 C=O stretch.

13-Amino-6,7-dihydro-[5H]-benzo-[3,4]-cyclohepta-[1,2-b]-quinoline (183)

Anthranilonitrile (150) (1.0g, 8.5mmol), 2-benzosuberone (182) (1.5g, 9.4mmol) and boron trifluoride diethyl etherate (1M complex, 1.2ml, 9.8mmol) were treated according to the general procedure (pg. 183) to give the title compound (183) 0.7g, 30% (crude).

Recrystallised: m.p. 177 - 179°C (EtOH).

Found: C, 82.85; H, 6.4; N, 10.55; M^+, 260.1315. $C_{18}H_{16}N_2$ requires C, 83.05; H, 6.2; N, 10.75; M^+, 260.1314.

δ_H(250MHz,$CDCl_3$) 2.08 - 2.68 (5H, m), 2.82 - 2.90 (1H, m), 4.96 (2H, br s, exchanges with D_2O, N*H*$_2$), 7.34 - 7.66 (6H, m, aryl-*H*), 7.79 (1H, d, J = 8.35, aryl-*H*), 7.00 (1H, d, J = 8.43, aryl-*H*).

N-(3-oxocyclohexen-1-yl)-2-aminobenzonitrile (152)[145]

Anthranilonitrile (150) (5.0g, 42.3mmol), cyclohexan-1,3-dione (151) (5.6g, 50.0mmol) and *p*-toluenesulphonic acid (0.25g, 1.5mmol) were placed in toluene (25ml) and heated at reflux using a Dean and Stark apparatus for 2h. The reaction mixture was cooled to 0°C and the product was filtered and washed with cold toluene. The yellow solid was stirred with water for 1h, filtered and dried *in vacuo* to give the title compound (152) (6.9g, 81%).

Recrystallised: m.p. 187 - 189°C (CH_2Cl_2:*n*-hexane), (lit[145] 188 - 189°C).

δ_H(90MHz, $CDCl_3$) 2.10 (2H, q, J = 7.2, CH_2), 2.40 (2H, t, J = 5.4, CH_2), 2.62 (2H, t, J = 6.3, CH_2), 5.48 (1H, s, vinylic CH),
7.10 - 7.75 (5H, m, 4H, aryl-H, NH, 1H exchanges with D_2O).

ν_{max}/cm^{-1} 3200 NH, 2250 C N, 1550 conj. C=O.

9-Amino-3,4-dihydro-[2H]-acridin-1-one[145](153)

N-(3-oxocyclohexenyl)-2-aminobenzonitrile (152) (3.0g, 14.2mmol) was suspended in THF (50ml). Anhydrous potassium carbonate (0.5g, 3.6mmol) and cuprous chloride (0.1g, 0.74mmol) were added and the mixture was heated at reflux for 5h. The hot mixture was then filtered into *n*-hexane (50ml). The resultant precipitate was filtered, washed with water and dried to give the title compound (153) (2.1g, 68% crude).

Recrystallised: m.p. 234 - 236°C (EtOH), (lit.[145] 236 - 238°C).

δ_H(90MHz, DMSO-d_6) 2.00 - 2.35 (2H, m, CH_2), 2.79 (2H, t, J = 7.2, CH_2), 3.11 (2H, t, J = 5.4, CH_2), 7.4 - 7.9 (3H, m, aryl-H), 8.45 (1H, d, J = 9.0, aryl-H), 10.3 (2H, br s, exchanges with D_2O, H-bonded NH_2).

ν_{max}/cm^{-1} 3260 NH_2, asymm. str, 3100 NH_2, symm. str.
1625 C=O stretch.

9-Amino 1,2,3,4-tetrahydro-acridin-1-ol maleate (154)[145]

9-amino-3,4-dihydro-[2*H*]-acridin-1-one (153) (5.0g, 23.6mmol) was suspended in THF (100ml) and a solution of lithium aluminium hydride (0.9g, 23.8mmol) in anhydrous diethyl ether (50ml) was added dropwise over 20min. The reaction was stirred for 2h then quenched by the addition of 10% hydrochloric acid (50ml). The mixture was then made strongly basic by the addition of sodium hydroxide (2M, 100ml) giving the free base of the product which was subsequently filtered. The solid was placed into *iso*-propanol with maleic acid (3.3 equivalents, 6.0g, 51.7mmol) and heated at reflux for 2h. The solution was filtered and the solvent evaporated *in vacuo* to give the salt of the title compound (154) (3.7g, 72%)

Recrystallised: m.p. 173 - 175°C (EtOAc/MeOH) (lit.[145] 171 - 173°C).

δ_H(90MHz, DMSO-d_6) 2.10 (br s, 4H), 2.6 (br s, 1H), 3.10 (br s, 2H), 4.95 (br s, 2H), 6.15 (s, 2H, maleic acid), 7.50 - 8.05 (m, 3H), 8.4 - 8.7 (m, 3H, aryl-*H*, maleic acid).

Found: C, 61.45; H, 5.6; N, 8.35. Calculated for $C_{13}H_{14}N_2O.C_4H_4O_4$ C, 61.8; H, 5.5; N, 8.5%.

1-Amino-2-cyano-cyclopent-1-ene (207) [148]

In a three-necked round-bottomed flask, fitted with a stirring bar, was placed potassium hydride (35% disp. in mineral oil, 5.7g, 49.9mmol), under a dry nitrogen atmosphere. The oil was replaced with THF (30ml) and the mixture placed in a water bath at 20°C. 1,4-Dicyanobutane (206) (5.7ml, 50.0mmol) was added in one

portion and the reaction mixture stirred for 4h, after which it was cooled to 0°C and quenched with H_2O (caution!). The organic layer was separated, dried and the solvent evaporated *in vacuo* to give the title compound (207) (2.3g, 42%).
M.p. 145 - 147°C (lit.[148] 147 - 148°C).

δ_H(90MHz, DMSO-d_6) 1.8 (2H, m, CH_2), 2.4 (4H, m, 2CH_2),
6.35 (2H, br s, exchanges with D_2O, NH_2).

Attempted preparation of 9-amino-2,3,5,6,7,8-hexahydro-[1H]-cyclopenta-[b]-quinoline (208)

1-Amino-2-cyanocyclopent-1-ene (207) (1.0g, 9.3mmol), cyclohexanone (116) (1.0g, 10.2mmol) and boron trifluoride diethyl etherate (1M, 1.3ml, 10.2mmol) were treated according to the general procedure (pg 183) to give black solids which could not be characterised.

9-Amino-1,4-methano-1,2,3,4-tetrahydroacridine (211)

Anthranilonitrile (150) (5.0g, 42.3mmol), norcamphor (210) (5.0g, 45.5mmol) and boron trifluoride diethyl etherate (1M, 5.1ml, 41.7mmol) were treated according to the general procedure (pg. 183) to give the title compound (211) 2.5g, 29% crude).

Recrystallised: m.p. 186-187°C (EtOH) (lit[172] 186 - 188°C)

M^+, 210.1158, $C_{14}H_{14}N_2$ requires M^+, 210.1157.

δ_H(400MHz, $CDCl_3$) 1.31 - 1.36 (1H, m, CH_2), 1.42 - 1.46 (1H, m, CH_2), 1.66 (1H, dt, J = 9.04, 1.52, CH_2), 1.87 - 1.90 (1H, m, CH_2), 1.96 - 2.08 (2H, m, CH_2), 3.49 (2H, d, J = 11.44, CH_2), 4.54 (2H, br s, exchanges with D_2O, NH_2), 7.39 (1H, t, J = 7.00, aryl-*H*), 7.57 (1H, t, J = 6.96, aryl-*H*), 7.71 (1H, d, J = 8.32, aryl-*H*), 7.96 (1H, d, J = 8.44, aryl-*H*).

$\delta_{(C-H)}$ (100MHz, $CDCl_3$) 26.46 (CH_2), 27.03 (CH_2), 38.73 (*C*H), 46.18 (*C*H), 46.85 (CH_2), 119.15 (*C*, aryl-*C*), 119.65 (*C*, aryl-*C*), 120.32 (*C*H, aryl-*C*), 124.19 (*C*H, aryl-*C*), 128.26 (*C*H, aryl-*C*), 129.34 (*C*H, aryl-*C*), 140.70 (*C*, aryl-*C*), 147.86 (*C*, aryl-*C*), 170.78 (*C*, aryl-*C*).

8-Amino-6,7-dihydro-5-N-tosyl-[3,2-b]-quinolino-[4,5-d]-5H-1-benzazepine (230)

Anthranilonitrile (150) (2.0g, 16.9mmol), 1,2,3,4-tetrahydro-1-N-tosyl-benzazepin-5-one (227)[174] (5.9g, 18.9mmol) and boron trifluoride diethyl etherate (1M, 2.3ml, 18.8mmol) were treated according to the general procedure (pg. 183) to give the title compound (230) (5.4g, 76% crude).

Recrystallised: m.p. >250°C, (EtOH/MeOH).

Found: C, 69.6; H, 5.1; N, 10.0; S, 7.7; M^+, 415.1347. $C_{24}H_{21}N_3O_2S$ requires C, 69.4; H, 5.1; N, 10.1; S, 7.0%; M^+, 415.1355.

δ_H(400MHz, DMSO-d_6) 1.76 (3H, s, Ts-CH_3), 2.30 (1H, br s, CH_2), 3.10 (1H, br s, CH_2), 3.91 (1H, br s, CH_2), 4.20 (1H, br s, CH_2), 6.45 (2H, br s, exchanges with D_2O, NH_2), 6.51 (2H, d, J = 7.9, Ts-aryl-*H*), 6.92 (2H, dt, J = 8.3, 2.0, Ts-aryl-*H*), 7.36 (1H, ddd, J = 6.7, 1.3,

aryl-*H*), 7.39 - 7.41 (1H, m, aryl-*H*), 7.51 - 7.58 (3H, m, aryl-*H*), 7.69 (1H, dd, *J* = 8.4, 0.7, aryl-*H*), 7.72 - 7.74 (1H, m, aryl-*H*),
8.06 (1H, dd, *J* = 8.3, 0.8, aryl-*H*).

$\delta_{(C-H)}$ (100MHz, DMSO-*d6*) 20.58 (*C*H_3), 24.50 (*C*H_2),
53.70 (*C*H_2), 108.20 (*C*, aryl-*C*), 117.82 (*C*, aryl-*C*), 122.06 (*C*H, aryl-*C*), 123.53 (*C*H, aryl-*C*), 125.63 (2*C*H, aryl-*C*), 128.17 (*C*H, aryl-*C*),
128.36 (2*C*H, aryl-*C*), 128.72 (*C*H, aryl-*C*), 128.97, (*C*H, aryl-*C*),
129.23 (*C*H, aryl-*C*), 129.74 (*C*H, aryl-*C*), 131.79 (*C*H, aryl-*C*),
135.16 (*C*, aryl-*C*), 136.37 (*C*, aryl-*C*), 140.98 (*C*, aryl-*C*), 141.92 (*C*, aryl-*C*),
147.33 (*C*, aryl-*C*), 147.74 (*C*, aryl-*C*), 157.00 (*C*, aryl-*C*).

8-Amino-6,7-dihydro-7-N-tosyl-[3,2-b]-quinolino-[4,5-d]-[5H]-3-benzazepine (231)

Anthranilonitrile (150) (0.7g, 6.3mmol), 1,2,3,4-tetrahydro-3-N-tosyl-benzazepin-5-one[175] (229) (2.0g, 6.4mmol) and boron trifluoride diethyl etherate (1M, 0.8ml, 6.5mmol) were treated according to the general procedure (pg. 183) to give the title compound (231) (1.6g, 63% crude).

Chromatography of a sample on neutral alumina with chloroform (100%), chloroform/methanol (1:1), and then ethanol (100%) as eluents yielded a pure amount of the title compound (231).
M.p. 227-229°C.

Found: C, 69.3; H, 5.05; N, 10.0; S, 7.7; M^+, 415.1329. $C_{24}H_{21}N_3O_2S$ requires C, 69.4; H, 5.1; N, 10.1; S, 7.70%; M^+, 415.1355.

δ_H(400MHz, $CDCl_3$) 2.32 (3H, s, Ts-CH_3), 2.58 (1H, dd, J = 14.24, 4.48, CH_2), 2.71 (1H, td, J = 13.78, 6.28, CH_2), 4.14 - 4.18 (1H, m, CH_2), 4.47 (1H, td, J = 13.68, 5.32, CH_2), 5.61 (2H, br s, exchanges with D_2O, NH_2), 6.83 (2H, d, J = 8.24 aryl-*H*), 6.98 (3H, dt, J = 8.36, 1.8, aryl-*H*), 7.04 (1H, td, J = 7.52, 1.2, aryl-*H*), 7.13 (1H, td, J = 7.42, 1.52, aryl-*H*), 7.17 (1H, dd, J = 7.51, 1.4, aryl-*H*), 7.49 (1H, ddd, J = 7.62, 1.36, aryl-*H*), 7.69 (1H, ddd, J = 7.7, 1.32, aryl-*H*), 7.84 (1H, dd, J = 8.42, 0.8, aryl-*H*), 8.01 (1H, dd, J = 8.44, 0.72, aryl-*H*).

$\delta_{(C-H)}$(100MHz, $CDCl_3$) 21.64 (*C*H_3), 29.90 (*C*H_2), 32.21 (*C*H_2), 111.97 (*C*, aryl-*C*), 118.62 (*C*, aryl-*C*), 121.30 (*C*H, aryl-*C*), 125.28 (*C*H, aryl-*C*), 127.02 (2*C*H, aryl-*C*), 127.46 (*C*H, aryl-*C*), 127.91 (*C*H, aryl-*C*), 128.90 (*C*H, aryl-*C*), 128.97 (*C*H, aryl-*C*), 129.51 (2*C*H, aryl-*C*), 130.37 (*C*H, aryl-*C*), 135.58 (*C*, aryl-*C*), 136.44 (*C*, aryl-*C*), 139.77 (*C*, aryl-*C*), 142.87 (*C*, aryl-*C*), 148.01 (*C*, aryl-*C*), 150.85 (*C*, aryl-*C*), 159.18 (*C*, aryl-*C*).

7-Amino-3-chloro-dibenzo-[b,h][1,6]-naphthyridine (220)

Anthranilonitrile (150) (0.3g, 2.5mmol), 4-amino-7-chloro-1,2,3,4-tetrahydroquinoline[173] (219) (0.5g, 2.8mmol) and boron trifluoride diethyl etherate (1M, 0.34ml, 2.8mmol) were treated according to the general procedure (pg. 183) to give the title compound (220) (0.15g, 21% yield crude).

Recrystallised: m.p. >220°C (MeOH).

Found: C, 68.5; H, 4.7; N, 12.9; Cl, 12.2. $C_{16}H_{10}N_3Cl$ requires C, 68.70 H, 3.6; N, 15.0; Cl, 12.7%.

δ_H(400MHz, DMSO-d_6) 7.49 (1H, ddd, J = 8.24, 1.24, aryl-*H*),
7.66 (1H, dd, J = 8.7, 2.2, aryl-*H*), 7.82 (1H, ddd, J = 8.4, 1.3, aryl-*H*),
7.98 (2H, m, aryl-*H*), 8.43 (2H, br s, exchanges with D_2O, N*H*$_2$),
8.51 (1H, dd, J = 8.5, 0.8, aryl-*H*), 9.04 (1H, d, J = 8.7, aryl-*H*),
9.76 (1H, s, aryl-*H*).

$\delta_{(C-H)}$ (100MHz, DMSO-d_6) 104.53 (*C*, aryl-*C*), 115.60 (*C*, aryl-*C*), 123.33
(*C*H, aryl-*C*), 123.41 (*C*H, aryl-*C*), 123.53 (*C*, aryl-*C*),
126.01 (*C*H, aryl-*C*), 126.42 (*C*H, aryl-*C*), 127.41 (*C*H, aryl-*C*),
128.60 (*C*H, aryl-*C*), 131.85 (*C*H, aryl-*C*), 134.47 (*C*, aryl-*C*),
146.93 (*C*, aryl-*C*), 149.00 (*C*, aryl-*C*), 151.09 (*C*H, aryl-*C*), 152.64 (*C*, aryl-*C*).

7-Amino-3-chloro-5,6-dihydro-5-N-tosyl-dibenz-[b,h][1,6]-naphthyridine (221)

Anthranilonitrile (150) (0.6g, 5.4mmol), 4-amino-7-chloro-1,2,3,4-tetrahydro-N-tosyl-quinoline[173] (218) (2.0g, 5.9mmol) and boron trifluoride diethyl etherate (1M, 0.7ml, 5.7mmol) were treated according to the general procedure (pg. 183) to give the title compound (221) (3.4g, 60% crude).

Recrystallised: m.p. 246 - 248°C (EtOH).

Found: C, 63.3; H, 4.05; N, 9.4; S, 6.75; Cl, 8.6; M^+, 435.0805.
$C_{23}H_{18}N_3O_2SCl$ requires C, 63.4; H, 4.2; N, 9.6; S, 7.35;
Cl, 8.1%; M^+, 435.0808.

δ_H (250MHz, DMSO-d_6) 1.70 (3H, s, C*H*$_3$), 4.94 (2H, s, C*H*$_2$),

6.68 (2H, d, J = 8.26, Ts-H, aryl-*H*), 6.87 (2H, br s, exchanges with D_2O, NH_2), 7.06 (2H, d, J = 8.19, Ts-H, aryl-*H*), 7.47 - 7.68 (5H, m, aryl-*H*), 8.11 (1H, d, J = 8.35, aryl-*H*), 8.21 (1H, d, J = 8.43, aryl-*H*).

9-Amino-5,6,7,8-tetrahydro-5-N-tosyl-[3,2-b]-quinolino-[5,6-e]-1-benzazocine (233)

Anthranilonitrile (150) (0.5g, 4.5mmol), 2,3,4,5-tetrahydro-N-tosyl-[*1H*]-benzazocin-6-one[166] (232) (2.0g, 4.6mmol) and boron trifluoride diethyl etherate (1M, 0.56ml, 4.6mmol) were treated according to general procedure (pg. 183) to give the title compound (233) 0.7g, 35% (crude).
Recrystallised: m.p. >250°C (EtOAc/EtOH).

M^+ 429.1507. $C_{25}H_{12}N_3O_2S$ requires M^+ 429.1511.

δ_H(400MHz, $CDCl_3/CD_3OD$) 1.52 - 1.75 (2H, m, CH_2),
1.93 (3H, s, Ts-CH_3), 2.06 (1H, ddd, J = 14.2, 10.2, 1.4, CH_2),
2.59 (1H, dd, J = 7.5, CH_2), 3.06 - 3.17 (1H, m, CH_2),
4.32 (1H, dd, J = 15.0, 4.8, CH_2), 6.48 (1H, d, J = 7.9, aryl-*H*),
6.91 (2H, dt, J = 8.5, 2.0, Ts-H, aryl-*H*), 7.10 - 7.13 (1H, m, aryl-*H*),
7.26 - 7.37 (3H, m, aryl-*H*), 7.45 (1H, ddd, J = 6.8, 1.3, aryl-*H*),
7.49 - 7.52 (1H, m, aryl-*H*), 7.67 - 7.73 (1H, m, aryl-*H*).

$\delta_{(C-H)}$(100MHz, $CDCl_3/CD_3OH$) 20.99 (CH_3), 24.85 (CH_2),
26.46 (CH_2), 52.04 (CH_2), 113.24 (*C*, aryl-*C*), 117.78 (*C*, aryl-*C*),
120.40 (*C*H, aryl-*C*), 124.56 (*C*H, aryl-*C*), 126.45 (2*C*H, aryl-*C*).
127.30 (*C*H, aryl-*C*), 128.76 (3*C*H, aryl-*C*), 128.90 (*C*H, aryl-*C*),

130.13 (2*C*H, aryl-*C*), 130.25 (*C*H, aryl-*C*), 136.98 (*C*, aryl-*C*),
140.21 (*C*,aryl-*C*), 142.00 (*C*, aryl-*C*), 142.77 (*C*, aryl-*C*),
146.27 (*C*, aryl-*C*), 147.21 (*C*, aryl-*C*), 156.86 (*C*, aryl-*C*).

DETOSYLATION METHOD WITH SODIUM NAPHTHALENIDE[178]

Sodium naphthalenide was prepared by the addition, under nitrogen, of clean pieces of sodium metal to a stirred solution of naphthalene (1 equivalent) in anhydrous, degassed dimethoxyethane (DME). The suspension was stirred at room temperature for approximately 1.5h during which time the resultant solution had turned green-black. To this, the tosylated compound in dry DME was added and the solution stirred for 1h. Water (5ml) was added (caution!) and the reaction mixture was poured onto hydrochloric acid (10%, 250ml). This was washed thoroughly with dichloromethane to remove any remaining naphthalene. The aqueous layer was basified with sodium hydroxide (2M, 250ml) and extracted with dichloromethane. The organic extracts were then combined, dried and the solvent evaporated *in vacuo* to give the product.

8-Amino-6.7-dihydro-[5H]-[3,2-b]-quinolino-[4,5-d]-1-benzazepine (241)

Sodium (0.7g, 32.3mmol), naphthalene (4.1g, 32.0mmol) and degassed DME (120ml) were treated according to the above procedure (pg. 204), giving a solution of sodium naphthalenide to which was added the tosylated compound, 8-amino-6,7-dihydro-5-N-tosyl-[*5H*]-[*b*]-quino-[5,4]-1-benzazepine (230) (6.1g, 14.7mmol) in dry DME. After reaction the title compound (241) was isolated. (1.9g, 51.2% crude).

δ_H(250MHz, CD_3OD) 2.87 (2H, t, J = 6.09, CH_2),

3.64 (2H, t, J = 6.27, CH_2), 6.95 (1H, d, J = 7.86, aryl-*H*),

7.12 (1H, t, J = 7.54, aryl-*H*), 7.26 - 7.44 (2H, m, aryl-*H*),

7.59 (1H, t, J = 7.01, aryl-*H*), 7.74 (1H, d, J = 7.63, aryl-*H*),

7.90 (1H, d, J = 8.15, aryl-*H*), 8.07 (1H, d, J = 8.42, aryl-*H*).

M^+ 261.1259, $C_{17}H_{15}N_3$ requires M^+ 261.1266.

Hydrated hydrochloride salt formed for analysis, m.p. 308 - 313°C.

Found: C, 55.5; H, 5.9; N, 11.4; Cl, 19.7. $C_{17}H_{15}N_3.2HCl.\ 2H_2O$ requires C, 55.15; H, 5.7; N, 11.3; Cl, 19.15%.

δ_H(250MHz, CD_3OD/D_2O) 2.98 (2H, t, J = 6.56Hz, CH_2),

4.00 (2H, t, J = 7.23, CH_2), 7.72 - 8.00 (7H, m, aryl-*H*),

8.34 (1H, d, J = 8.51, aryl-*H*).

9-Amino-5,6,7,8-tetrahydro-[3,2-b]-quinolino-[5,6-e]-1-benzazocine (242)

Sodium (0.2g, 8.7mmol) and naphthalene (1.1g, 8.2mmol) in DME (60ml) were treated according to the above procedure (pg 204) giving a sodium naphthalenide solution to which was added the tosylated compound, 9-amino-5,6,7,8-tetrahydro-5-N-tosyl-[*b*]-quino-[6,5]-1-benzazocine (233) (0.7g, 1.6mmol) in dry DME. After reaction the title compound (242) was isolated (0.1g, 24% crude).

M.p. 237 - 240°C.

Found: C, 78.6; H, 6.4; N, 15.5; M^+, 275.1416. $C_{18}H_{17}N_3$ requires C, 78.50; H, 6.2; N, 15.3%; M^+, 275.1423.

δ_H(250MHz, CD_3OD) 1.63 - 1.72 (1H, m, CH_2), 1.83 - 1.94 (1H, m, CH_2),
2.85 - 3.17 (4H, m, $2CH_2$), 6.59 - 6.65 (2H, m, aryl-*H*),
7.07 (1H, t, $1J$ = 7.77, aryl-*H*), 7.16 (1H, d, J = 8.07, aryl-*H*),
7.40 (1H, t, J = 7.17, aryl-*H*), 7.57 (1H, t, J = 7.68, aryl-*H*),
7.85 (1H, d, J = 8.49, aryl-*H*), 8.04 (1H, d, J = 8.40, aryl-*H*).

Attempted detosylation of 7-amino-3-chloro-5,6-dihydro-5-N-tosyl-dibenz-[b,h][1,6]-naphthyridine (221)

Following the detosylation procedure (pg. 204) the reaction with 7-amino-3-chloro-5,6-dihydro-5-N-tosyl-dibenz-[*b,h*][*1,6*]-naphthyridine (221) (1.5g, 3.4mmol) gave 0.24g, 25% of the fully aromatic product *i.e.* compound (211) and not the dihydro product.

Attempted isolation of N-(3,6-methano-6,7,7-trimethyl-cyclohex-1-yl)-2-imino-benzonitrile (244)

(i) <u>with molecular sieves 5Å</u>

Anthranilonitrile (150) (5.0g, 42.3mmol) and R-(+)-camphor (213) (7.1g, 46.6mmol) were placed in a flask with anhydrous diethyl ether (40ml) and heated at reflux for 24h with molecular sieves 5Å (40g). The mixture was filtered and the solvent evaporated *in vacuo* to give 9.4g of a pale golden oil. Analysis showed only starting materials

(ii) <u>with titanium(iv) chloride</u>

Anthranilonitrile (7.0g, 59.2mmol) and R-(+)-camphor (3.0g, 19.7mmol) were placed in anhydrous toluene (120ml). Titanium(iv) chloride (1.1ml, 9.8mmol)

was added and the reaction heated at reflux for 24h. After cooling, the reaction mixture was filtered through kieselguhr and the solvent evaporated *in vacuo* to give a yellow oil. Tlc analysis showed only starting materials.

Attempted preparation of 9-amino-1,4-methano-1,2,3,4-tetrahydro-4,11,11-trimethyl-acridine (214)

(i) with lithium di-isopropylamide

Anthranilonitrile (150) (3.0g, 25.4mmol) and R-(+)-camphor (213) (3.8g, 25.4mmol) were placed in a reaction vessel containing anhydrous THF (70ml). Lithium diisopropylamide (LDA) was synthesised by placing di-isopropylamine (3.5ml, 25.4mmol) in anhydrous THF (10ml) under nitrogen. *n*-Butyllithium (1.6M solution in hexanes, 15.8ml, 25.4mmol) was added at -78°C dropwise *via* syringe and stirred at -78°C for 0.5h before warming to RT. The reaction vessel containing the starting materials was cooled to -78°C and the LDA was then added *via* syringe. The reaction was stirred while warming to RT for 24h. A sample of the reaction was taken, quenched with water and on analysis (TLC) showed only starting material. Another equivalent of LDA was prepared in the same manner as before and added to the reaction at -78°C. After 45min the reaction was warmed to RT and stirred for 48h. On quenching with water (25ml) the organic layer was separated, dried and the solvent evaporated *in vacuo* to give 7.9g of a brown oil. Tlc analysis showed starting materials.

(ii) with the general procedure (pg. 183)

Anthranilonitrile (150) (5.0g, 42.3mmol) and R-(+)-camphor (213) (7.0g, 46.7mmol) and boron trifluoride diethyl etherate (1M complex, 5.6ml, 45.8mmol)

were treated according to the general procedure (pg. 183). On tlc analysis, the reaction mixture consisted only of starting materials.

(iii) with titanium(iv) chloride[150]

Anthranilonitrile (150) (7.0g, 59.2mmol) and R-(+)-camphor (213) (3.0g, 19.7mmol) were placed in anhydrous toluene (100ml). Titanium(iv) chloride (1.1ml, 9.8mmol) was added dropwise *via* syringe and the reaction heated at reflux for 24h. On cooling the reaction mixture was filtered through kieselguhr and the solvent evaporated *in vacuo* to give a golden oil. Tlc analysis showed starting materials.

9-Amino-1,4-methano-1,2,3,4-tetrahydro-4,11,11-trimethylacridine (214)

Procedure with R-(+)-camphor

Anthranilonitrile (150) (5.0g, 42.3mmol) and R-(+)-camphor (213) (7.0g, 46.7mmol) were placed in toluene (70ml). Molecular sieves 5Å (50g) were added and the reaction was heated at reflux for 1h. On cooling, the mixture was filtered and boron trifluoride diethyl etherate (1M, 7.7ml, 63.0mmol) was added and the reaction heated at reflux for 24h. The toluene was decanted and the remaining solids were heated at reflux with sodium hydroxide (2M, 100ml) for 24h. On cooling, the organic components were extracted with chloroform, the extracts combined, dried and the solvent evaporated *in vacuo* to give a golden oil. On trituration with petroleum ether (60° - 80°C) this gave a white precipitate which was removed by filtration to give the title compound (214) (0.4g, 3.8%).

M.p. 150° - 154°C.

Found: C, 80.8; H, 8.3; N, 10.9; M^+, 252.1631. $C_{17}H_{20}N_2$ requires C, 80.9;

H, 8.0; N, 11.1%; M^+, 252.1626.

δ_H(250MHz, $CDCl_3$) 0.63 (3H, s, CH_3), 0.83 - 1.43 (8H, m, $2CH_3$, 2H),
1.90 (1H, td, J = 9.66, 2.99), 2.07 - 2.17 (1H, m),
2.91 (1H, d, J = 3.81), 4.43 (2H, br s, exchanges with D_2O, NH_2),
7.39 (1H, td, J = 6.88, 1.27, aryl-*H*), 7.56 (1H, td, J = 6.91, 1.46, aryl-*H*),
7.71 (1H, dd, J = 8.23, 1.32, aryl-*H*), 8.02 (1H, dd, J = 8.39, 1.13, aryl-*H*).

Procedure with S-(-)-camphor

As before with anthranilonitrile (150) (7.1g, 59.8mmol), S-(-)-camphor (10.0g, 65.8mmol), toluene (50ml), molecular sieves 5Å and boron trifluoride diethyl etherate (1M, 10.9ml, 89.7mmol) to give a yellow solid.(14g, 7.6%). Tlc analysis showed this to contain the product contaminated with starting material. A sample of the solid was taken up in methanol and an attempt at purification was carried out by column chromatography [40% ethyl acetate in petroleum ether (60° - 80°C)]. Only anthranilonitrile was isolated and no product was recovered.

Attempted preparation of N-(3,4-benzoycyclopent-1-yl)-2-imino-benzonitrile (251)

(i) with boron trifluoride diethyl etherate

Anthranilonitrile (150) (3.25g, 27.5mmol) and indan-2-one (245) (4.0g, 30.3mmol) were suspended in toluene (100ml). Boron trifluoride diethyl etherate (1M complex, 5.0ml, 40.9mmol) was added and the reaction heated at reflux for 1h. This resulted in intractable black solids which could not be purified.

(ii) with molecular sieves 5Å

Anthranilonitrile (150) (5.0g, 42.3mmol) and indan-2-one (245) (6.1g, 46.6mmol) were suspended in toluene (70ml) and molecular sieves 5Å (60g) were added. On heating at reflux for 3h, the reaction turned black and showed no discernable products upon tlc analysis.

Attempted preparation of N-(3,4-benzo-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (252)

(i) with molecular sieves 5Å

Anthranilonitrile (150) (6.6g, 55.9mmol), indan-1,3-dione (247) (9.0g, 61.6mmol) were suspended in anhydrous toluene (100ml). Molecular sieves 5Å (60g) were added and the reaction was heated at reflux for 2h. On filtration of the sieves the solvent was evaporated *in vacuo* to give black solids. Tlc analysis showed decomposition products.

(ii) with *p*-toluenesulphonic acid

Anthranilonitrile (150) (4.0g, 33.8mmol), indan-1,3-dione (247) (5.5g, 37.4mmol) and *p*-toluenesulphonic acid (0.5g, 2.6mmol) were suspended in anhydrous toluene (250ml). The mixture was heated at reflux in a Dean and Stark apparatus for 2h. The mixture was cooled and washed with water. The organic components were extracted with chloroform, the extracts combined, dried and the solvent evaporated *in vacuo* to give black solids. In an attempt to purify the solid it was taken up in chloroform with a few drops of ethanol, and then heated at reflux with activated charcoal. This failed to purify the solid, tlc analysis showed decomposition products.

N-(4-methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) and N-(2-methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263b)

Anthranilonitrile (150) (5.0g, 42.3mmol), 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) (254a) (5.2g, 46.6mmol) and *p*-toluenesulphonic acid (1.0g, 5.8mmol) were placed in a three-necked round-bottomed flask fitted with a magnetic stirrer and a Dean and Stark apparatus. Toluene (150ml) was added and the reaction heated at reflux for 40min. On cooling, the solvent was evaporated *in vacuo* to give a dark red oil which solidified on standing. Chromatography on silica gel with ethyl acetate - petroleum ether (60°-80°C) (1:4) then (1:3) gave two products.

(a) N-(4-methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a)

(7.2g, 80%). Recrystallised: m.p. 79 - 81°C [diethyl ether - petroleum ether (60°-80°C) (3:1)].

Found: C, 73.6; H, 5.9; N, 13.1; M^+, 212.0952. $C_{13}H_{12}N_2O$ requires C, 73.6; H, 5.7; N, 13.2%; M^+, 212.0950.

δ_H(250MHz, $CDCl_3$) 1.25 (3H, d, J = 7.48, CH_3), 2.23 (1H, ddd, J = 18.15, 2.11, 1.00, CH_2), 2.49 (1H, quintet of d, J = 7.43, 2.08, CH_2), 2.95 (1H, ddd, J = 18.15, 3.31, CH_2), 6.78 - 6.81 (2H, m, 1H exchanges with D_2O, N*H*, to give t, 1H, J = 3.22, CH_2), 6.93 (1H, td, J = 7.56, 0.98, aryl-*H*), 7.32 (1H, d, J = 9.09, aryl-*H*), 7.50 (2H, t, J = 9.73, aryl-*H*).

ν_{max}/cm^{-1} 3361 NH stretch, 2213 C N stretch, 1753 C=O stretch

(b) N-(2-methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263b)

(0.7g, 7%). Recrystallised: m.p. 96° - 98°C [diethyl ether - petroleum ether (60°-80°C) (3:1)].

Found: C, 73.3; H, 6.3; N, 11.6; M^+, 212.0921. $C_{13}H_{12}N_2O$ requires C, 73.6; H, 5.7; N, 13.2%; M^+, 212.0950.

δ_H(250MHz, $CDCl_3$) 1.98 (3H, s, C*H*$_3$), 2.50 - 2.67 (4H, m, 2C*H*$_2$), 5.91 (1H, br s, exchanges with D_2O, N*H*), 6.52 (1H, d, J = 8.31, aryl-*H*), 6.83 (1H, td, J = 7.51, 0.97, aryl-*H*), 7.36 (1H, td, J = 7.89, 1.63, aryl-*H*), 7.46 (1H, dt, J = 7.80, 0.35, aryl-*H*).

ν_{max}/cm^{-1} 3350 NH stretch, 2220 C N stretch, 1750 C=O stretch

Attempted cyclisation of N-(4-methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a)

(i) <u>with potassium carbonate</u>

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (1.0g, 4.7mmol), potassium carbonate (1.0g, 7.2mmol) and cuprous chloride (0.1g, 0.7mmol) were suspended in anhydrous THF (90ml). The reaction was heated at reflux for 9h, cooled then filtered through a pad of kieselguhr. The solvent was removed *in vacuo* to give a black oil showing decomposition by tlc analysis.

(ii) with sodium hydride

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (1.0g, 4.8mmol) was suspended in anhydrous toluene (25ml). Sodium hydride (35% dispersion in mineral oil, 0.19g, 4.8mmol) was added at O°C and stirred for 15min, then quenched with water (20ml). The analysis indicated starting material. The experiment was also carried out with two equivalents of sodium hydride with the same result.

(iii) with zinc chloride etherate

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (1.7g, 8.1mmol) was suspended in toluene (100ml). Zinc chloride diethyl etherate (1M, 1.3ml, 1.3mmol) was added *via* syringe and the reaction heated at reflux for 3h. The toluene was decanted and the remaining brown gum was heated at reflux with sodium hydroxide (2M, 100ml) for 15min. On cooling, the organic components were extracted with chloroform, then the solvent evaporated *in vacuo* to give 0.22g of black solid. The toluene layer was examined and found to contain starting material.

9-Amino-2,3-dihydro-2,3-dimethyl-3-hydroxy-[1H]-cyclopenta-[1,2-b]-quinoline (269)

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (4.0g, 18.9mmol) was placed in anhydrous THF (75ml) in a three-necked round-bottomed flask under nitrogen. This was cooled to -78°C while stirring. Methyllithium (1.5M solution in diethyl ether, complexed with LiBr, 31.5ml, 47.2mmol) was added dropwise *via* syringe and the reaction left to stir and warm up to RT over 24h.

Water (30ml) was added to quench the reaction. The organic layer was separated, dried and the solvent evaporated *in vacuo* to give the title compound (4.2g, 99% crude*).

*(Yield of reaction when toluene replaced THF, 58%)

Recrystallised: m.p. (dec.) 229° - 230°C (MeOH/Et_2O).

Found: C,73.4; H, 7.2; N, 12.2; M^+, 228.1265. $C_{14}H_{16}N_2O$ requires C, 73.65; H, 7.1; N, 12.3%; M^+, 228.1263.

δ_H(250MHz, CD_3OD) 1.19 (3H, d, J = 6.95, C*H*$_3$), 1.16 (3H, s, C*H*$_3$), 2.25 (1H, q, J = 7.07, C*H*$_2$), 2.51 (1H, q, J =7.99, C*H*$_2$), 2.93 (1H, q, J = 7.59, C*H*$_2$), 7.37 (1H, td, J = 6.81, 1.26, aryl-*H*), 7.56 (1H, td, J = 6.83, 1.43, aryl-*H*), 7.90 (1H, dd, J = 8.50, 0.66, aryl-*H*), 8.03 (1H, dd, J = 8.46, 1.02, aryl-*H*).

9-Amino-3-n-butyl-2,3-dihydro-3-hydroxy-2-methyl-[1H]-cyclopenta-[1,2-b]-quinoline (268)

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (1.0g, 4.7mmol) was placed in anhydrous THF in a three-necked round-bottomed flask under nitrogen. The reaction was cooled to -78°C and *n*-butyllithium (1.6M solution in hexanes, 7.4ml, 11.8mmol) was added dropwise *via* syringe and the reaction left to stir and warm up to RT over 24h. Water (20ml) was then added to quench the reaction. The organic layer was separated, dried and the solvent evaporated *in vacuo* to give the title compound (0.67g, 52%* crude).

*(Yield when toluene replaced THF 39%.)

Recrystallised: m.p. 172 - 173°C ($MeOH/Et_2O$).

Found: C, 75.2; H, 8.5; N, 10.2; M^+, 270.1733.

$C_{17}H_{22}N_2O$ requires C, 75.55; H, 8.2; N, 10.4%; M^+, 270.1732.

δ_H(250MHz, $CDCl_3$) 0.87 (3H, t, J = 6.76, CH_3), 1.13 - 1.36 (7H, m, $CH_2CH_2CH_3$), 1.85 - 2.08 (2H, m, CH_2), 2.43 - 2.89 (2H, m, CH_2), 2.96 (1H, q, J = 9.81), 7.36 (1H, td, J = 6.79, 1.26, aryl-*H*), 7.55 (1H, td, J = 6.82, 1.41, aryl-*H*), 7.90 (1H, dd, J = 8.51, 0.66, aryl-*H*), 8.03 (1H, dd, J = 8.42, 0.91, aryl-*H*).

Attempted preparation of 9-amino-3-t-butyl-2,3-dihydro-3-hydroxy-2-methyl-[1H]-cyclopenta-[1,2-b]-quinoline (270, R = t-Bu)

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-amino-benzonitrile (263a) was placed in dry THF in a three-necked round-bottomed flask under nitrogen. The reaction was cooled to -78°C and *t*-butyllithium (1.7M solution in pentane, 6.2ml, 10.6mmol) was added dropwise *via* syringe and the reaction left to stir and warm up to RT over 24h, then quenched with water (20ml). The organic layer was separated, dried and evaporated *in vacuo* but no product could be isolated from the resultant golden oil which contained predominantly starting material.

Attempted preparation of 9-amino-2,3-dihydro-3-hydroxy-3-phenyl-[1H]-cyclopenta-[1,2-b]-quinoline (270, R = Ph)

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (1.4g, 6.6mmol) was placed in anhydrous THF in a three-necked round-bottomed flask under nitrogen. The reaction was cooled to -78°C and phenyllithium (1.8M solution in cyclohexane/diethyl ether, 9.2ml, 16.5mmol) was added. The reaction stirred overnight while warming to RT, then quenched with water (30ml). The layers were separated, the organic layer dried and the solvent evaporated *in vacuo* to give a pale golden oil consisting mainly of starting material. No product could be isolated.

Attempted preparation of 9-amino-2,3-dimethyl-[1H]-cyclopent-2-enyl-[1,2-b]-quinoline (271)

(i) 9-Amino-2,3-dihydro-2,3-dimethyl-3-hydroxy-[*1H*]-cyclopenta-[*b*]-quinoline (269) (0.5g, 2.2mmol) was suspended in toluene (25ml) with a catalytic amount of *p*-toluenesulphonic acid. The reaction was heated at reflux for 24h but evaporation of the solvent *in vacuo*, starting material was recovered.

(ii) 9-Amino-2,3-dihydro-2,3-dimethyl-3-hydroxy-[*1H*]-cyclopenta-[*b*]-quinoline (269) (0.5g, 2.2mmol) was suspended in toluene (15ml) with *p*-toluenesulphonic acid (0.63g, 3.3mmol) and the reaction heated at reflux for 3h. The solution was then basified with sodium hydroxide (2M), filtered and the organic components extracted with chloroform. The organic extracts were combined, dried and the solvent evaporated *in vacuo* to give starting material.

9-Amino-2,3-dimethyl-[1H]-cyclopent-2-enyl-[1,2-b]-quinoline (271)

9-Amino-2,3-dihydro-2,3-dimethyl-3-hydroxy-[*1H*]-cyclopenta-[*b*]-quinoline (269) (4.3g, 18.6mmol) was placed in a round-bottomed flask fitted with a reflux condenser. Sulphuric acid (30%, 150ml) was added and the solution heated at reflux for 24h. On cooling, the solution was basified with sodium hydroxide (2M) and the organic components extracted with chloroform. The organic extracts were combined, dried and the solvent evaporated *in vacuo* to give the title compound (271) (3.9g, 99% yield, crude).

Recrystallised: m.p. >320°C (MeOH).

Found: C, 79.10; H, 7.25; N, 13.25; M^+, 210.1161. $C_{14}H_{14}N_2$ requires C, 79.95; H, 6.70; N, 13.30; M^+, 210.1157.

δ_H(250MHz, CD_3OD) 2.02 (3H, s, C*H*$_3$), 2.05 (3H, s, C*H*$_3$),
3.05 *(1.5H, br s, C*H*$_2$), 7.32 (1H, td, *J* = 6.87, 1.23, aryl-*H*),
7.52 (1H, td, *J* = 6.87, 1.39, aryl-*H*), 7.89 (1H, d, *J* = 8.49, aryl-*H*),
7.99 (1H, d, *J* = 8.33, aryl-*H*).
* This signal disappeared after several days exchanging with D_2O.

9-Amino-3-n-butyl-2-methyl-[1H]-cyclopenten-2-yl-[1,2-b]-quinoline (272)

9-Amino-3-*n*-butyl-2,3-dihydro-3-hydroxy-2-methyl-[*1H*]-cyclopenta-[*b*]-quinoline (268) (1.3g, 4.9mmol) was placed in a round-bottomed flask fitted with a reflux condenser. Sulphuric acid (98%, 30ml) was added and the solution heated at reflux for 24h. On cooling, the solution was basified with sodium hydroxide (2M)

and extracted with chloroform. The organic extracts were combined, dried and the solvent evaporated *in vacuo* to give the title compound (272) (0.89g, 72%, crude).

M^+ 252.1628; $C_{17}H_{20}N_2$ requires M^+ 252.1626; Microanalysis unsatisfactory.

δ_H(250MHz, CD_3OD) 0.97 (3H, t, J = 7.15, CH_3), 1.40 - 1.59 (4H, m, $2CH_2$), 2.27 (3H, s, CH_3), 2.70 (2H, t, J = 7.23, CH_2), 3.34 (1H, s, CH_2), 3.47 (1H, s, CH_2), 7.60 (1H, t, J = 7.28, aryl-*H*), 7.84 (1H, t, J = 8.26, aryl-*H*), 8.03 (1H, d, J = 8.84, aryl-*H*), 8.27 (1H, d, J = 8.40, aryl-*H*).

2-methoxy-3-methyl-2-cyclopenten-1-one[198] (273)

Dimethyl sulphate (4.3ml, 45mmol) was added slowly over 30min to a stirred suspension of 2-hydroxy-3-methyl-2-cyclopenten-1-one (254a) (5.0g, 45mmol) and potassium carbonate (6.4g, 46mmol) and then heated at reflux for 2.5h. After cooling the acetone was evaporated *in vacuo* to give a pale orange oil. This was purified by Kugelröhr distillation (65°C, 0.5 mmH) to give the title compound (273) (3.7g, 66%).

δ_H(250MHz, $CDCl_3$) 1.86 (3H, m, CH_3), 2.01 - 2.23 (2H, m, CH_2), 2.28 - 2.32 (2H, m, CH_2), 3.72 (3H, s, CH_3).

Attempted preparation of N-(5-methoxy-4-methyl-1,3-cyclopentadienyl)-2-aminobenzonitrile (274)

(i) with molecular sieves

Anthranilonitrile (150) (2.0g, 17mmol) and 2-methoxy-3-methyl-2-cyclopenten-2-one (273) (2.35g, 18.6mmol) were placed in toluene (60ml). Molecular sieves 5Å (50g) were added and the reaction heated at reflux for 5h. After filtration, the solvent was evaporated *in vacuo* to give a brown oil which, on tlc analysis, showed decomposition products.

(ii) with *p*-toluenesulphonic acid

Anthranilonitrile (150) (2.6g, 21.6mmol) and 2-methoxy-3-methyl-2-cyclopenten-1-one (273) (3.0g, 23.8mmol) were placed in toluene (120ml). *p*-Toluenesulphonic acid (0.25g, 1.5mmol) was added and the reaction heated at reflux in a Dean and Stark apparatus for 2.5h. The reaction was cooled and washed with water. The organic layer was separated, dried and the solvent evaporated *in vacuo* to give a brown oil which, on tlc analysis, showed decomposition products.

3-Methyl-2-t-butyl-dimethyl-silyloxy-2-cyclopent-1-one (275)

2-Hydroxy-3-methyl-cyclopenten-1-one (254a) (1.25g, 11.2mmol), *t*-butyl-dimethylsilyl chloride (2.0g, 13.0mmol) and imidazole (1.9g, 27.5mmol) were placed in a round-bottomed flask with dry DMF [2.5ml, (2ml/g of starting material)] and stirred at 36°C for 24h. Evaporation of the solvent *in vacuo* gave an orange oil which contained both the product and imidazole. The title compound was removed by filtration of the oil through a pad of silica gel with 25% ethyl acetate in

petroleum ether (60° - 80°C). The solvent was removed *in vacuo* to give the title compound (275) in 99% yield.

Attempted preparation of N-(4-methyl-5-t-butyl-dimethylsilyloxy-1,3-cyclopentadienyl)-2-aminobenzonitrile (276)

Anthranilonitrile (150) (1.0g, 8.8mmol) and 3-methyl-2-*t*-butyl-dimethylsilyloxy-2-cyclopenten-1-one (275) (2.0g, 8.8mmol) were placed in toluene (75ml). Molecular sieves 5Å (40g) were added and the reaction heated at reflux for 24h. Tlc analysis showed starting materials.

Attempted preparation of N-(4-methyl-5-(1,3-dioxolano)-cyclopenten-1-yl)-2-aminobenzonitrile (277)

N-(4-Methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (263a) (1.0g, 4.7mmol) and ethane-1,2-diol (2.6ml, 47.0mmol) were placed in benzene (80ml) with *p*-toluene-sulphonic acid (0.5g, 2.6mmol). The reaction was heated at reflux for 1.5h in a Dean and Stark apparatus. The resultant solution was washed with water (100ml), the organic layer dried and the solvent evaporated *in vacuo* to give a brown oil. Tlc analysis showed decomposition products.

Attempted preparation of 6-amino-2,3-benzo-1,4-dihydro-1a,4a,-dimethyl-[5H]-cyclopenta-[b]-quinoline (279)

9-Amino-2,3-dimethyl-[*1H*]-cyclopenten-2-yl-[*b*]-quinoline (271) (2.0g, 9.5mmol) was placed in xylenes (75ml). 3-Sulpholene (280) (1.24g, 10.4mmol) was added and the reaction was heated at reflux (170°C) for 24h. On cooling, the solvent was

evaporated *in vacuo* to give a brown oil from which a yellow precipitate formed on standing. Analysis showed this to consist of starting material.

2,4,6-Tri-isopropylbenzenesulphonyl hydrazide [202b]***(297)***

To a cooled, stirred solution of 2,4,6-tri-isopropylbenzenesulphonyl chloride (295) (30.0g, 99.2mmol) in anhydrous THF (100ml) at -10°C, hydrazine hydrate (296) (6.8ml, 218.2mmol) was added over 7 min. The flask warmed up to 0°C and the reaction was stirred for 3h at this temperature. Water (10ml) was added to dissolve the precipitated solids. The organic layer washed was with ice cold brine (50ml), dried and the solvent evaporated *in vacuo*. The solids were partially dissolved in petroleum ether (60 - 80°C) and triturated with water. The precipitating solids were filtered off and dried over phosphorus pentoxide then dried *in vacuo* to give the title compound (297) (24.63g, 83.5%).
M.p. 107 - 109°C (lit.[202b] 118 - 120°C).

δ_H(250MHz, $CDCl_3$) 1.40 - 1.56 (18H, m, $CH(CH_3)_2$), 3.10 (1H, *psuedo*-heptuplet, $CH(CH_3)_2$), 4.23 - 4.39 (2H,s 1H, s, both exchange with D_2O, N*H*, NH_2), 7.38 (2H, s, aryl-*H*).

Acetone-2,4,6-Tri-isopropylbenzenesulphonyl hydrazone (298)

Acetone (2.5ml, 33.6mmol) was added to a stirred suspension of 2,4,5-tri-isopropylbenzenesulphonyl hydrazide (297) in methanol (100ml). The solution became clear, then after a few minutes a white precipitate was formed, and the vessel was cooled overnight. The precipitate was filtered off and the remaining liquors were concentrated *in vacuo* and again on cooling, a white precipitate was

formed. This was filtered and combining the material, the solvent was removed *in vacuo* to give the title compound (298) (6.67g, 59%).

M.p. 128 - 134°C (dec)

Found: C, 64.0; H, 9.2; N, 8.2; S, 9.5. $C_{18}H_{30}N_2O_2S$ requires C, 63.9; H, 8.9; N, 8.3; S, 9.5%.

δ_H(250MHz, $CDCl_3$) 1.29 - 1.38 (18H, m, $CH(CH_3)_2$), 1.90 (3H, s, N CCH_3), 2.02 (3H, s, N CCH_3), 2.97 - 3.14 (1H, m, NH), 4.30 - 4.41 (2H, m, $CH(CH_3)_2$), 7.48 (2H, s, aryl-H).

Attempted preparation of 9-amino-2-methyl-3-hydroxy-3-(2-propen-1-yl)-cyclopenta-[b]-quinoline (283)

Acetone-2,4,6-tri-isopropylbenzenesulphonyl hydrazone (298) (6.6g, 19.6mmol) was placed in a solution of tetramethylethylenediamine (TMEDA) in *n*-hexane (10%v/v, 60ml) with stirring. The reaction was cooled to -78°C and *n*-butyllithium (2.5M, ml, 39.2mmol) was added dropwise *via* syringe. After stirring for 15min at this temperature the reaction was allowed to warm to 0°C and stirred until nitrogen evolution ceased. N-(4-methyl-5-oxocyclopenten-1-yl)-2-aminobenzonitrile (2.1g, 9.8mmol) in anhydrous THF (30ml) was added *via* syringe, keeping the reaction temperature at 0°C. This was stirred for 24h then quenched with water (25ml). The aqueous layer was removed and repeatedly extracted with diethyl ether. Combining this ethereal layer with the THF layer the bulk solution was dried and the solvent evaporated *in vacuo*. The resulting brown oil was triturated with ethyl acetate which gave a yellow powder (0.51g). Tlc

analysis showed a one-spot baseline product, but the NMR seemed to indicate a mixture of compounds. With the remaining oil purification with column chromatography was attempted with ethyl acetate:petroleum ether (60° - 80°C) (3:2) as eluent. The solid isolated in this manner was not identifiable by NMR.

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