REFERENCE

The Synthesis of Novel Indazole Alkaloids With Potential Medicinal Properties

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I would like to thank all the people who encouraged and supported me during my studies. I owe to them what I have achieved till now. I dedicate this to my parents without whose care and support I could not be here. Most of all, I would particularly like to give a lot of thanks to my supervisor Dr Akram Khan for his excellent supervision, patience and advice during the work and his much appreciated understanding of some of the problems I had experienced. I would like to thank my second supervisor Professor K.D. Rainsford for his supportive role during my research here. I would also like to thank the technical staff and in particular Kevin Osborne for his excellent, energetic and enthusiastic technical support throughout the project. Lastly but not least I would like to thank all my friends at Sheffield Hallam University.

ABSTRACT

The black seeds of *Nigella sativa* Lin. are universally accepted as a panacea. In particular the black seed oil extract has been shown to have bronchodilatory, antidiabetic, antibacterial, antifungal, analgesic, anti-inflammatory, and immunopotentiang activities. Four principal alkaloids Nigellicine **1**, Nigellimine-*N*-oxide **2**, Nigellimine **3** and Nigellidine **4** have been isolated from *Nigella sativa* Linn. The isoquinoline alkaloids **2** and **3** have recently been synthesised in these laboratories.

The aim of this research programme was to prepare the other two compounds, by two alternate routes I and II, so that their pharmacological properties could be elucidated subsequently. Route I represented a novel approach in which four β-monoacylated acid hydrazides **7a-d** were prepared for cyclisation into indazoles **8a-d**. Reaction of phenylhydrazine with benzoyl chloride, 4-benzyloxy benzoyl chloride and 4-nitrobenzoyl chloride gave hydrazides **7a-c** whilst reaction of and 2-ethylphenyl hydrazine with 4-nitrobenzoyl chloride produced **7d**. All the four acid hydrazides **7a-d** failed to cyclise to yield indazoles **8a-d** in polyphosphoric acid (PPA), polyphosphate ester and conc.sulphuric acid at a variety of reaction temperatures (60-160°C). However, 4-nitrobenzoyl 2-ethylphenylhydrazide with tosic acid cyclised in refluxing xylene to give 7-ethyl-3-(4'-nitrophenyl)-*1H*-indazole **8c** in 37.5% yield.

By route II the indazole **47** was prepared from 2,4,6-trimethoxyacetophenone and hydrazine hydrate in PPA in only 6-16% yield (lit. 65% yield). The reaction of 2,4,6-trimethoxyphenyl α-ketoester **65** and 2,4,6-trimethoxy phenyl α-ketoacid **66** with hydrazine hydrate and benzylhydrazine dihydrobromide failed to produce indazole products on heating in glacial acid, PPA, DMF and xylene.

Retrosynthesis of Nigellidine required trimethoxyphenyl 4-benzyloxyphenyl ketone **53** as a starting material which failed by Friedel-Crafts acylation of 1,3,5-trimethoxybenzene with 4-benzyloxybenzoyl chloride. 2,4,6-Trimethoxy benzaldehyde and 4-benzyloxyphenyl magnesium bromide reacted in THF to give the alcohol **52** which resisted oxidation to ketone **53** by several methods. Oxidation of the alcohol with TEMPO successfully gave the ketone **53** albeit in a low yield.

While it was not possible to obtain Nigellicine **1** and Nigellidine **4** the present studies have progressed understanding of the synthetic pathways. Some strategies are proposed to enable future work to be undertaken to obtain these alkaloids.

DECLARATION

No part of the work discussed in this thesis has been submitted in support of an application for another degree or qualification of this at any other learning institution.

El-sanoussi Elhmalye

December 2005

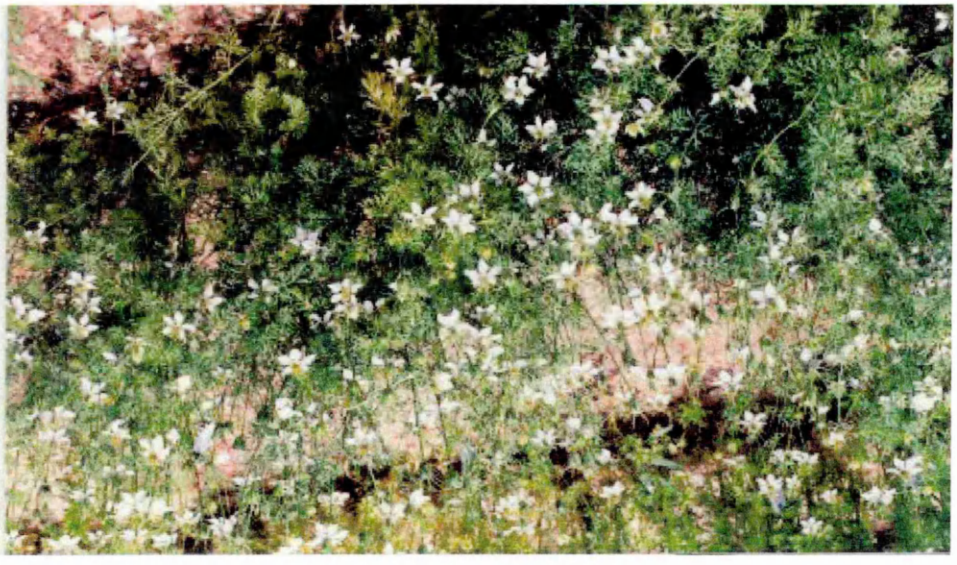
CONTENTS

Chapter 1

General Introduction

1 Introduction

Nigella sativa Linn. belonging to the Buttercup or Ranunculaceae family is an annual herbaceous plant that is native to the Mediterranean region and is now cultivated in other parts of the world including Middle East, North Africa and Asia. The plant which grows to a maximum height of about 60 cm, has finely divided foliage and blue-white flowers from which are produced small caraway-type black seeds as shown in the picture below[1].



The spicy black coloured seeds from this plant, which are sometimes called Black Cumin, have had medicinal usage dating back to the ancient Egyptians, Greeks and Romans[2]. The black seeds are considered to be among the more potent healing herbs and are universally accepted as a panacea. In Islamic medicine, however, the use of the seeds is recommended in daily use because it has prophesised "cure for all known diseases"[3]. This herb has been used for centuries in folklore medicine to enhance the immune system, clean the body, purify the blood, protect against irritants and to support health and longevity.

In recent times considerable research effort has been devoted worldwide to investigate the black seeds for their historically acclaimed medicinal properties. In particular the

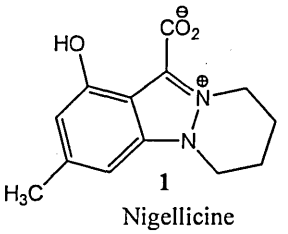
black seed oil extract has been examined and shown to have bronchodilatory[4], antidiabetic[5], antibacterial[6], antifungal[7], analgesic[8], anti-inflammatory[9], and immunopotentiang activities[10].

Apart from the mineral content (see Appendix) other constituents identified in the black seed include fixed oils, saponins, volatile or essential oils, alkaloids and amino acids[2]. The fixed oils, constitute 37% of the seed and may be subdivided into triglycerides and sterols. Sterols are steroids containing 27 or more carbon atoms with an OH [hydroxyl] group. A remarkable number of 23 sterols have been identified in *Nigella sativa* seed.

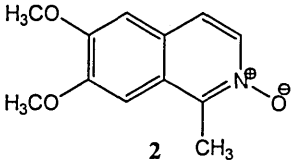
The varied use of *Nigella sativa* in the folk medicine has encouraged many investigators to isolate the possible active components and to conduct *in vi*vo studies in laboratory animals and on human beings as well as *in vitro* studies in order to investigate their pharmacological properties. Among those reported to be due to some of the components are immune stimulation, anti-inflammatory, anti-cancer, anti-microbial, anti-parasitic, anti-oxidant, and hypoglycemic effects[2].

Although the existence of alkaloids in *Nigella sativa* Linn. has been proposed since the turn of the twentieth century, their isolation and structural elucidation has not been achieved until very recently. Alkaloids are plant constituents which contain atoms of nitrogen in their structure and are capable of affecting human physiology. Four principal alkaloids have been isolated from *Nigella sativa* Linn. cultivated in Pakistan and these have been identified as Nigellicine **1**[11] Nigellamine-*N*-oxide **2**[12], Nigellimine **3**[13] and Nigellidine **4**[14].

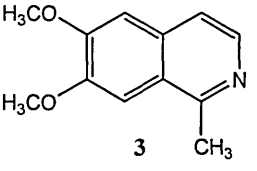
The compounds Nigellicine **1** and Nigellidine **4** are indazole alkaloids which are extremely rare in nature because of the inability of micro organism to make an N-N bond and these two compounds represent the only examples of indazole alkaloids in nature. The other two alkaloid Nigellimine *N*-oxide **2** and Nigellimine **3** have already been synthesised in these laboratories[18] (Scheme 1).



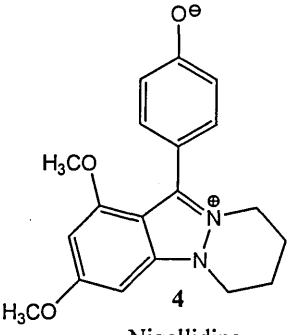
1
Nigellicine



2
Nigellimine *N*-Oxide



3
Nigellimine

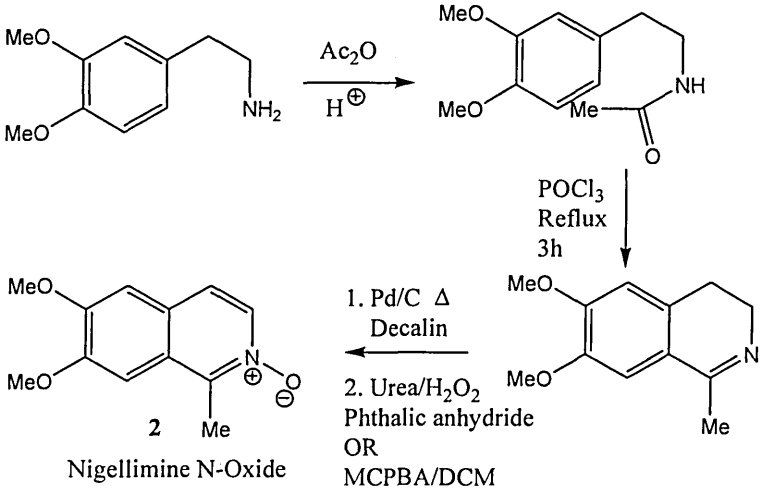


4
Nigellidine

The aims of this research were firstly to investigate the chemical synthesis of the two hitherto unknown indazole alkaloids Nigellicine **1** and Nigellidine **4** so that it will be possible to determine their pharmacological activities in future.

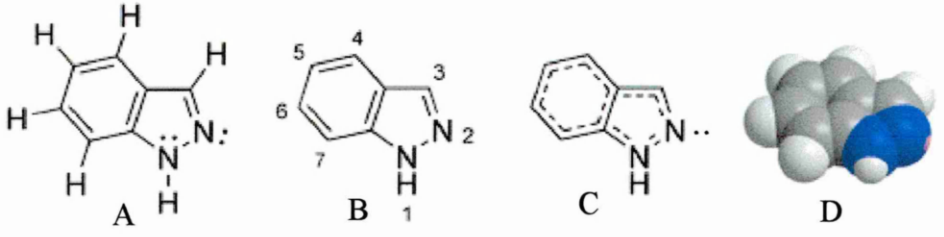
Little is known about the individual therapeutic properties of the indazole alkaloids. However, compounds containing the indazole nucleus have recently received attention as antifertility [15] and antiarthritic agents[16]. A number of indazole analogues have been synthesised for their biological activity[17]. On the other hand the alkaloid Nigellamine-*N*-oxide, an Isoquinoline derivative, is structurally related to quinolone, and could therefore exert the possible effects of this group which includes its use in the treatment of malaria and for the prophylactic treatment of cardiac arhythmiasis.

Scheme 1



Nigellimine N-Oxide

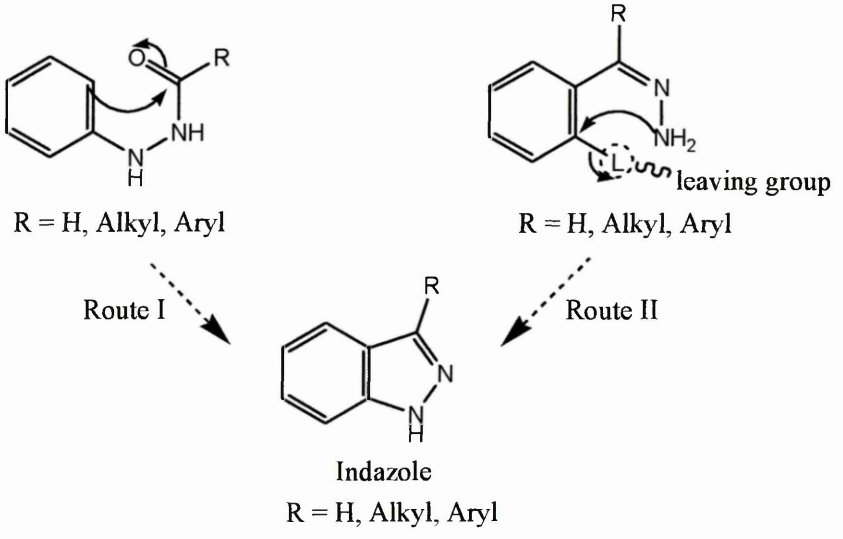
The indazole molecule is an example of an heterocyclic aromatic system which obeys the Huckle rule for aromaticity by having 10 π electrons. The indazole molecule consists of a benzene ring fused with a pyrazole ring (structure A). The most basic nitrogen atom of the two is the middle nitrogen atom (N-2 in structure B) whose lone pair of electrons is orthoganol to the pyrazole ring system and does not participate in the aromaticity of the system. The delocalisation of electrons in indazole is shown in structure C in which the lone pair of electrons on the middle nitrogen atom (N-2) is still available for reaction. The lone pair of electrons on the nitrogen atom of the pyrazole ring that is directly linked to the benzene ring (N-1 in structure B) is involved in the aromaticity of the pyrazole system and is very similar to the nitrogen atom in pyrrole. In the puckered model (structure D) of indazole the two nitrogen atoms are shown in blue colour.



Synthetic Strategy

The strategic synthesis of an indazole molecule[19] can be envisaged by two alternate routes I and II in which the pyrazole ring is constructed involving intramolecular ring closure reactions as shown in Scheme 2.

Scheme 2



Route I involves the intramolecular electrophilic aromatic substitution reaction to form a carbon-carbon bond whilst route II involves intramolecular nucleophilic aromatic substitution reaction to form a carbon-nitrogen bond. Route I to the best of our knowledge represents a novel approach and will be discussed in Chapter 2 of this thesis. Route II is the frequently used method reported in the literature for indazole synthesis which will be discussed in greater detail in chapter 3.

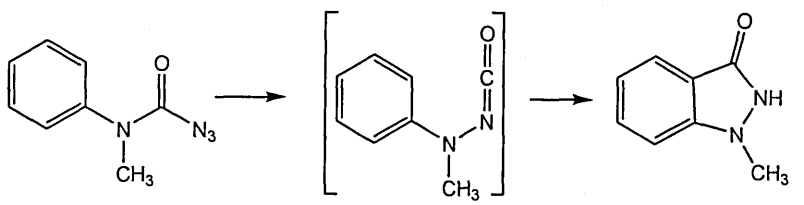
Chapter 2

Synthesis of indazoles by electrophilic aromatic substitution (cyclodehydration) reactions of diaryl acid hydrazides

2.1 Introduction

Not much is reported in the literature regarding synthesis of *1H*-indazoles by the route I intramolecular ring closure reactions. One example is the synthesis of 1-methyl-*1H*-indazolone by an intramolecular ring closure reaction. Thus methyl phenyl carbamyl azide on heating in xylene gave 1-methylindazole in 60% yield as shown in Scheme 3. In this reaction the intermediate is believed to undergo a Curtius type of rearrangement[20].

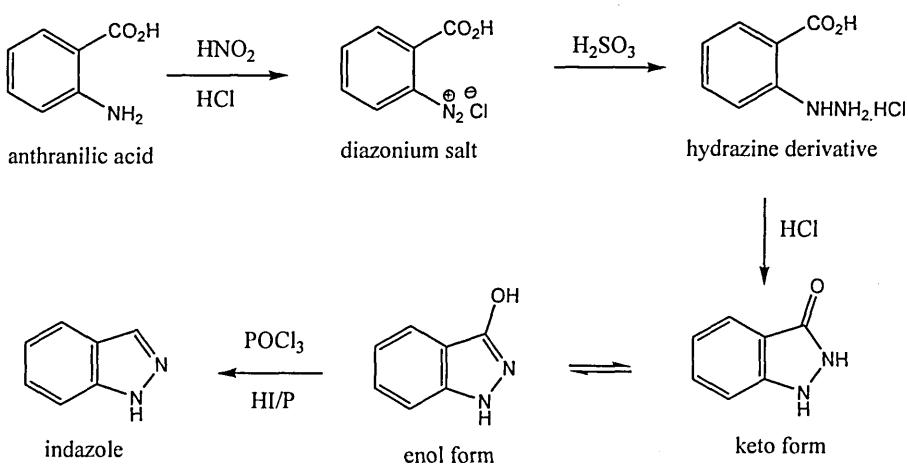
Scheme 3



methyl phenyl carbamyl azide — intermediate — 1-methylindazolone

The parent molecule *1H*-indazole has been made by two classical syntheses[21-22]. One such synthesis is that shown in Scheme 4 in which anthranilic acid as a starting material is transformed into indazole by four classical reactions involving diazotization, reduction and cyclisation[21].

Scheme 4

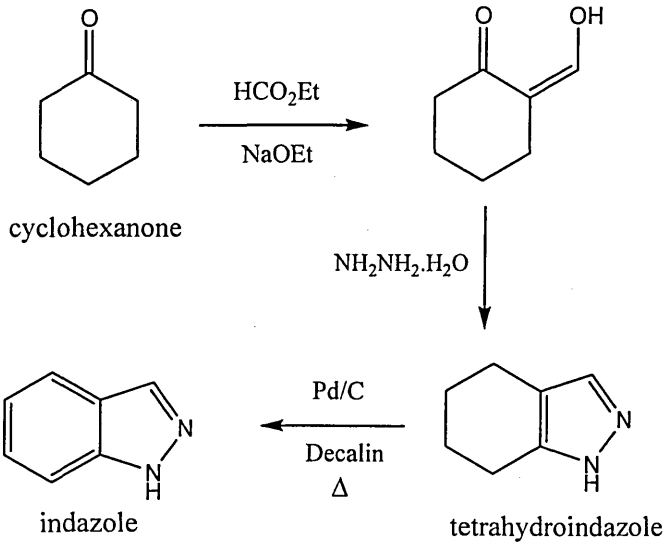


anthranilic acid — diazonium salt — hydrazine derivative

indazole — enol form — keto form

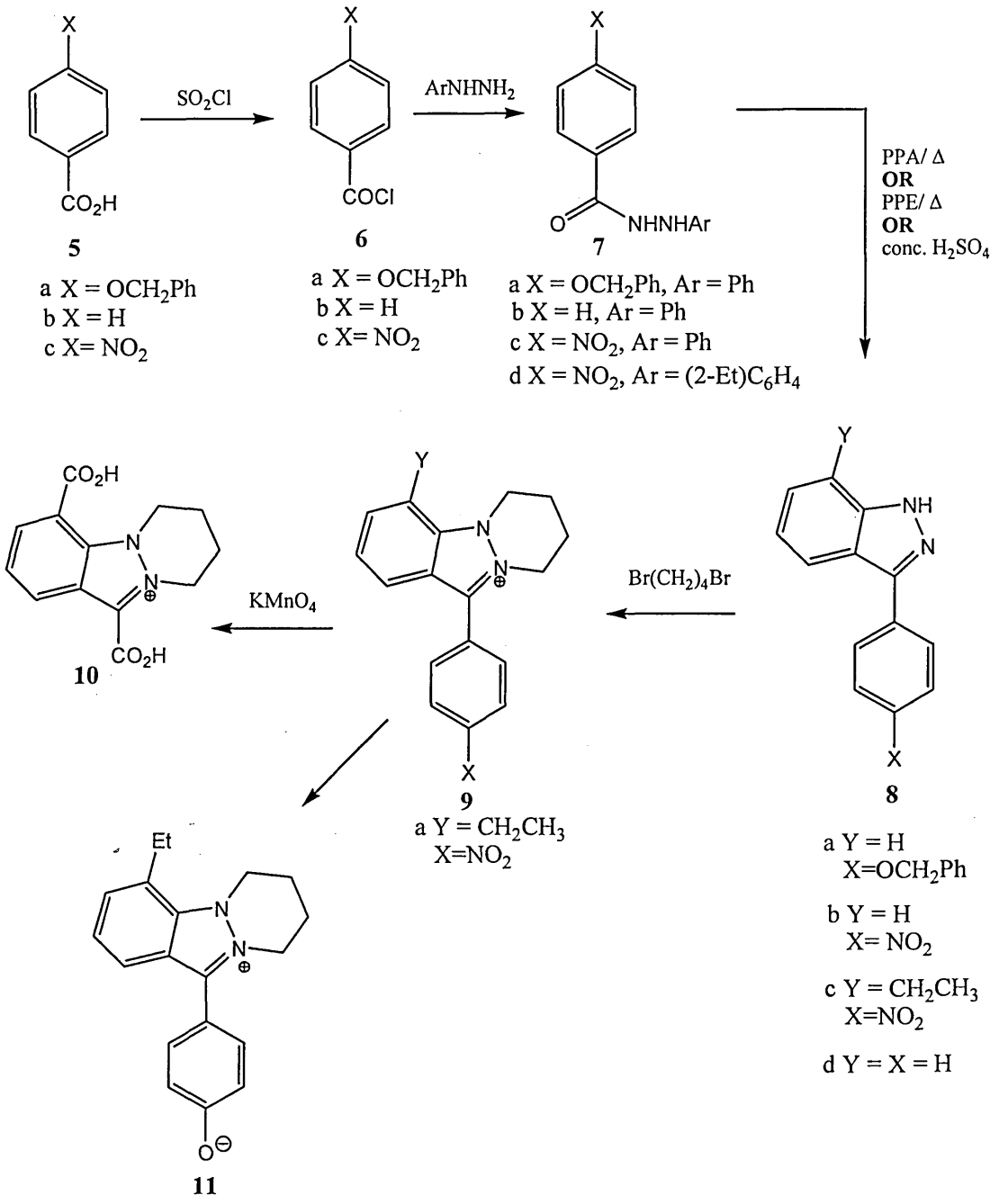
In the second classical synthesis *1H*-indazole has been prepared from cyclohexanone in three steps as shown in Scheme 5 . The Aldol condensation product from ethyl formate and cyclohexanone underwent a condensation and ring closure reaction to afford a tetrahydroindazole which was then oxidised with Pd-charcoal catalyst in refluxing decalin to indazole[22].

Scheme 5



In this section of the work we will discuss our attempted synthesis of indazoles by intramolecular cyclisation of diaryl acid hydrazides **7a-d**. We envisaged the synthesis of the indazole alkaloids **1** and **4** through the route I carbon-carbon bond formation strategy outlined earlier in scheme 2. In this strategy we proposed to synthesise the diaryl acid hydrazides **7a-d** by the reaction of appropriate acyl chlorides with arylhydrazines in pyridine. Acid catalysed intramolecular cyclisation of the diaryl acid hydrazides was expected to produce the required indazoles **8a-d** (Scheme 6).

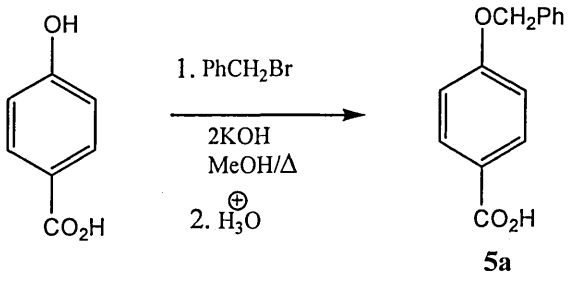
Scheme 6


X
SO_2Cl
$ArNHNH_2$
CO_2H
COCl
O
NHNHAr
PPA/ Δ
OR
PPE/ Δ
OR
conc. H_2SO_4
5
6
7
a X = OCH_2Ph
b X = H
c X= NO_2
a X = OCH_2Ph, Ar = Ph
b X = H, Ar = Ph
c X = NO_2, Ar = Ph
d X = NO_2, Ar = $(2\text{-Et})C_6H_4$
Y
NH
N
$Br(CH_2)_4Br$
$KMnO_4$
CO_2H
CO_2H
10
9
8
a Y = CH_2CH_3
X=NO_2
a Y = H
X=OCH_2Ph
b Y = H
X= NO_2
c Y = CH_2CH_3
X=NO_2
d Y = X = H
Et
O
11

2.2 Results and Discussion

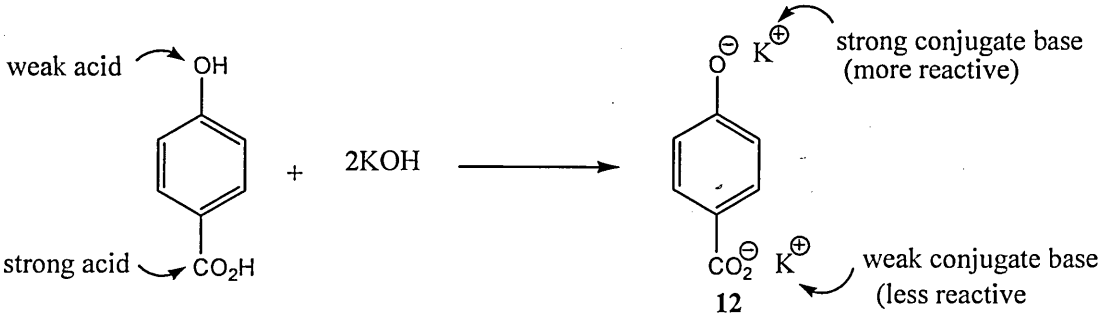
The acid hydrazides **7a-d** required the compounds **5a-c** as starting materials of which **5a** was commercially unavailable and was prepared from the reaction of 4-hydroxy benzoic acid with benzyl bromide in the presence of two molar equivalents of potassium hydroxide in methanol as shown in scheme 7. The protecting group in **5a** was chosen as benzyl due to its ease of formation and deprotection under mild conditions with hydrogen gas and Pd-C catalyst.

Scheme 7

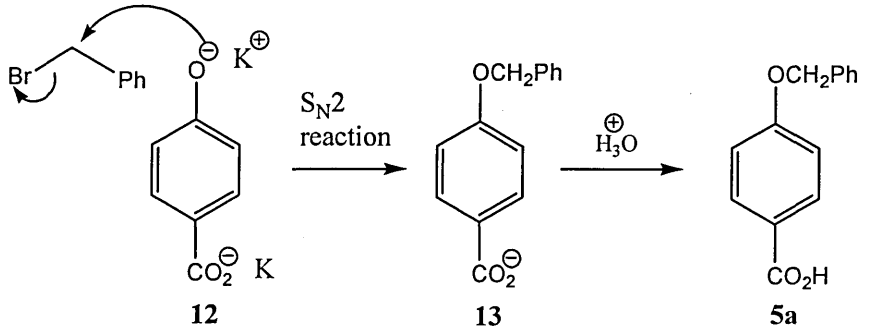


5a

Mechanism



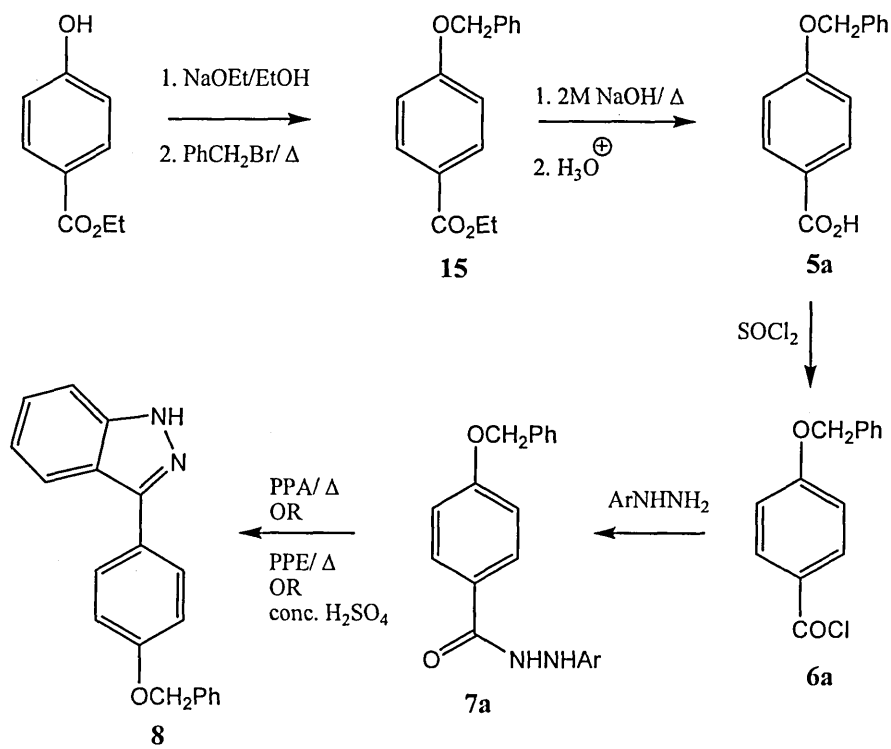
12



12 **13** **5a**

The reaction mixture was refluxed for 22h in which time the KOH being a strong base reacted with both the phenolic group and the carboxylic acid to produce the double salt **12**. The phenoxide anion in **12** is more reactive towards the electrophile benzyl bromide because it is the conjugate base of a weak acid (phenol) in contrast to the carboxylate anion which is the conjugate base of a strong acid, the benzoic acid. Nucleophilic substitution reaction of the phenoxide anion with benzyl bromide furnished the intermediate ether **13** which on acidification with hydrochloric acid gave the corresponding 4-benzyloxybenzoic acid **5a** in 52 % yield. The IR spectrum of **5a** showed a broad hydrogen bonded OH peak between 2500-3300 cm^{-1} whilst its ^1H-NMR spectrum displayed two doublets of an aromatic AB system at δ 7.10 and δ8.00 which along with the mass spectrum showing a molecular ion at m/z 228 that characterised the molecule. The 4-benzyloxy carboxylic acid **5a** was also synthesised starting with ethyl 4-hydroxybenzoate as the starting material and after hydrolysing the intermediate ester **15**[29] to the acid using dilute sodium hydroxide solution (70 % yield) as shown in scheme 9

Scheme 9



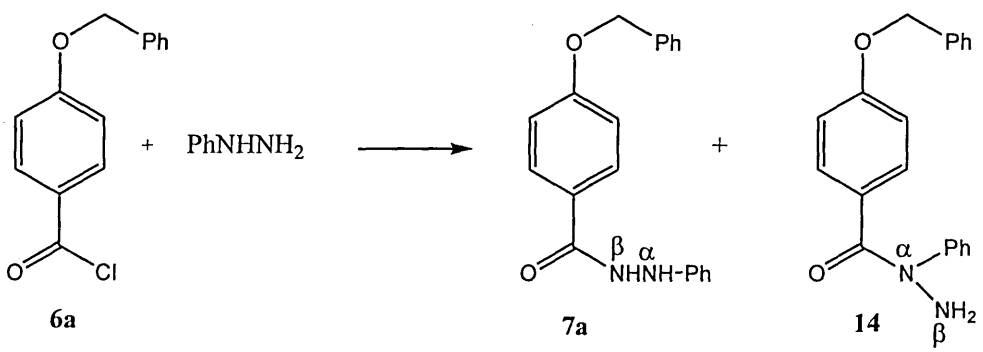
Whilst precursors **6b** and **6c** were commercially available compounds, **6a** was prepared in the laboratory from **5a** by chlorination with thionyl chloride. This was achieved by refluxing a solution of **5a** in excess thionyl chloride to afford the acid

chloride **6a**[30] in 90% yield after washing with petrol on the Buchner funnel and was used as such for the next stage. That the crude acid chloride **6a** was fairly pure was evaluated from its melting point which showed a narrow range and from its ^{1}H NMR spectrum which showed clean peaks at δ 5.20 for the benzylic protons and an AB system of two doublets at δ 7.05 and 8.10 for the para disubstituted aromatic ring. Thionyl chloride is a good chlorinating agent because the by-products of the reaction are all gaseous and the product is easily isolated from the excess thionyl chloride by evaporation on the rotary evaporator. The IR spectrum of the acid chloride **6a** revealed the absence of the OH group in the 3100-4000cm^{-1} and the presence of a strong carbonyl peak at 1770 cm^{-1} characteristic of acid chlorides and indicating completion of the reaction. The proton magnetic resonance spectrum of **6a** showed two doublets of an AB system for the 1,4-disubstituted aromatic ring and a multiplet for the aromatic ring of the benzyloxy group along with a singlet at δ 4.85 for the benzylic protons. The mass spectrum showed a molecular ion peak at m/z 246.

The acid chlorides **6a-c** were reacted with phenylhydrazine in pyridine to give the acid hydrazides **7a-c** (60-80 % yield). Reaction of 2-ethyphenylhydrazine with 4-nitrobenzoyl chloride **6c** yielded the acid hydrazide **7d** in purified 80% yield.

It is worthy to note that the reaction of the acid chlorides with phenylhydrazine could in principle produce two possible products. For example reaction of acid chloride **6a** with phenylhydrazine could yield the two hydrazides **7a** and **14** as shown in scheme 8.

Scheme 8



6a **7a** **14**

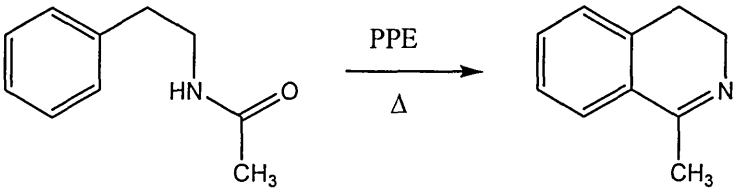
Literature precedence[23-24] suggests that acylation of monoalkylhydrazines usually produce mixtures of α- and β-monoacyl derivatives whereas monoarylhydrazines preferentially acylate in the β-position to give products of the desired type **7a**.

All the hydrazides **7a-d** were easily purified from small amounts of the undesired α-isomer **14** by passing dry HCl gas through their solution in chloroform at ice temperature for 1-2 hours which caused the hydrochlorides of the desired product to precipitate out. The hydrochloride salts were washed with saturated sodium bicarbonate solution to generate the neutral compounds which were further purified by recrystallisation from chloroform and petroleum ether. The indicated structures of the acid hydrazide **7a-d** were supported by the literature precedence[23-24] and illucidated by analysis of their IR spectra which confirmed the presence of an NH absorption in the region 3200-3300 cm^{-1} and a carbonyl peak in the region 1640-1650 cm^{-1}. ^{1}H-NMR spectral analysis also revealed the correct structure and the mass spectrum showed ions corresponding to the molecular ions. Thus the elctron impact mass spectra of **7b-d** showed molecular ions at m/z 212 (32%), 257 (43%) and 285 (13%) respectively whilst the electrospray mass spectrum of **7a** showed an M+1 ion at m/z 318. The degradation ions observed in the EI mass spectra of **7b-d** complemented the proposed structures. Noticeable features in the ^{1}H-NMR spectra of **7a** and **7c-d** were the presence of two doublets corresponding to the para-disubstituted aromatic rings.

The acid hydrazides **7a-d** were heated with polyphosphoric acid (PPA) in order to cause a cyclodehydration to form the indazoles **8a-d**. At a variety of reaction temperatures (60 -160°C) and reaction times (1-5 h) the cyclodehydration reaction did not afford the desired indazole product **8.** In these reactions using PPA product isolation proved difficult and in most instances the starting material **7** was recovered in each case albeit in low yield. The IR spectrum of the products from these reactions had a prominent carbonyl peak at 1650 cm^{-1}.

The cyclodehydration reaction of the acid hydrazide **7b** into the indazole **8d** was considered analogous to the Bischler-Napieralski reaction (shown below) in which a variant of PPA called polyphosphate ester (PPE) has been quite successfully used. However, the compound **7b** upon refluxing in freshly prepared PPE for a variety of reaction times did not show any conversion into the indazole **8d.**

Bischler-Napieralski Reaction



In one reaction when the reaction mixture was heated for 24 h considerable amount of decomposition leading to side products had occurred after monitoring the reaction progress by TLC (ethyl acetate: petrol, 1:2). Work-up had always yielded recovered starting material albeit in small yields on occasions.

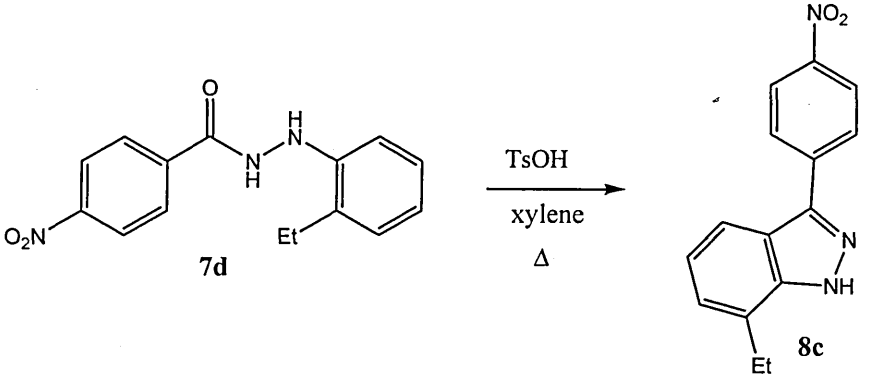
The failure of **7a** to cyclise was linked to electronic effects. It was argued that the carbonyl group in **7a** had reduced electrophilic character due to the presence of the benzyoxy group at 4-position of the aromatic ring and the linked hydrazide group both of which are strongly electron releasing groups. Perhaps what was needed was an electron withdrawing group at 4-position of the aromatic ring of the hydrazoic acid. This hypothesis was tested by synthesising the acid hydrazides **7b** and **7c** from the commercially available benzoic acid and 4-nitrobenzoic acid **5b** and **5c** respectively as shown in scheme 5. Both of the hydrazides **7b** and **7c** failed to cyclise in PPA once again under a variety of conditions and in refluxing PPE. Thus, it was demonstrated that the quality of the electrophilic character of the carbonyl group in the hydrazides was not responsible for the lack of ring closure to give the indazole molecules. Other reasons which could be related to the β-nitrogen atom of the hydrazide causing the problem since aromatic amides have been reported to readily undergo cyclisation as in the Bischler-Napieralski reaction.

The hydrazoic acid derivative **7d** was made from 4-nitrobenzoyl chloride **6c** and 2-ethylphenylhydrazine in 80% yield. The ethyl group in 2-ethylphenylhydrazine was expected to enhance the nucleophilicity of the aromatic ring towards intramolecular attack on the carbonyl group. The structure of the acid hydrazide was confirmed from its ^{1}H-NMR spectrum which showed two doublets constituting an AB system of the 4-nitrophenyl aromatic ring at δ 7.98 and δ 8.25. The aromatic protons of the ethyl phenyl group appeared as two sets of multiplets between δ 6.84 and δ 7.16. The ethyl group gave the typical quartet and triplet pattern at δ 2.65 and δ1.35 respectively. The

amino group of the amide functional group resonated as a broad singlet at δ 8.60 and the other amino group resonated upfield as a very broad singlet at δ 5.05. This information was complemented by the IR spectral information which showed an amino absorption at 3263 cm^{-1} and a carbonyl group absorption at 1694 cm^{-1}. Complementary evidence from the mass spectrum indicated a molecular ion of m/z 285 and degradation pattern given in the experimental section that was consistent with the correct molecular structure.

Since cyclisation of **7d** in PPA and PPE gave a very poor yield of reaction products an alternate medium was sought for cyclising this compound. We reasoned that an acid catalyst was essential for the intramolecular cyclisation and decided to try an organic sulphonic acid such as tosic acid in xylene. When compound **7d** together with an excess of tosic acid was stirred and heated in xylene at 150-160°C overnight, a reaction had occurred which according to TLC (1:3, ethyl acetate:petrol) consisted of two main products (R_f.0.55 and R_f 0.20) with the complete disappearance of **7d** (R_f 0.34) (scheme 10).

Scheme 10

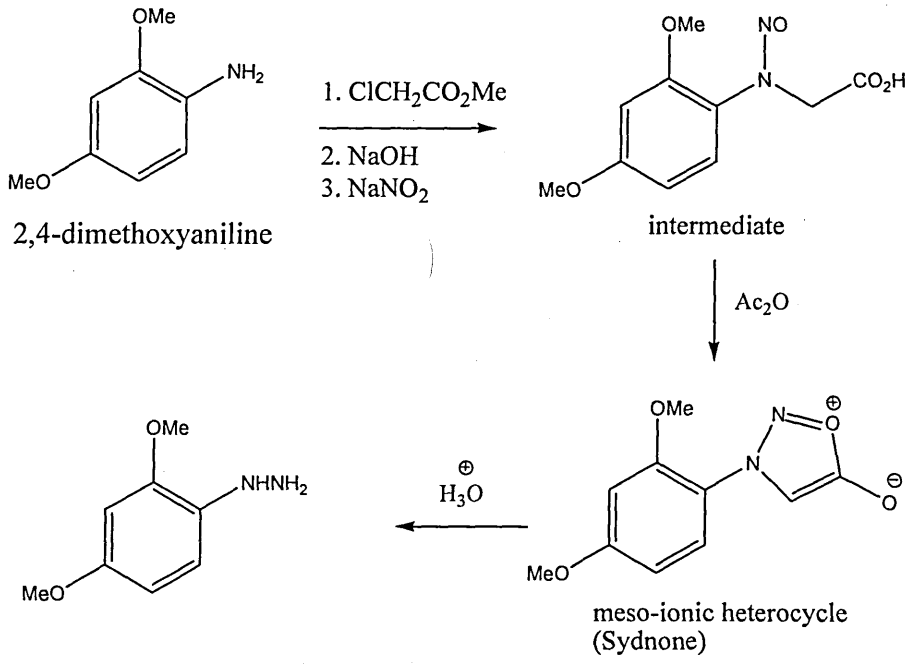


The two main products in the reaction mixture were separated by flash chromatography. The most polar product (R_f 0.20) was the desired indazole product **8c** which was characterised by ^1H NMR and MS spectral analysis. The electrospray mass spectrum of the compound gave a molecular ion m/z 268 (M+1). By proton magnetic resonance spectroscopy the ethyl group typically resonated as a triplet (δ 1.11) and a quartet (δ 2.37). The aromatic region showed two doublets (δ 7.69 and 7.28) of an AB system indicative of the 4-nitrophenyl group and two multiplet signals (δ 6.59-6.62) and (δ 6.49-6.52) of integration 2H and 1H respectively. There was a

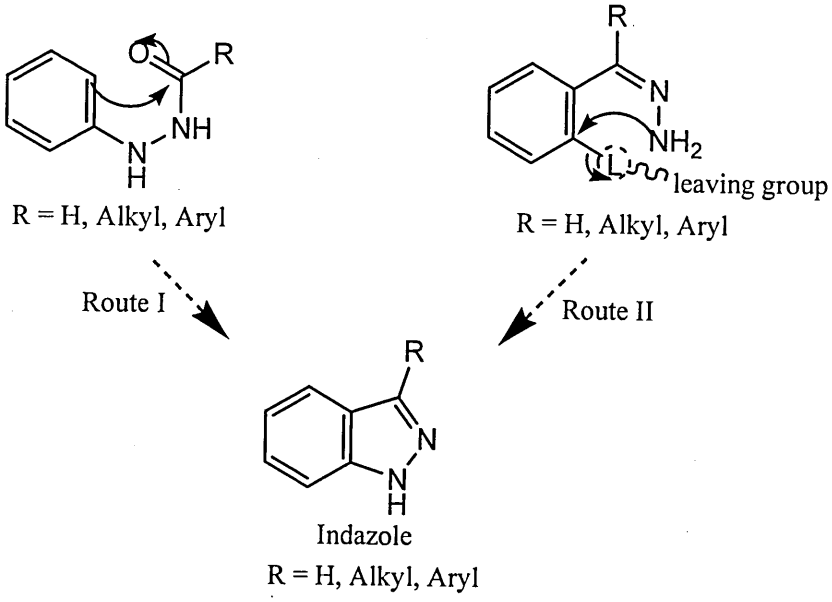
broad signal at δ 3.50 due to the NH proton. The ^{1}H NMR spectrum of the other compound showed the absence of 4-nitrophenyl group and was identified as 2-ethylphenylhydrazine.

The amount of product **8c** isolated from the reaction was insufficient to alkylate with 1,4-dibromobutane in order to make the salt **9a** (scheme 5). Conditions for alkylating indazole as a model substrate with 1,4-dibromobutane were tried with later and are discussed in chapter 2 of this thesis. Moreover, the application of this novel approach to the synthesis of Nigellicine **1** would require the starting materials 2,4-dimethoxyhydrazine or 2,4,6-trimethoxyhydrazine which are not commercially available and would have to be synthesised. A literature search suggested that these type of alkoxyphenylhydrazines can be made from the corresponding substituted anilines via the meso-ionic sydnone intermediates[25-27] (scheme 11).

Scheme 11



However, this method for making indazoles was postponed due to the commercial inavailability of the starting compound 2,4-dimethoxyaniline and it was decided to adopt another strategy altogether for the synthesis of these compounds. This new strategy involved intramolecular C-N bond forming ring closure reaction conforming to route II methodology as depicted below. This chemistry is discussed in chapter 3 of this thesis.


O
R
NH
N
H
R = H, Alkyl, Aryl
R
N
NH2
L
leaving group
R = H, Alkyl, Aryl
Route I
Route II
R
N
N
H
Indazole
R = H, Alkyl, Aryl

2.3 Experimental Procedures

General Directions

Melting points (m.p.s) are uncorrected and were determined on Stuart Scientific SMP3 electrothermal apparatus. Infrared (IR) spectra were recorded with an ATI Mattson Genesis series FTIR spectrophotometer. 1H and ^{13}C NMR spectra were recorded in deuterochloroform (unless otherwise stated) using a Brucker AC 250 spectrometer operating at 250 and 62.9 MHz respectively. Chemical shifts (δ) are in ppm downfield from tetramethylsilane as internal standard, J-values are given in Hz. Electron impact mass spectra (EIMS) were recorded with a VG 7070 Analytical mass spectrometer. Electrospray mass spectra (ESMS) were recorded with a Micromass Platform single quadrupole mass spectrometer fitted with a Harvard syringe driver. High resolution mass spectra (HRMS) were recorded on Applied Biosystem/ MDS Sciex. Q-Star Pulsar-i hybrid quadrupole time of flight mass spectrometer fitted with an O-MALDI2 source.

All solvents were dried and distilled by standard techniques. All organic solutions were dried over anhydrous magnesium sulphate. Petrol refers to a light petroleum fraction boiling in the range 40-60°C. EtOAc and DCM refer to ethyl acetate and dichloromethane.

Analytical thin layer chromatography (TLC) was performed on commercially available (Merck) aluminium sheets with a coating of silica gel and fluorescent material $60F_{254}$ for detection of compounds under a uv lamp. Detection of compounds was also done by spraying the plates with a solution of alkaline potassium. permanganate. Flash column chromatography was performed using Apollo Scientific Ltd. silica gel of mesh size 35-70 micron.

Preparation of 4-benzyloxybenzoic acid **5a**[28]

4-Hydroxybenzoic acid (90.5g, 0.66 moles) and methanol (400 ml) were added to a one litre three-necked round-bottomed flask equipped with a reflux condenser, an addition funnel, an oil bath and a magnetic stirrer. A solution of potassium hydroxide (105g, 1.9 moles) in distilled water (125 ml) and the solution stirred rapidly. Benzyl bromide (126.5g, 0.74 moles) was added dropwise over a period of one hour and the resulting mixture was heated at 80°C overnight. A thick white precipitate had formed.

The methanol was removed by simple distillation and water (1 litre) was added to the cooled mixture to produce a clear solution. Acidification with concentrated hydrochloric acid produced the a white precipitate which on Buchner filtration produced the crude product **5a** (78g, 52%) as a white solid, m.p. 180-185°C (Lit.[28] 186°C), IR (KBr) ν_{max} (KBr) 3300-2500 (OH), 1680 (>C=O), 1600 cm^{-1} (>C=C<) ; ^{1}H-NMR δ 8.00 (2H, d, J = 7.5 Hz, AB system ortho to CO_2H), 7.50 (5H, m, Ph), 7.10 (2H, d, 7.5 Hz, AB system ortho to OCH_2Ph), 5.20 (2H, s, -CH_2-); EIMS m/z 228 (M^+).

Preparation of Ethyl 4-benzyloxybenzoate **15**[29]

Sodium (6.90g, 0.30 mole-atoms) was added to dry ethanol (120 ml) contained in a dry three-necked round bottomed flask equipped with an addition funnel, a reflux condenser with a drying tube and an oil bath on a magnetic stirrer hotplate. After all the sodium had reacted to form sodium ethoxide, a solution of ethyl 4-hydroxybenzoate (49.8g, 0.30 moles) in dry ethanol (30 ml) was added slowly and the reaction mixture was stirred for 5 minutes. Benzyl bromide (52g, 0.30 moles) was added dropwise and the resulting mixture heated under reflux overnight. After removing the ethanol on the rotary evaporator, water (80 ml) was added and the mixture extracted with ethyl acetate (200 ml). The organic solution after drying ($MgSO_4$) was filtered and evaporated to yield the product **15** (73.2g, 95%), IR (KBr) ν_{max} 1720 cm^{-1} (>C=O); ^{1}H-NMR δ7.96 (2H, d, J = 7.5 Hz, AB system ortho to CO_2Et), 7.20-7.50 (5H, m , Ph), 7.10 (2H, d , J = 7.5 Hz, AB system ortho to -OCH_2-), 5.10 (2H, s , -OCH_2-Ph), 4.20 (2H, q , J = 7.8 Hz, -CH_2-), 1.30 (3H, t , J = 7.8 Hz, -CH_3); EIMS m/z 256 (M^+, 8%), 91 (100%).

Hydrolysis of ester **15** *to give the acid* **5a**

Into a one litre flask equipped with a reflux condenser was placed a solution of ethyl 4-benzyloxybenzoate **15** (73g, 0.268 moles) in methanol (200 ml). Dilute sodium hydroxide solution (2M, 300 ml) was added and the resulting mixture was refluxed overnight in a heating mantle. Most of the methanol was removed by rotary evaporation and the residue after cooling in ice was acidified with concentrated hydrochloric acid using blue limus to check the end point. Water (200 ml) was added and the precipitated white solid was filtered on a Buchner funnel and dried in the

oven to yield acid **5a** (39.7g, 60.7%) which was identical to the previously obtained compound.

Preparation of 4-benzyloxybenzoyl Chloride **6a**[30]

4-Benzyloxybenzoic acid **5a** (60.5g, 0.27 moles), an excess of Thionyl chloride (90 ml, 0.76 moles) and dimethylformamide (10 drops) were placed into a 250 ml round-bottomed flask equipped with a reflux condenser, a $CaCl_2$ drying tube, a magnetic stirrer and an oil bath. The stirred mixture was stirred at room temperature overnight and then heated at 60°C for 4 h. The excess Thionyl chloride was removed on the rotary evaporator at 50°C to give the acid chloride **6a** which was purified by washing with petrol on the Buchner funnel to give a cream coloured solid (65.5g, 92.4%), m.p. 97-100°C (Lit.[30] 102°C), IR (KBr) ν_{max} (KBr) 1770 (>C=O) cm^{-1}; ^1H NMR δ 5.20 (2H, s, O-CH_2), 7.05 (2H, d, J = 7.5 Hz), 7.3-7.5 (5H, m, Ph), 8.10 (2H, d, J = 7.5 Hz),; EIMS m/z 246 (M^+), 91 (100%).

A general procedure for the preparation of acid hydrazides is illustrated by the synthesis of compound **7a** *as follows*: An ice-cold mixture of 4-benzyloxybenzoyl chloride **6a** (11.7g, 0.11 moles) in dry pyridine (60 cm^3) was prepared. Phenyl hydrazine (16.0g, 0.11 moles) was added slowly with stirring before allowing the mixture to come to room temperature. An orange/red coloured mixture developed after leaving the reaction mixture stirring overnight. Treatment with water (50 cm^3) and crushed ice (50g) yielded an orange precipitate upon suction filtration. The precipitate was stirred with dilute sulphuric acid (500 cm^3), filtered through a sinter funnel on a Buchner flask and washed with aqueous saturated sodium bicarbonate solution followed by plenty of water to yield the crude product. Purification[24]: The dried crude product was dissolved in chloroform (90ml) and placed in an ice bath. Dry hydrogen chloride gas was passed through the solution in the fumehood for 2 h and the precipitated hydrochloride salt was removed by filtration on a sintered Buchner funnel. The product was transferred into a beaker and stirred with saturated sodium bicarbonate solution (200ml) and then re-filtered on the Buchner funnel. The dried acid hydrazide **7a** was recrystalised from chloroform and petroleum ether to yield the pure product *4-benzyloxybenzoyl phenyl hydrazide* **7a** (15.4 g, 66%) as a creamy-white coloured solid, m.p. 136-140°C; IR (KBr) ν_{max} (KBr) 3360 and 3260 (-NH-),

1650 (>C=O), 1600 cm^{-1} (>C=C<); ^{1}H NMR δ 4.20 (1H, broad s, NH), 5.20 (2H, s, OCH_2-), 6.66 – 6.75 (3H, m, Ar), 7.00 (2H, d of AB system, J = 7.5 Hz, O-Ar), 7.18 – 7.20 (7H, m, Ar), 7.86 (2H, d of AB system, J = 7.5 Hz, CO-Ar), 8.20 (1H, broad s, CONH-) ; EIMS m/z 318 (M^+); HRMS: m/z calcd. for $C_{20}H_{16}N_2O_2$ +1: 317.3692. Found: m/z, .

Benzoyl phenyl hydrazide **7b** was prepared from benzoyl chloride (16.0g, 0.11 moles), dry pyridine (60 ml) and phenylhydrazine (11.70g, 0.11 moles) as a light yellow powder (14.0g, 60 % yield) after purification[24], m.p.157-158°C; IR (KBr) ν_{max} 3325 and 3244 (NH), 3054 (C-H), 1643 cm^{-1} (>HNC=O); ^{1}H NMR δ 8.16 (1H, broad s, NHCO), 7.85 (2H, d, J = 7.5 Hz, *ortho* PhCONH-), 7.42-7.63 (3H, m, *meta* and *para* PhCONH-), 7.19-7.29 (2H, m, *meta* PhNH-), 6.89-6.98 (3H, m, *ortho* and *para* PhNH-)4.30 (1H, broad s, NHPh) ; EIMS m/z 212 (M^+, 32%), 105 (PhCO, 100%), 77 (Ph, 69%), 51 (17%).

4-Nitrobenzoyl phenyl hydrazide **7c** was prepared from 4-nitrobenzoyl chloride **6c** (19.0g, 0.1 moles), dry pyridine (100 ml) and phenylhydrazine (12.0g, 0.11 moles) as a light orange coloured solid (20.6g, 80%) after purification[24], m.p. 185-189°C; IR (KBr) ν_{max} 3292 (NH), 3104 (C-H), 1640 (>C=O), 1601 (>C=C<), 1520 cm^{-1} (NO_2); ^{1}H-NMR δ 8.37 (2H, d, J = 7.5 Hz, ortho to $ArNO_2$ group), 8.21 (2H, d, J = 7.5 Hz, ortho to ArCO group), 7.18 (3H, m, meta and para to ArNH group), 6.66 (2H, d, J = 7.5 Hz, ortho to ArNH), 4.00 (1H, br s, NH); EIMS m/z 257 (M^+, 43%), 150 (M-NHNHPh, 97%), 107 (NHNHPh, 100%), 77 (Ph, 65%).

4-Nitrobenzoyl 2-ethylphenyl hydrazide **7d** was prepared from 4-nitrobenzoyl chloride **6d** (10.72g, 0.058 moles), dry pyridine (100 ml) and 2-ethylphenylhydrazine (10.0g, 0.058 moles) as a dark orange coloured solid (14.5g, 80%) after purification[24], m.p. 137-140°C; IR (KBr) ν_{max} 3301 and 3263 (NH), 3104, 3075, 2970 and 2878 (C-H), 1650 (>C=O), 1602 (>C=C<), 1531 cm^{-1} (NO_2); ^{1}H NMR δ 8.60 (1H, Br s, NHCO), 8.25 (2H, d, J = 7.5 Hz, ortho to ArNO2 group), 7.98 (2H, d, J = 7.5 Hz, ortho to ArCO group), 7.0-7.16 (2H, m, H-3 and H-5 of ArNH-), 6.84-6.97 (2H, m, H-4 and H-6 of ArNH-), 5.05 (1H, br s, NH), 2.65 (2H, q, J = 7.8 Hz, $-CH_2$), 1.35 (3H, t, J = 7.8 Hz, CH_3); ^{13}C NMR δ 13.1, 23.5, 112.3, 121.6, 123.7, 126.7, 128.3, 128.5, 129.2,

137.5, 144.5, 149.8 and 165.5; EIMS m/z 285 (M^+, 13%), 240 (M-HNO_2, 9%), 150 (NO_2-C_6H_4-Et, 100%), 133 (Et-C_6H_4-N_2, 22%), 105 (Et-C_6H_4-, 77%), 76 (C_6H_4, 57%); HRMS: m/z calcd. for $C_{15}H_{15}N_3O_3$ +1: 286.3119. Found: m/z, 285.3049.

Preparation of polyphosphate ester (PPE)

A mixture of phosphorous pentoxide (P_4O_{10}) (225g, 1.68 moles), dry diethyl ether (450 ml) and dry chloroform (225 ml) was refluxed for 30 h in an oil bath at 65-70°C to give a viscous liquid (EtO_3P , PPE) (200g).

Method A: Cyclization of diary hydrazides **7a-d** *to 3-arylindazole* **8a-d** *in PPA or PPE.*

(i) Benzoyl phenyl hydrazide **7b** (1.5g, 4.65 mmol) and polyphosphoric acid (15g) were heated with stirring for two hours at 100 ^{0}C. Water (100ml) was added to dissolve the poly-phosphoric acid and the solution was neutralised using solid potassium carbonate. The mixture was suction filtered and washed with water to yield a light brown solid (1.03g) which according to TLC (ethyl acetate: petrol, 1:2 v/v) and ^{1}H NMR was the starting hydrazide **7b**.

(ii) Benzoyl phenyl hydrazine **7b** (1.0g, 3.1 mmoles) and polyphosphate ester (PPE) (10g) were heated at 100^{0}C for 2 hours. The mixture was cooled and water/ice (40ml) was added to dissolve the PPE and extracted with dichloromethane (30 ml. The organic layer was separated and the solvent removed *in vacuo* to yield a brown solid (1.0g) which according to TLC (ethyl acetate: petrol, 1:2 v/v) and ^{1}H NMR was the starting hydrazide **7b**.

(iii) Benzol phenyl hydrazide **7b** (1.0g, 3.1 mmol) and (PPE) (10g), were heated for 5 hours at 130°C. The mixture was worked up as above to yield a brown solid (0.78 g) which was identified as the starting hydrazide **7b**.

(iv) Benzoyl phenyl hydrazide **7b** (1.0g, 3.1 mmol) and PPE (10g) were heated for 5 hours at 80 ^{0}C. The mixture was worked up as above to yield a brown solid (0.90 g) which was identified as the starting hydrazide **7b**.

(v) Benzoyl phenyl hydrazide **7b** (1.0g 3.1 mmol) and PPE (10g) were heated with stirring for 5 hours at 160 ^{0}C. The mixture was worked up in the above manner to yield a brown solid (0.8g) which was identified by TLC and ^{1}H NMR as the starting hydrazide **7b**.

(vi) Benzoyl phenyl hydrazide **7b** (1.0g, 3.1 mmol) and PPE (10g) were heated with stirring for 5 hours at 60 ^{0}C which after standard work up yielded a brown solid (0.50 g) that was identified as the starting hydrazide **7b**.

(vii) 4-Nitrobenzoyl phenyl hydrazide **7c** (2.0g) in PPE (20g) was heated for 2 hours at 100 ^{0}C. To the cooled mixture was added ice-water (40ml) and extracted with dichloromethane (30 ml). After drying ($MgSO_4$) the organic layer was evaporated to yield a product which was recovered starting material **7c**.

(viii) 4-Nitrobenzoyl phenyl hydrazide **7c** (1.0g) in PPE (10g) was heated for 5 hours at 100 ^{0}C. Similar work up to above yielded recovered starting material **7c**.

(ix) 4-Nitrobenzoyl phenyl hydrazide **7c** (1.0g) in polyphosphoric acid (10g) was heated for 1 hour at 80 ^{0}C. Standard work up to above yielded a very small amount of product which was recovered starting material **7c**.

(x) 4-Nitrobenzoyl phenyl hydrazide **7c** (1.0g) in poly phosphoric acid (10g) was heated for 4 hours at 100 ^{0}C. Work up yielded a small amount of product identified as recovered starting material **7c**.

(xi) 4-Nitrobenzoyl 2-ethylphenyl hydrazide **7d** (1.0g) in poly phosphoric acid (10g) was heated for 4 hours at 100 ^{0}C. Standard work up yielded negligible amount of crude reaction product.

(xii) 4-Benzyloxybenzoyl phenyl hydrazide **7a** (2.20g, 10 mmol) and poly-phosphoric acid (35g) were heated with stirring for two hours at 100 ^{0}C. Water (200ml) was added to dissolve the polyphosphoric acid and the solution was neutralised using solid potassium carbonate. The mixture was suction filtered and washed with water to yield a light brown solid (2.0g) which according to TLC (ethyl acetate: petrol, 1:2 v/v) and ^{1}H NMR was the starting hydrazide **7a**.

(xiii) 4-Benzyloxybenzoyl phenyl hydrazine **7a** (2.20g, 10 mmol) in polyphosphate ester (PPE) (30g) was heated at 120^{0}C for 4 hours. The mixture was cooled and water/ice (100ml) was added to dissolve the PPE and extracted with dichloromethane (80 ml). Work up in the usual way gave a brown solid (2.0g) which according to TLC (ethyl acetate: petrol, 1:2 v/v) and ^{1}H NMR was the starting hydrazide **7a**.

(xiv) 4-nitrobenzoyl 2-ethylphenyl hydrazide **7d** (1.00g, 3.5mmol) in PPA (10g) was heated at 120^{0}C for 2 hours after which it was worked up in the usual way to give a brown solid (0.5g) which was identified as starting hydrazide **7d** from its ^{1}H NMR spectrum.

Method B: Cyclization of benzoyl phenyl hydrazide **7b** *to 3-phenylindazole* **8a** *in Conc. H_2SO_4.*

(i) Benzoyl phenyl hydrazide **7b** (2.0g, 6.2 mmol) in concentrated sulphuric acid (15.0 ml) was heated with stirring for 2 hours at (105-110 ^{0}C). The mixture was poured into ice (150g) and neutralised by adding solid potassium carbonate until slight alkaline. Extraction with dichloromethane (60ml) gave a solution which on evaporation did not yield any product.

(ii) Benzoyl phenyl hydrazide **7b** (2.0g, 6.2 mmol) in concentrated sulphuric acid (10.0 ml) was heated with stirring for 30 mints at 60 ^{0}C. Work up similar to above showed the absence of any reaction product.

Method C: Cyclisation of 4-nitrobenzoyl 2-ethylphenyl hydrazide **7d** *in xylenes*

A mixture of the hydrazide **7d** (1.00g, 3.5 mmol), tosic acid (0.70g, 3.5mmol) in xylenes (20 ml) was heated under stirring in an oil bath at 150-160°C overnight. The solvent was removed by distillation under reduced pressure and the residue was dissolved in dichloromethane (100 ml) and washed with saturated $NaHCO_3$ solution (2 x 40 ml). After drying ($MgSO_4$) the solvent was evaporated to yield a residue which was examined by TLC [ethyl acetate:petrol, 1:2] to consist of two major products (R_f 0.55 and R_f 0.20) along with a few other minor polar and non-polar components. The reaction mixture was separated by flash chromatography using the eluent [ethyl acetate:petrol,1:2] to afford firstly the product (R_f 0.55 on TLC) (0.20g) as a dark oil identified as 2-ethylphenylhydrazine; secondly the product (R_f 0.20 on TLC) (0.35g, 37.5%) also as a dark thick oil which partially solidified and identified as the indazole **8c**, IR (tlf) ν_{max} 3300 (NH), 3102, 3065, 2968 and 2875 (C-H), 1600 (>C=C<), 1529 cm^{-1} (NO_2); ^{1}H NMR δ 7.69 (2H, d, J = 7.8 Hz, ortho to $ArNO_2$ group), 7.29 (2H, d, J = 7.8 Hz, ortho to Ar), 6.59-6.62 (2H, m, H-4 and H-5), 6.48-6.50 (1H, m, H-6), 3.50 (1H, broad s, NH), 2.37 (2H, q, J = 7.2 Hz, -CH_2), 1.35 (3H, t, J = 7.2 Hz, CH_3); ESMS m/z 268 (M+1)(20%).

2.4 Conclusions

In this chapter a novel method in the form of route I was tried for making *1H*-indazoles. 4-Benzyloxybenzoyl chloride **6a** was prepared in two steps fron 4-hydroxybenzoic acid by protection with benzyl bromide and acylation using thionyl chloride. Phenyhydrazine was reacted with benzoyl chloride **6b**, 4-benzyloxy benzoyl chloride **6a** and 4-nitrobenzoyl chloride **6c** in pyridine to give the desired β-monoacylated acid hydrazides **7a-c** that were contaminated with a minor amount of α-monoacylated derivatives and were purified by passing HCl gas through their solution in chloroform at 0°C. Similarly reaction of 2-ethylphenylhydrazine with 4-nitro benzoyl chloride in pyridine yielded 4-nitrobenzoyl 2-ethylphenylhydrazide **7d .**

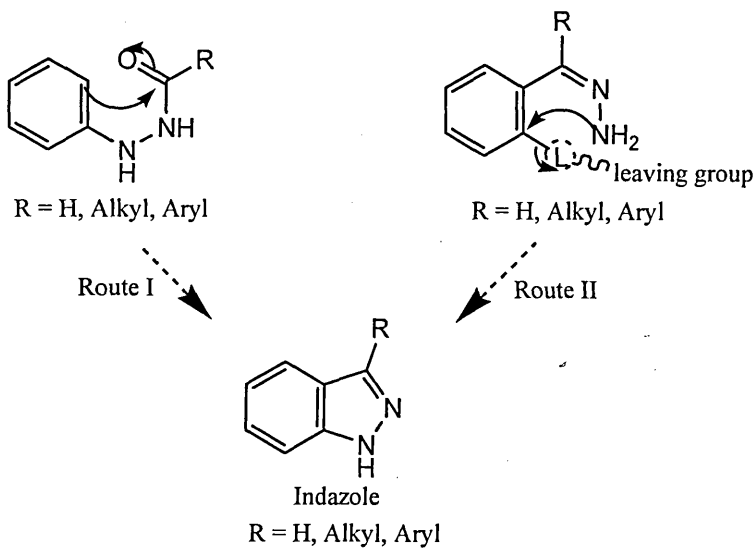
The acid hydrazides **7a-d** failed to cyclise to yield the indazole products **8a-d** on heating in polyphosphoric acid (PPA), polyphosphate ester (PPE) and concentrated sulphuric acid at various temperatures (60-160°C). However, the hydrazide **7d** underwent cyclisation in xylene containing an equivalent amount of tosic acid at 150-160°C to produce the desired indazole **8c** in 37.5 % yield along with ethylphenylhydrazine as a cleavage product. This result showed that an electron releasing group was essential on the phenylhydrazine component of the acid hydrazide and an electron withdrawing group was necessary on the benzoyl component. More examples are needed to confirm the hypothesis and in particular 2,4-dimethoxyhydrazine derived acid hydrazide would be very interesting to try to cyclise under these reaction conditions.

Chapter 3

Synthesis of Indazoles by Nucleophilic Substitution Reactions of Diaryl and Aryl Alkyl Hydrazones

3.1 Introduction

As discussed out in chapter 1 the synthesis of indazole molecule can be envisaged by two alternate routes I and II in which the pyrazole ring is constructed involving intramolecular ring closure reactions as shown below.



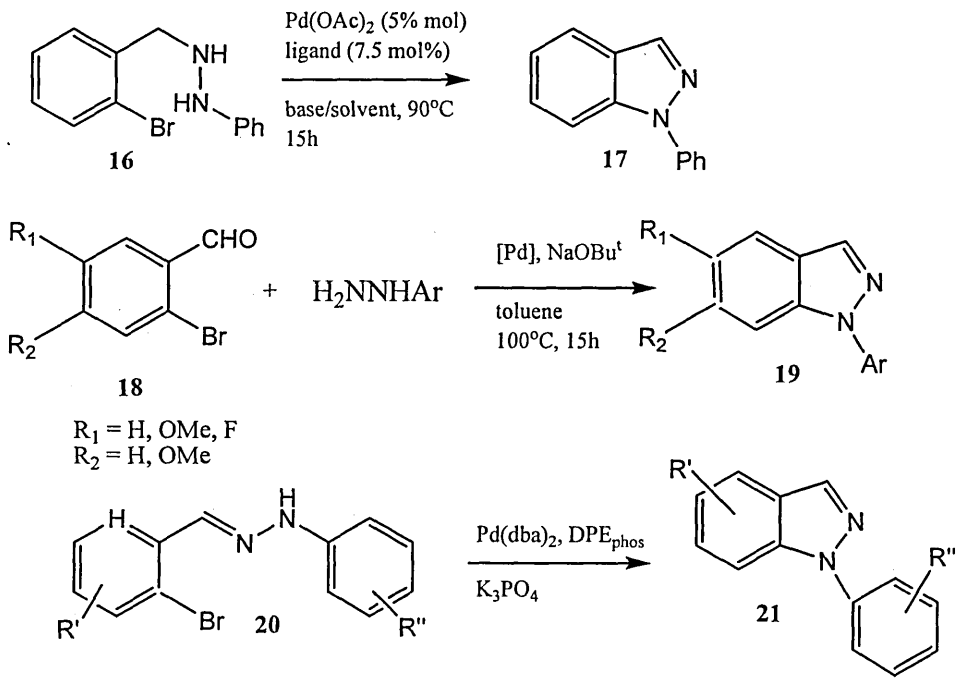
Route I involves the intramolecular electrophilic aromatic substitution reaction to form a carbon-carbon bond whilst route II involves intramolecular nucleophilic aromatic substitution reaction to form a carbon-nitrogen bond. The novel approach of route I was examined in chapter one. In this chapter formation of indazoles by the approach of route II will be discussed.

The indazole subunit is a frequently found motif in medicinal compounds having important biological properties such as antifertility[31], antidepressants[27], anti-inflammatory[31] anti-tumor and anti-HIV activities[32]. New methods for the synthesis of

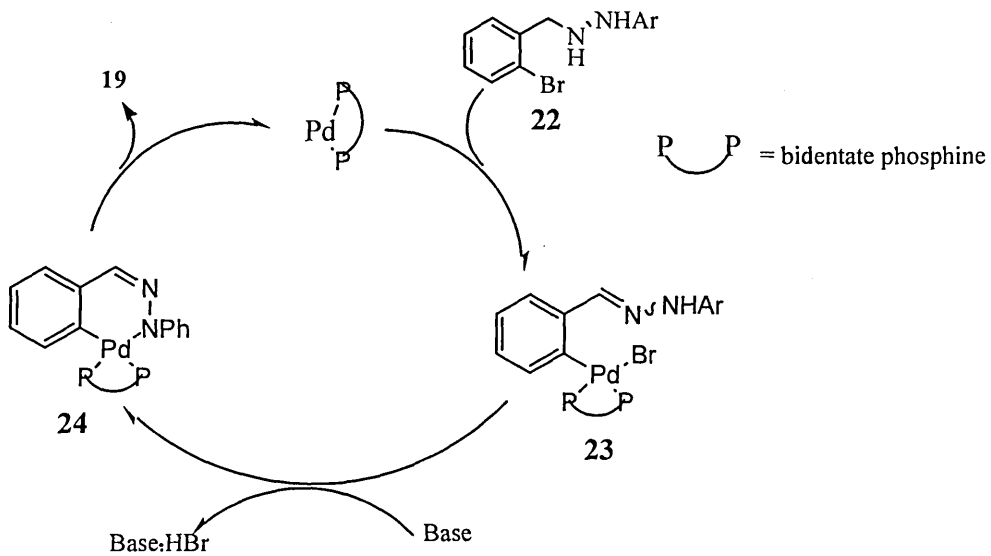
indazoles are therefore essentially important. Methods of synthesis which form the C-N bond are of importance in indazole chemistry and a large number of examples exist in the literature for this particular strategy[33-54].

In particular, palladium-catalysed aromatic C-N bond forming reaction by the cross-coupling of aryl halides (or triflates) and amines has received considerable attention

Scheme 1



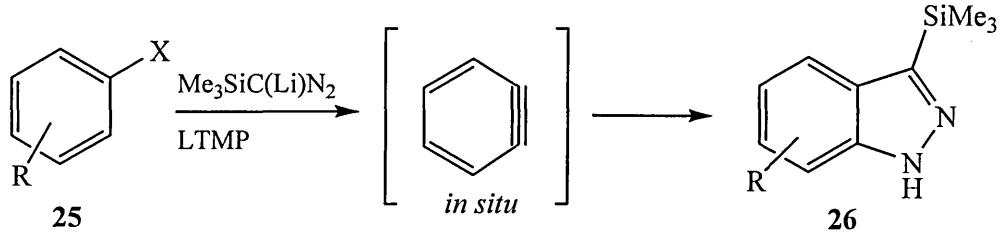
Plausible Reaction Mechanism (compound **19**)



by several research groups to synthesise 1-aryl-1*H*-indazoles[55]. For example, 2-bromobenzaldehyde **18** reacted with arylhydrazine in toluene at 100°C in the presence of a palladium catalyst and phosphorous chelating ligands along with sodium t-butoxide to furnish the 1-aryl-1*H*-indazole **19** in 84% yield (scheme 1). A plausible mechanism has been suggested in which oxidative addition of the carbon-bromide bond of hydrazone **22**, formed *in situ*, to Pd(0) produce an arylpalladium (II) complex **23**. This is followed by an intramolecular closure to give a palladacycle **24** which affords the 1-aryl-1*H*-indazole **19** by reductive elimination as shown in scheme 1. Substrates **16** and **20** yielded the corresponding indazoles **17** and **21** respectively by a similar mechanism[55].

In another synthesis of 1*H*-indazoles **26** a [3+2] cycloaddition reaction of lithium trimethylsilyldiazomethane with benzynes, generated from halobenzenes **25**, has been reported[56]. In this approach the lithium trimethylsilyldiazomethane has been prepared from stable and safe compound trimethylsilyldiazomethane and butyllithium or lithium diisopropylamide (LDA) (scheme 2).

Scheme 2

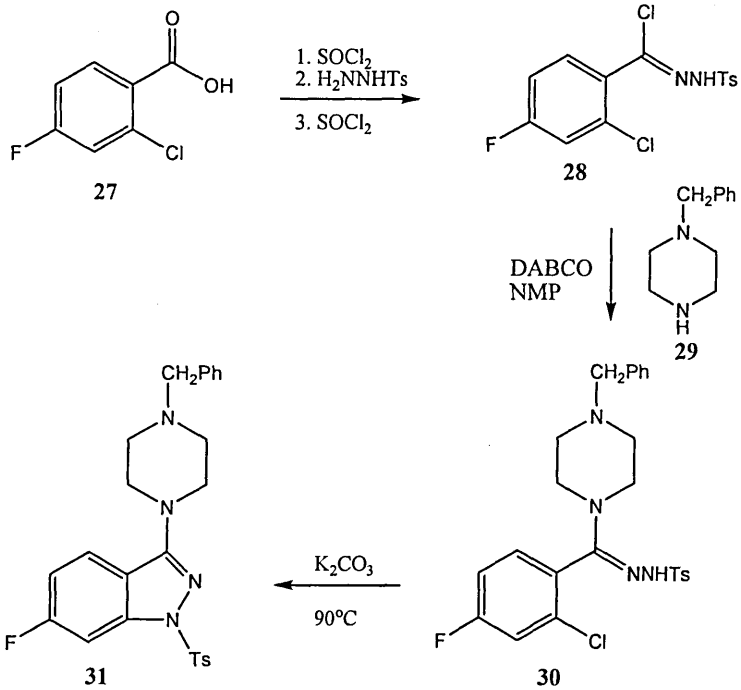


25 **26**

X = F, Cl, Br, I

3-Aminoindazole derivatives have been identified as potent dopamine receptor antagonists for use in antipsychotic treatment, as well as non-steroidal anti-inflammatory compounds with analgesic properties. The synthesis of a potential antipsychotic agent **31**, required for pre-clinical evaluation, was devised starting with the commercially available acid **27**. The acid **27** was converted into the imidoyl chloride **28** which on reaction with piperazine **29** in the presence of DABCO in N-methyl-2-pyrrolidinone yielded the imidate **30** that cyclised on treatment with potassium carbonate into the target compound **31** (scheme 3)[57].

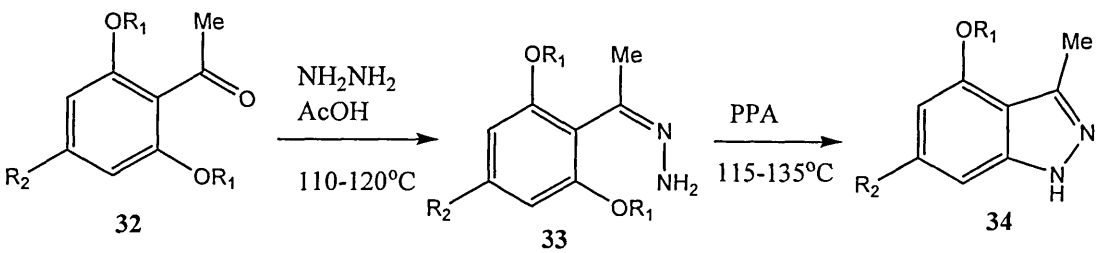
Scheme 3



27 28 29 30 31

A new and facile synthesis of indazoles has been reported in which cyclisation of 2,6-dialkoxyacetophenone hydrazones was obtained in the presence of polyphosphoric acid (PPA)[58]. Treatment of 2,6-dialkoxy (hydroxy) acetophenones **32** with hydrazine hydrate and catalytic amount of acetic acid at 110-120°C resulted in hydrazones **33** which without isolation were heated in PPA at 110-135°C to furnish the final indazole products **34** in reasonably good yields. Presumably the mechanisms of the cyclisation reactions involve nucleophilic aromatic substitution reaction with the displacement of a protonated alkoxy group as a leaving group in each case (scheme 4).

Scheme 4



32 33 34

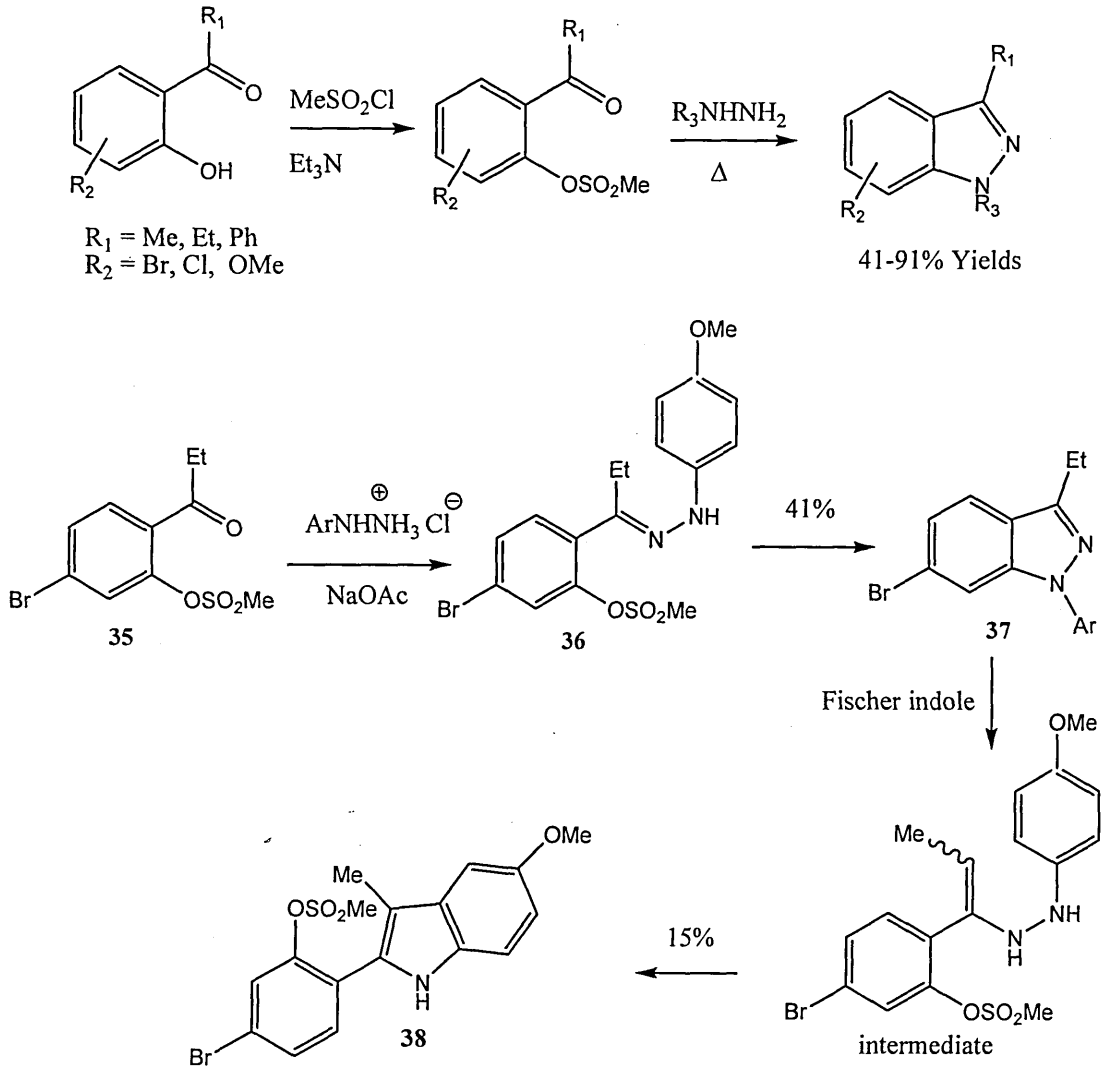
R_1 = H, Me, Et, Pr, Bu
R_2 = H, OMe

34-87% Yields

Another facile synthesis of indazoles reported in the literature involves the formation and cyclisation of arylhydrazones possessing a leaving group in the *Ortho* position[59]. This method involves a two-step, one pot sequence in which the hydrazones are

generated and cyclised to the desired 3-substituted indazoles in a single operation as outlined in scheme 5.

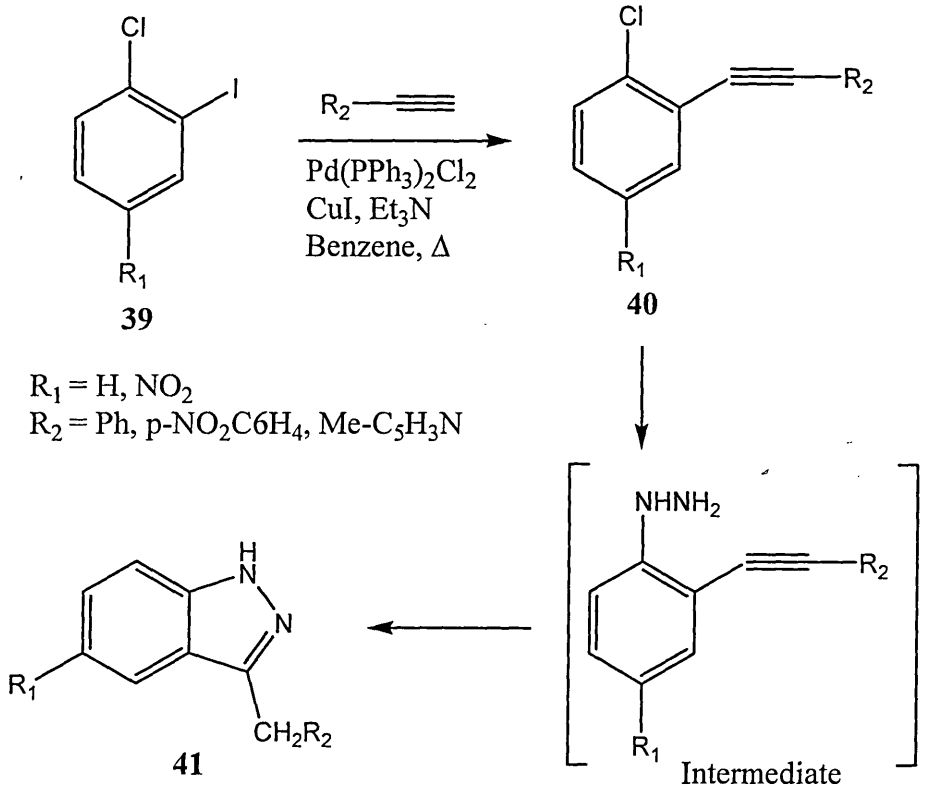
Scheme 5



In all of the reactions the Fischer-indole synthesis operated as a competitive process alongside the indazole synthesis. It was found that with 4-methoxyphenylhydrazine and the mesylate **35,** the hydrazone **36** formed gave the corresponding indazole **37** more rapidly than the competing Fischer indole synthesis product **38**. It was concluded that the only substituted hydrazines produced the indazole products and no indazoles were obtained with either hydrazine hydrate or hydrazine hydrochloride. The two outlines from the literature shown in schemes 4 and schemes 5 appear contradictory. In scheme 4 the researchers obtained indazole derivatives using hydrazine hydrate whereas in scheme 5 the authors claim that unless the hydrazine was substituted no indazole products could be obtained.

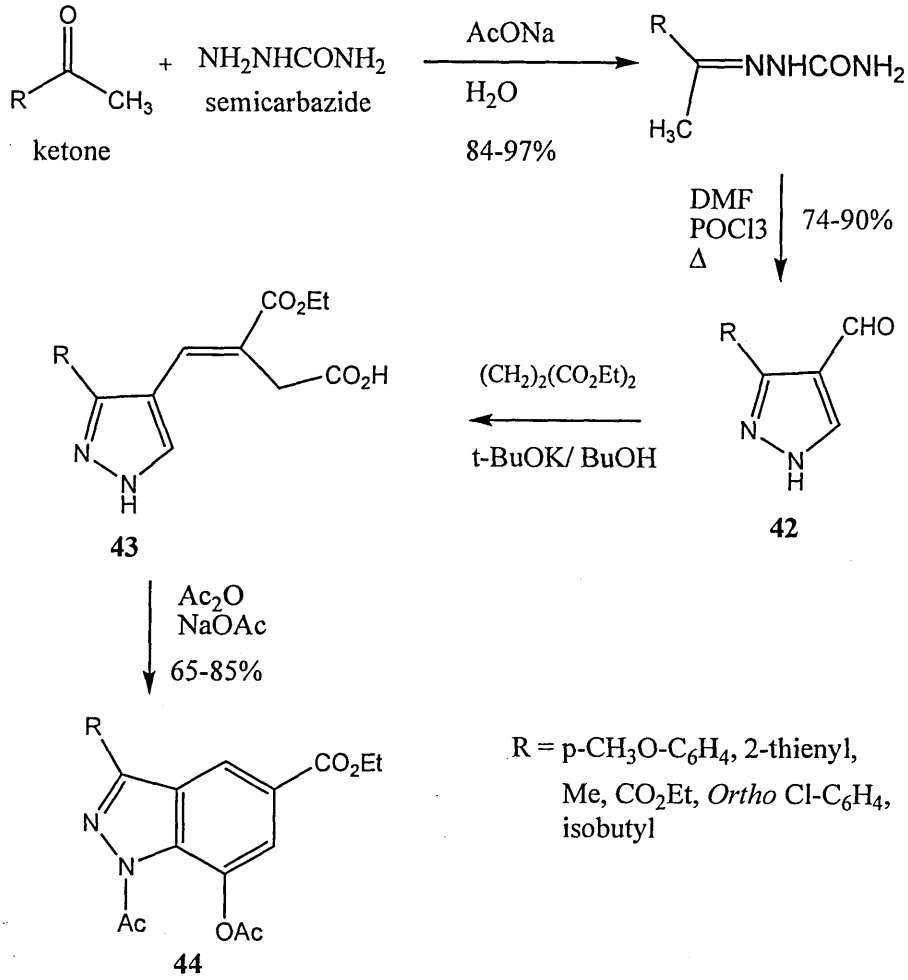
A novel route to substituted indazoles involving cyclocondensation of activated *Ortho*-chloroarylacetylenes with hydrazine hydrate has been reported[60]. In this methodology the essential ingredients are the *Ortho*-chloroiodoarenes (**39**) which presumably must have been synthesised from the corresponding *Ortho*-nitroarenes *via* reduction, diazotization and the Sandmeyer reaction. The aryl iodides were condensed with terminal acetylenes in the presence of $(PPh_3)_3PdCl_2$, CuI and Et_3N to give the *Ortho*-chloroacetlenes (**40**) in 75-85% yields. The ortho-chloroacetylenes (**40**) were reacted with hydrazine hydrate in refluxing butanol to yield the substituted indazoles (**41**) in 65-88% yields (scheme 6).

Scheme 6

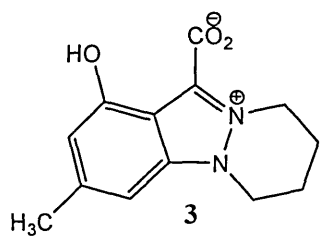


In all of the above syntheses the pyrazole ring of the indazole has been created last. However, there is a recent synthesis reported in the literature which actually constructs the pyrazole ring first and constructs the aromatic ring system last[61]. In this new synthetic approach a Stobbe condensation reaction has been performed between 3-alkyl or aryl-4-formylpyrazoles **42** and diethyl succinate in the presence of potassium t-butoxide followed by intramolecular ring closure of the Stobbe products **43** using acetic anhydride and sodium acetate to afford the corresponding indazole derivatives **44** in 65-85% yields as illustrated in scheme 7.

Scheme 7

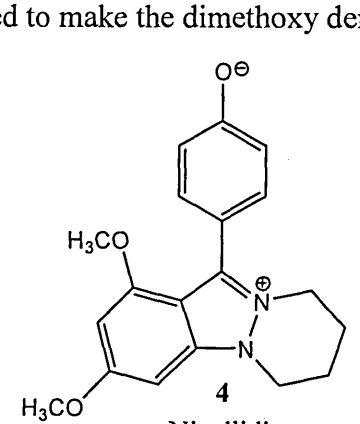


Examining all the literature synthetic methods[55-61] for the synthesis of 1*H*-indazoles it became apparent that for our alkaloids **1** and **4** the most suitable approach was that shown in scheme 4 above[59]. It was decided to make the dimethoxy derivative **49**



3

Nigellicine



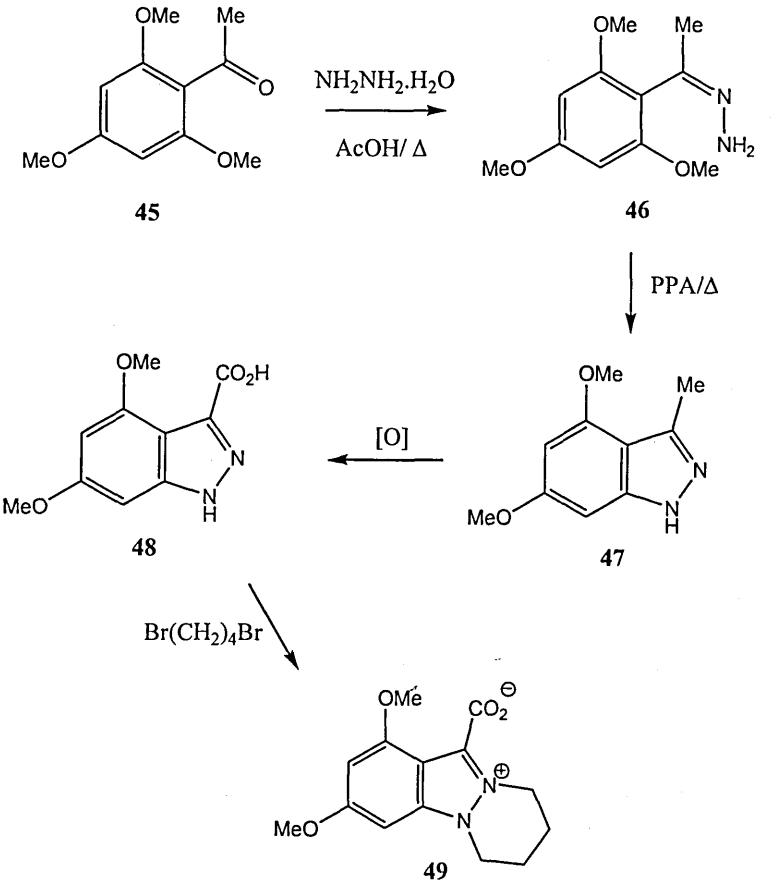
4

Nigellidine

of Nigellicine **1** by the chemistry shown in scheme 8 in which it was envisaged that the reaction of the starting ketone **45** to form the hydrazone **46** with hydrazine hydrate would cyclise it to the indazole **47** in PPA. Oxidation of the methyl group at 3-

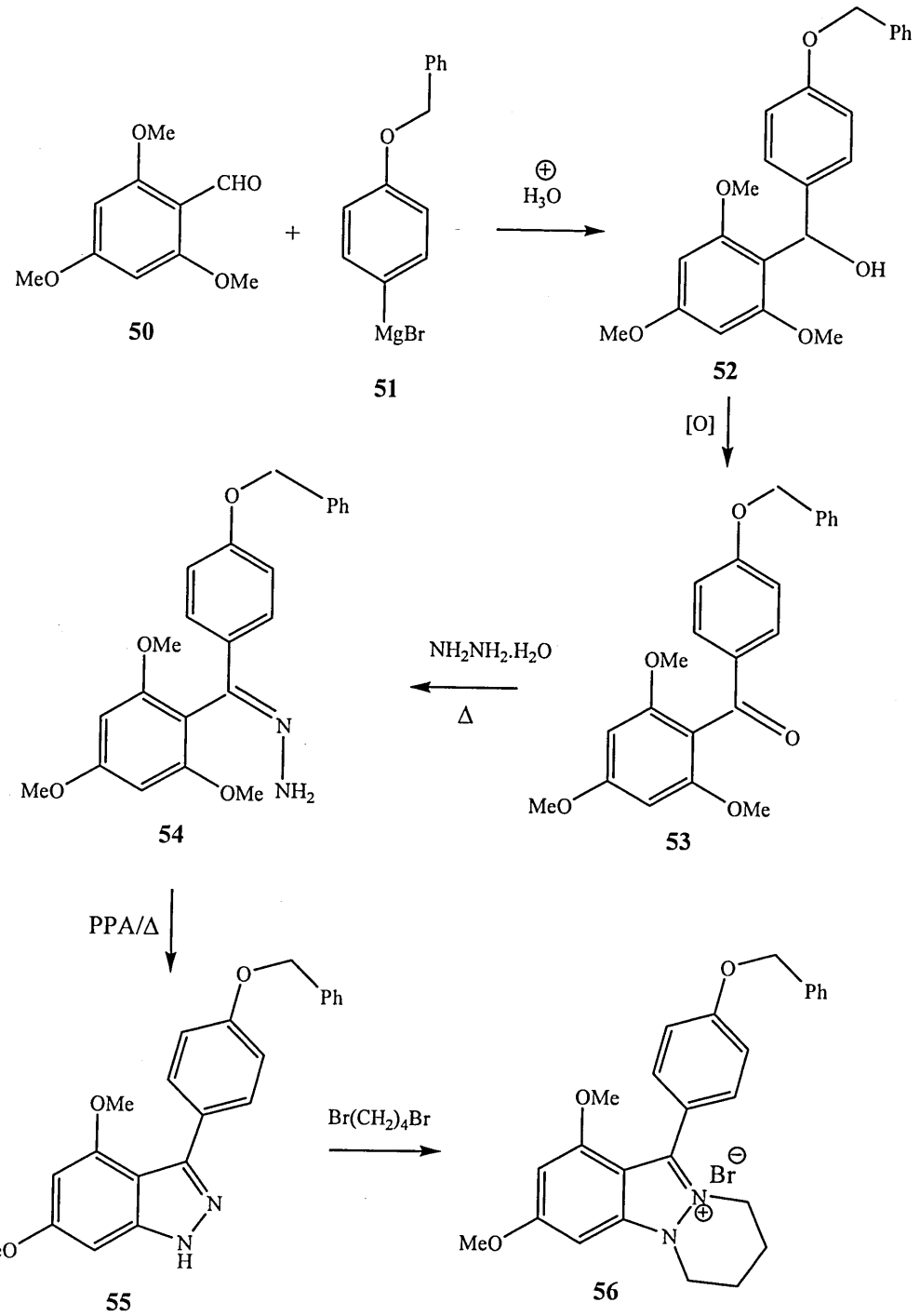
position of the indazole **47** was to be affected using known literature procedures and the six-membered ring was to be introduced by reaction of the indazole **48** with 1,4-dibromobutane to give compound **49** which is an analogue of Nigellicine **3** (scheme 8).

Scheme 8



For the synthesis of Nigellidine **4** similar strategy was envisaged involving the formation of an intermediate hydrazone **54** which on cyclisation was expected to yield the corresponding indazole **55** and consequently a protected form of Nigellidine **56** (scheme 9). Deprotection of **56** by hydrogenolysis would then yield Nigellidine **4**.

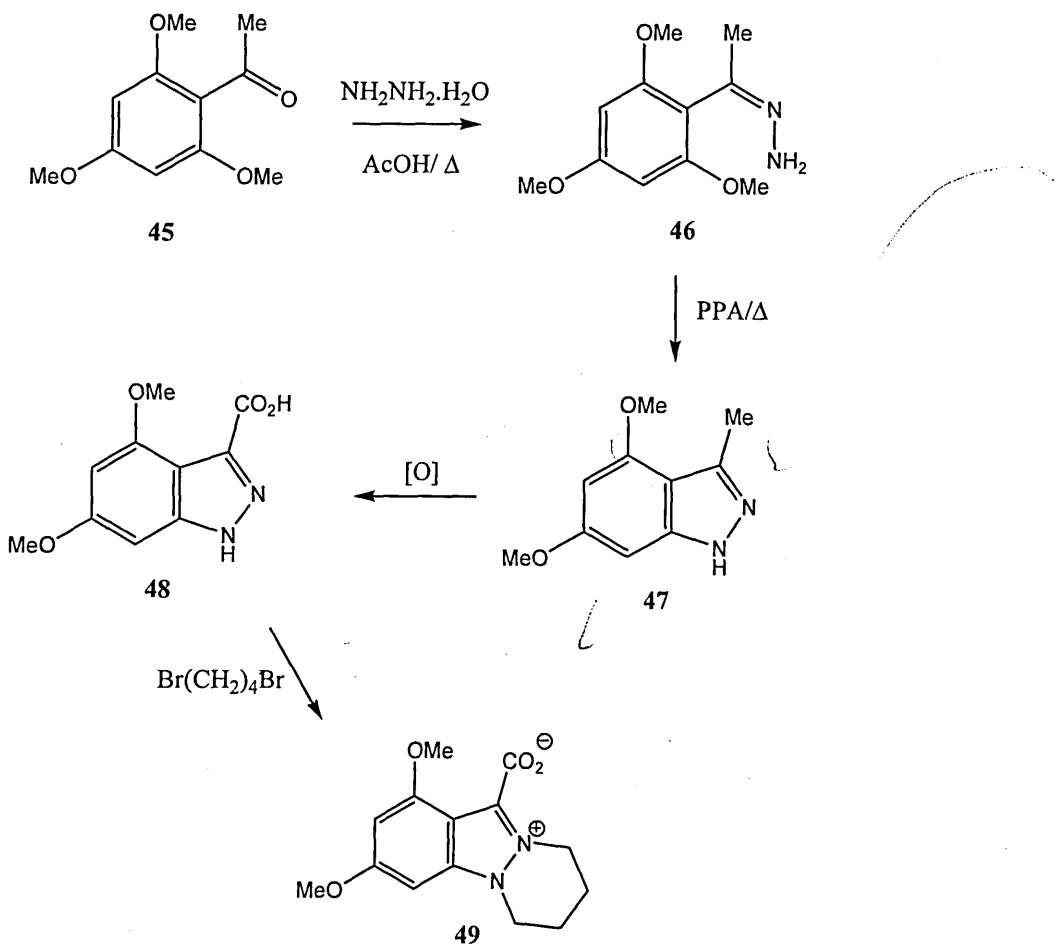
Scheme 9


OMe
CHO
MeO
OMe
50
Ph
O
MgBr
51
⊕
H_3O
Ph
O
OMe
OH
MeO
OMe
52
[O]
Ph
O
OMe
O
MeO
OMe
53
$NH_2NH_2.H_2O$
Δ
Ph
O
OMe
N
MeO
OMe
NH_2
54
PPA/Δ
Ph
O
OMe
N
N
H
MeO
55
$Br(CH_2)_4Br$
Ph
O
OMe
Br
⊖
N
⊕
N
MeO
56

3.2 Results and Discussion

The commercial availability of required starting material dictated which synthetic strategy was to be adopted. In this regard the availability of the commercial compound 2,4,6-trimethoxyacetophenone **45** was considered a good starting material for the chemistry outlined in scheme 8.

Scheme 8

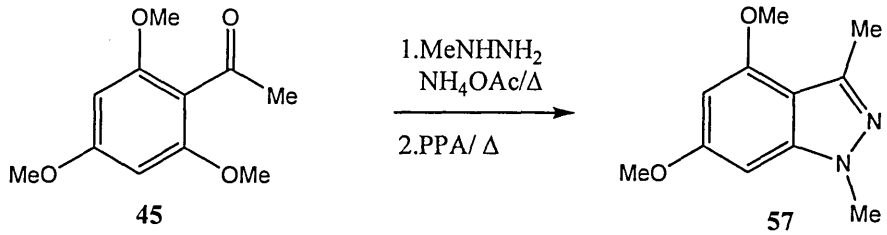


The 'one-pot method' of Zhenqi *et.al*[59] was followed in which 2,4,6-trimethoxy-acetophenone **45** was treated with hydrazine hydrate in the presence of glacial acetic acid at 110-120°C. The resulting hydrazone **46** without isolation was subjected to heating in PPA at 110-135°C under stirring for 30 min. to give the indazole **47** in a low yield of 20% (lit. yield 65%). The reaction was repeated several times using fresh commercial PPA but the yield could not be improved to match that reported in the literature. The reaction was next performed using PPE instead of PPA but the product formation deteriorated considerably under these conditions. Product isolation from the reaction mixtures involving PPA proved particularly difficult. Although Zhenghi *et.al*[59] have reported the isolation of their products from the PPA mixture without

neutralisation of the aqueous phosphoric acid with a base, it was considered necessary at the end of the reaction to neutralise the aqueous PPA with either solid sodium hydrogen carbonate or concentrated ammonia solution since the indazole product has basic nitrogen atoms which are likely to form salts with the aqueous phosphoric acid. The reaction mixtures were extracted with the recommended solvent, ethyl acetate, as well as other solvents such as dichloromethane and chloroform. Interestingly, it was not possible to isolate any product at all if the reaction mixture was not neutralised as suggested in the literature. The hydrazone **46** was formed in excellent yield and was characterised by ^{1}H-NMR and IR spectroscopy. The ^{1}H-NMR spectrum showed the methyl group resonating at higher field (δ 1.0) compared with the methyl in the ketone **45** (δ 2.20) and the appearance of a broad signal at δ 8.10 which presumably was the amino group. Its IR spectrum showed two sharp peaks at 3260 cm^{-1} and 3350 cm^{-1} indicative of the amino group as well as the absence of the carbonyl group at 1690 cm^{-1} and the appearance of a peak at 1670 cm^{-1} indicative of the imine bond.

In view of the low yield obtained for the 1*H*-indazole **47** attention was turned to another method of ring closure reported in the literature[55]. Using this method the ketone **45** was reacted with hydrazine hydrate and ammonium acetate in xylene at 135°C for 24 h. TLC analysis [ethyl acetate: petrol; 1:3 v/v] of the reaction mixture showed a new spot with very little of the starting material left. The xylene was removed by simple distillation and the product mixture was separated by flash chromatography [ethyl acetate: petrol; 1:3 v/v] to yield the product compound identified as the hydrazone **46** (89%). This method did not offer any greater advantage over the previous method.

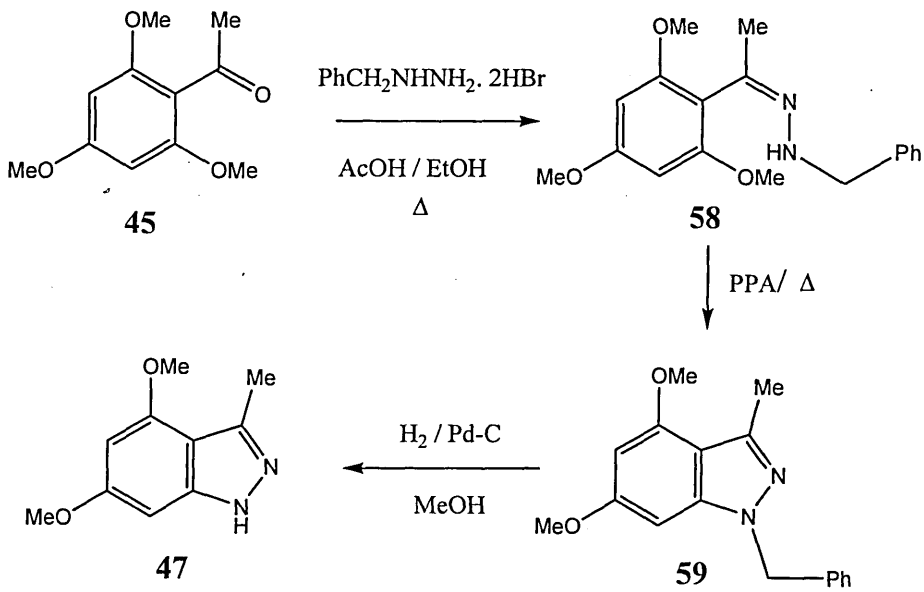
Hydrazines with electron releasing groups such as alkyl hydrazines have been reported to give best results in the ring closure process to form the indazoles. In this regard initial experiments were conducted with methylhydrazine which is commercially available. The reaction of methylhydrazine with 2,4,6-trimethoxyacetophenone using catalytic amount of glacial acetic acid in PPA yielded the corresponding 1,3-dimethylindazole albeit in low yield (18%). This was a disappointing result once again.



45 57

The next consideration was whether the hydrophobic content of the expected indazole could be increased by using benzylhydrazine instead of hydrazine and if by so doing the molecule would precipitate out better at the end of the reaction. Benzylhydrazine is commercially available as the dihydrochloride or dihydrobromide salt. The other advantage of benzylhydrazine is that the benzyl group can be removed from the anticipated indazole under mild conditions such as by hydrogenolysis involving hydrogen gas and Pd/C catalyst in an alcoholic solvent (scheme 10).

Scheme 10

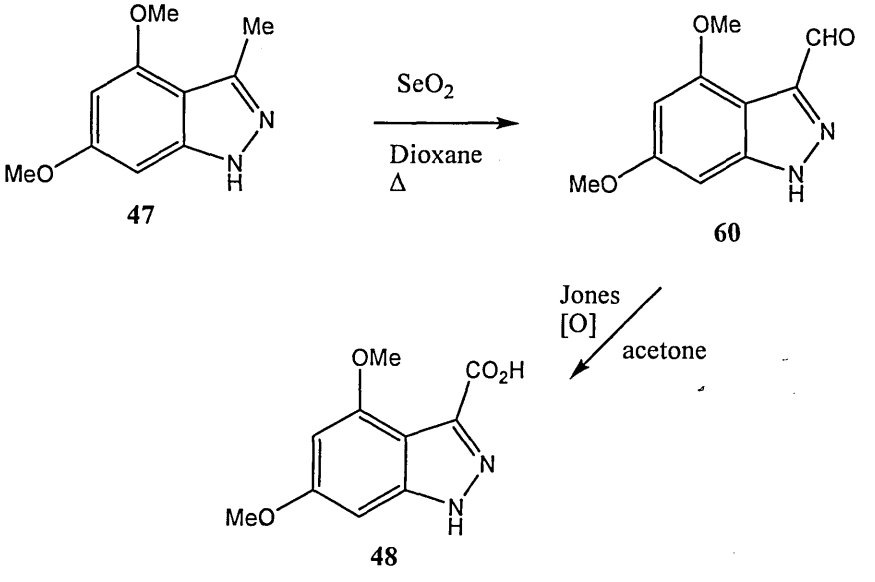


45 58 47 59

Benzylhydrazine dihydrobromide was reacted with 2,4,6-trimethoxyacetophenone in refluxing ethanol to form the hydrazone which after removal of the solvent was subjected to cyclisation in PPA at 120-135°C. Disappointingly the isolated crude product was shown to consist of a mixture of many compounds and separation of the mixture by flash chromatography yielded the expected product in very low yield (16%). The ^{1}H NMR spectrum showed the benzyl group having its benzylic protons

resonating at δ 4.80 as a result of deshielding by the aromatic ring and the nitrogen atom and its phenyl group at δ 7.20-7.35. The methyl group in the 3-position resonated at the expected position of δ 2.75. Electrospray mass spectrum of the molecule showed an $[M+H]^+$ ion at m/z 283 consistent with the molecular structure. We next diverted our attention to oxidation of the methyl group at the 3-position in the 1*H*-indazole product **47**. Successful experience with oxidation of substituted 4-methylquinolines to the corresponding aldehydes in these laboratories using selenium dioxide in moist dioxane encouraged us to try the reagent for the oxidation of the 3-methylindazole system **47**.

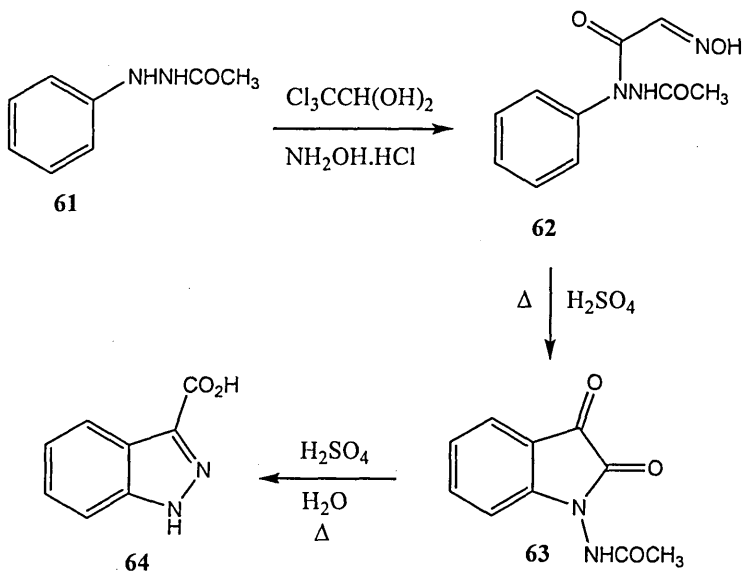
Scheme 11



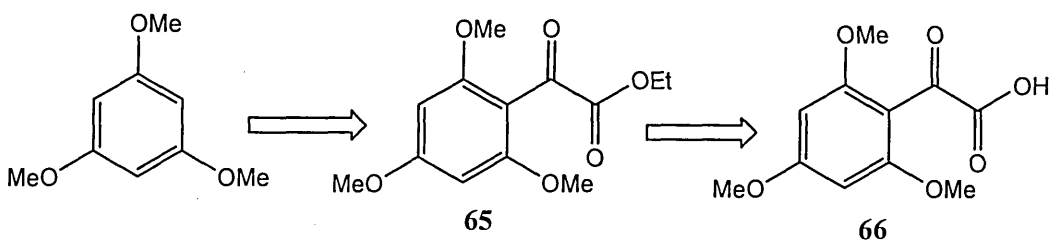
A literature search[63] had revealed that oxidising agents for a methyl group on aromatic systems include SeO_2 in pyridine and $KMnO_4$ solution. It was envisaged that the oxidation of **47** with SeO_2 would produce the aldehyde **60** which on further oxidation with Jones's reagent would produce the desired carboxylic acid **48** (scheme 11). However, when the chemistry outlined in scheme 11 was performed in the laboratory, the crude product isolated from the reaction was flash chromatographed [ethyl acetate: petrol; 1:3] to give recovered starting material **47** only. This result was surprising because as pointed out earlier oxidation of 4-methylquinolines with SeO_2 in wet dioxane produced the corresponding aldehydes in reasonable yields (50-60%).
A literature search on indazole-3-carboxylic acids revealed a synthesis that involved

reaction of β-acetylphenylhydrazine **61** with chloral hydrate and hydroxylamine hydrochloride in acidic medium to afford an intermediate *N*-acetylaminoisonitroso-acetanilide **62** in 80% yield[64]. Treatment of the intermediate with sulphuric acid afforded indazole-3-carboxylic acid **64** in 77% yield *via* compound **63** as shown in scheme 12. For this synthesis to be applicable to the alkaloids studied here would require 2,4,6-trimethoxyhydrazine, which is not commercially available.

Scheme 12

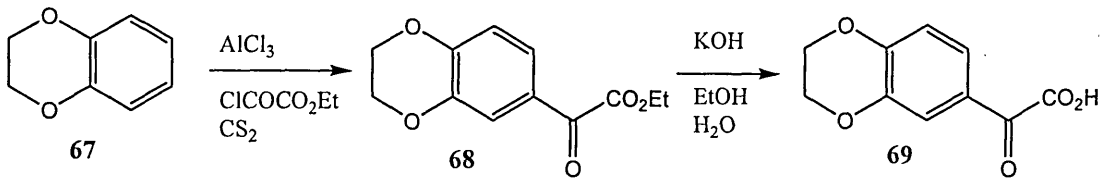


In view of the difficulty in making 2,4,6-trimethoxyphenylhydrazine required for making the indazole-3-carboxylic acid by the route outlined in scheme 12, it was decided to invent another novel strategy for making 4,6-dimethoxyindazole-3-carboxylic acid **48**. The new strategy involved making the trimethoxy keto ester **65** by Friedel-Crafts acylation of 1,3,5-trimethoxybenzene with ethyl oxalylchloride and hydrolysis of the keto ester **65**[65] to the corresponding keto acid **66**[65] required for the synthesis the indazole **48**.



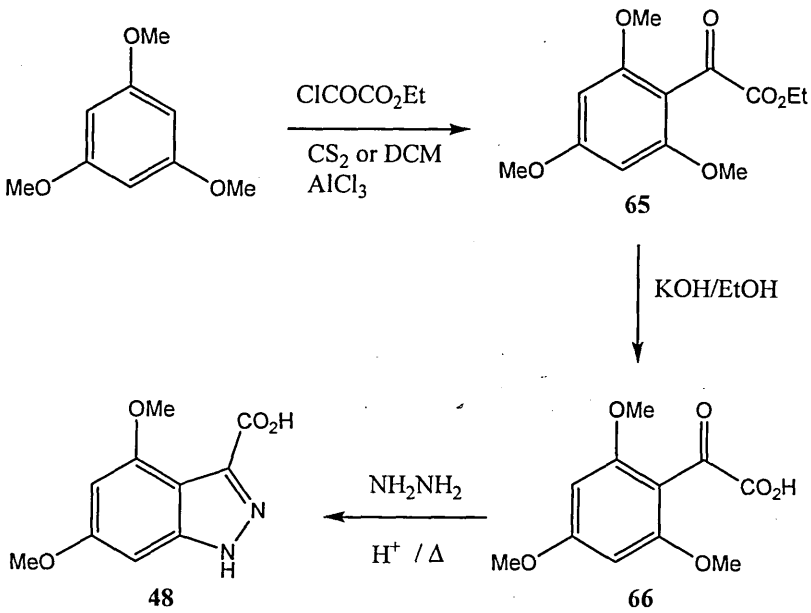
A literature search showed[66] a method for the synthesis of an α-ketoester **68** from 2,3-dihydro-1,4-benzodioxin **67** and hydrolysis of the α-ketoester **68** to the corresponding α-ketoacid **69** as shown in scheme 13.

Scheme 13



This strategy was adopted for our system as outlined in scheme 14. The literature

Scheme 14

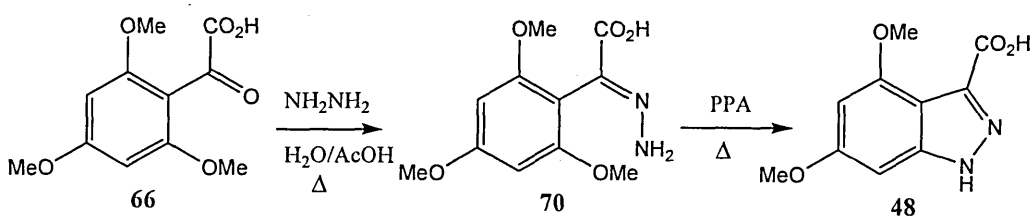


procedure[66] for making α-ketoester **68** used a small amount of anhydrous $AlCl_3$ but we found that a large amount of anhydrous $AlCl_3$ was essential for obtaining good yields of the α-ketoester **65.** It was also found that the hazardous solvent CS_2 could be substituted with dry DCM without any difference in yield of the product **65**. The α-ketoester **65** was purified by flash chromatography and obtained as a white crystalline solid in excellent yield (94-97%). The α-ketoester **65** was spectroscopically characterised. Its IR spectrum displayed two carbonyl frequenceies at 1725 and 1678 cm^{-1} whilst its proton magnetic resonance spectrum showed a typical ethyl pattern of a triplet at δ 1.33 for the methyl group and a quartet at δ 4.30 for the methylene group. The two methoxy groups *Ortho* to the ketoester functional group resonated as a

singlet at δ 3.79 and the third methoxy group which is *Para* to the ketoester functional group resonated at slightly lower field at 3.84 ppm. The two aromatic protons showed as a singlet at 6.08 ppm. The electron impact mass spectrum of the compound gave the molecular ion peak at m/z 268 whilst the fragmentation ions at m/z 195 and m/z 168 corresponded to the loss of ethoxycarbonyl group and carbonylethoxycarbonyl group respectively. Hydrolysis of the α-ketoester **65** in a mixture (7:3) of ethanol and water produced, after acidification with 6M HCl acid, the α-ketoacid **66** as a grey-white solid in excellent yield (81-93%). The α-ketoacid **66** was readily soluble in water presumably due to its enhanced acidity due to the α-carbonyl group and hence greater dissociation in an aqueous medium. The IR spectrum of the α-ketoacid **66** showed typical features such as broad hydrogen bonded OH group at 2789-2690 cm^{-1} and two carbonyl absorptions at 1757 and 1724. ^1H NMR spectrum of the α-ketoacid **66** measured in deuterium oxide was very simple as expected. It showed the resonance of the three methoxy protons at δ 3.82 whilst the two aromatic protons resonated at δ 6.15. The carboxylic OH group could not be detected at lower field due to the fact that it underwent exchange with D_2O and as a result, however, a large singlet at δ 4.78 was observed which most probably was due to HDO. The electrospray mass spectrum displayed the molecular ion at m/z 141 corresponding to (M+1).

It was decided to react the α-ketoacid **66** with hydrazine hydrate in water containing a catalytic amount of acetic acid since both substrates were readily soluble in water.

Scheme 15



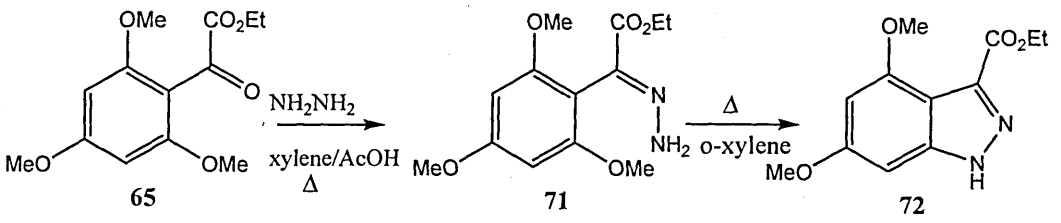
66 **70** **48**

The refluxed mixture was evaporated and the residue heated with PPA in order to induce intramolecular cyclisation. The isolated product, obtained in low yield, was examined by proton magnetic resonance spectroscopy in D_2O which showed a singlet that integrated to two protons at δ 1.90 and was assigned to an NH_2 group. The two methoxy groups *Ortho* to the hydrazone functional group resonated at slightly higher

field at δ 3.77 compared to the starting α-ketoacid **66** in which the three methoxy groups resonated at δ 3.82. The remaining methoxy group *Para* to the hydrazone functional group resonated at slightly lower field at δ 3.85. The two aromatic protons resonated as a singlet at slightly lower field at δ 6.35 compared to the α-ketoacid **66** in which the aromatic protons resonated at δ 6.15. The electrospray mass spectrum gave an ion at m/z 255 that corresponded to M+1 for the hydrazone **70**. The cyclised indazole **48** was not formed in this reaction.

When the reaction of the α-ketoester **65** was performed with hydrazine hydrate in dry DMF in the presence of catalytic amount of glacial acetic acid at 120-130°C the molecule underwent fragmentation to produce the isolated reaction product as 1,3,5-trimethoxybenzene according to the proton magnetic resonance spectrum of the compound. Similarly, when the was α-ketoester **65** and hydrazine hydrate were heated in o-xylene containing a small amount of tosic acid, the crude reaction product on examination by ^{1}H NMR spectroscopy showed the absence of an ethyl group and indicated the formation of 1,3,5-trimethoxybenzene. There was no evidence for the formation of hydrazone **71** or the indazole **72.**

Scheme 16

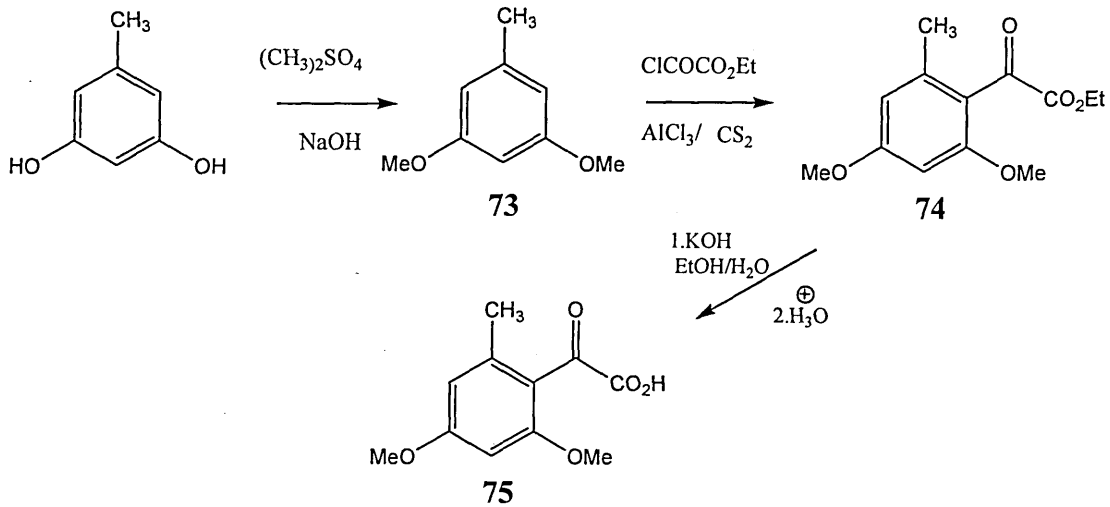


In previous experiments the reaction of the α-ketoacid **66** with benzylhydrazine dihydrobromide in o-xylene in the presence of sodium acetate at reflux temperature for 72 h was investigated. This reaction yielded a complex mixture of products which were separated by flash chromatography to yield five fractions that were examined by ^{1}H NMR spectroscopy. Fractions 4 was the cleanest spectrum indicative of benzylhydrazone formation and fractions five and six although they had elements of the correct resonances for the benzylhydrazone were actually less reliable. Repeating the experiment in dry DMF containing ammonium acetate at 100-120°C the reaction

mixture was once again quite complex and separation of the various products by flash chromatography generated five fractions which were examined by ^{1}H NMR spectroscopy. Fraction four was identified as 1,3,5-trimethoxybenzene whilst fraction five was interpreted to be benzylhydrazine. The other remaining fractions were difficult to interpret and make conclusions.

The α-ketoester **74** from 3,5-dimethoxytoluene was also synthesised by reaction with ethyl Oxalyl chloride under similar reaction conditions that were used for making the α-ketoester **65**. The 3,5-dimethoxytoluene **73** itself was made in 60% yield from the commercially available 3,5-dihydroxytoluene by alkylation with dimethyl sulphate using the Williamson ether synthesis. Treatment of 3,5-dimethoxytoluene **73** with ethyl Oxalylchloride in anhydrous CS_2 in the presence of $AlCl_3$ produced after purification by silica column chromatography the desired compound **73** in 60% yield as a solid compound. The ^{1}H NMR spectrum of the compound showed the ethyl resonance at δ 1.40 and δ 4.35 whilst the methoxy groups resonated in the expected positions of δ 3.80 and δ 3.85. The methyl group was observed to resonate at δ 2.45. The electro ionisation mass spectrum gave the correct molecular ion at m/z 252 (8%) and fragmentation pattern that was consistent with the correct structure.

Scheme 17

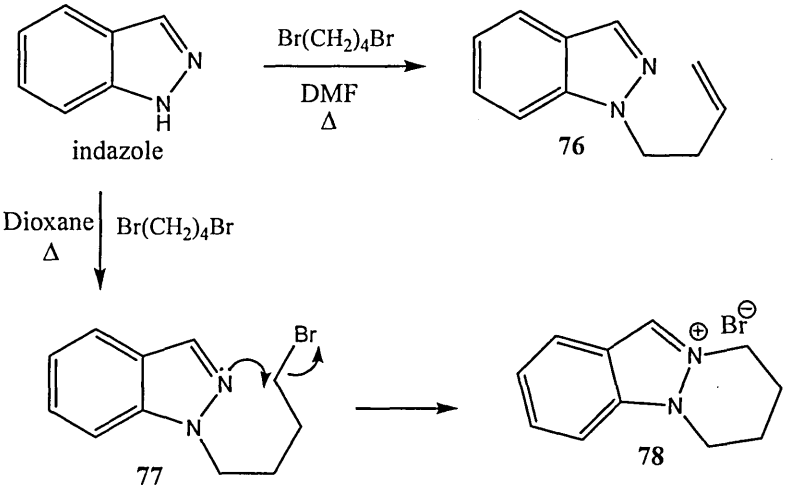


Hydrolysis[66] of the α-ketoester was carried out using the literature conditions of dilute aqueous ethanolic KOH which produced the α-ketoacid **75**[65] in 90% yield. The compound **75** was characterised spectroscopically. The IR spectrum showed a broad hydrogen bonded OH group at 2700-3700 cm^{-1} and a strong carbonyl absorption at

1754 cm^{-1}. Its ^1H NMR spectrum recorded in deuterium oxide showed a three proton singlet at 2.35 ppm for the methyl group and a six proton singlet at 3.78 ppm for the two methoxy groups. The H-3 aromatic proton resonated at 6.10 ppm whilst the H-5 aromatic proton resonated slightly lower field at 6.15 ppm.

The construction of the six membered ring on the indazole nitrogen was addressed next atoms and for this commercially available indazole was chosen as the model system for the investigation. Earlier work in these laboratories had shown that if the reaction between indazole and 1,4-dibromobutane was carried out in DMF under heating, the product of the reaction was the elimination product **76** (scheme 18). It appeared that in DMF, a polar solvent, the E2 reaction was favoured rather than the desired S_N2 reaction. An alternative solvent for the reaction was considered and it was decided to use 1,4-dioxane. Heating an equimolar solution of indazole and 1,4-dibromobutane in 1,4-dioxane at 150°C produced the iminium bromide **78** in 64% yield after trituration with dry ether and drying the product in the oven at 60°C.

Scheme 18

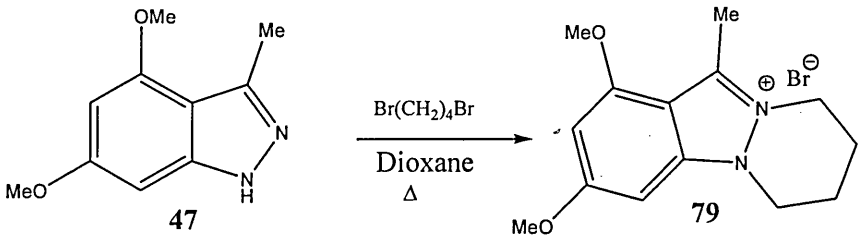


The compound was identified by ^1H NMR spectroscopy and mass spectrometry. The electron ionisation mass spectrum of the compound showed two nearly equal isotopic molecular ions at 252 (Br^{79}) and 254 (Br^{81}) (18%) and fragment ions at m/z 173 (53%) corresponding to the loss of a bromine atom. The ^1H NMR spectrum recorded in deuterochloroform showed a broad singlet at δ 1.90 which was probably water. A four proton multiplet at δ 2.35 was due to the two internal methylene groups while the other two methylene protons adjacent to the two nitrogens resonated lower field. A two protons triplet (J 6.3 Hz) resonating at δ 4.64 was assigned to the methylene proton attached to the N-1 of indazole whereas the other triplet (J 6.1 Hz) also

integrating to two protons resonated lower field at δ 5.25 was assigned to the methylene group attached to the positively charged iminium nitrogen atom which caused the deshielding. The indazole proton at position-3 resonated as a very low field singlet at δ 10.02 as a result of considerable deshielding by the positively charged iminium group. The four aromatic protons resonated discretely as four peaks. The triplet (J 8.1 Hz) resonating at δ 7.45 was assigned to H-5 which was relatively the most shielded of the aromatic protons as a result of being adjacent to the electron releasing tertiary nitrogen atom. The H-4 and H-7 protons resonated as two sets of doublet (J 8.1 Hz) at δ 7.65 and δ 8.25 respectively whilst the H-6 proton resonated as a triplet at δ 7.80.

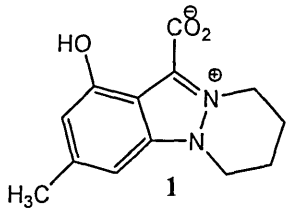
Having established the reaction conditions for constructing the six-membered heterocyclic ring we then proceeded to try it out on the 4,6-dimethoxy-3-methyl-1H-indazole **47**. Reaction of the indazole **47** with 1,4-dibromobutane in 1,4-dioxane at 150°C for 48 h produced the desired product **79** in 78% yield (scheme 19).

Scheme 19



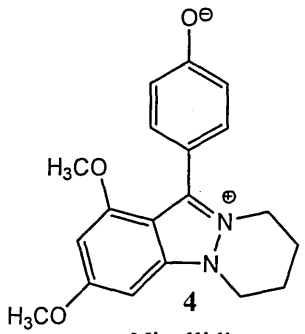
The structure of **79** was established from its ^{1}H nmr spectrum recorded in deuterochloroform containing a few drops of dimethyl sulphoxide in which the various peaks were broad compared to the spectrum of indazole **78**. Some characteristic features of the spectrum were that the internal methylene protons resonated as a four proton multiplet at δ 2.10 and the methyl group at position-3 resonated as a three proton singlet at δ 2.85. The methylene group attached to the nitrogen atom at position-1 of the indazole resonated as a multiplet at δ 4.28 whilst the other methylene group attached to the iminium nitrogen atom resonated as a multiplet at δ 4.40. This was in contrast with the resonance position of δ 5.25 for the methylene protons attached to the iminium nitrogen in indazole **78**. This upfield shift observed in **79** for the methylene protons attached to the iminium nitrogen is presumably as a result of the electron releasing methyl group which donates electrons

to the iminium group thus decreasing its electrophilic nature. The electrospray mass spectrum gave (M+1) ions at m/z 327/329 consistent with the molecular structure of **79**.



1

Nigellicine

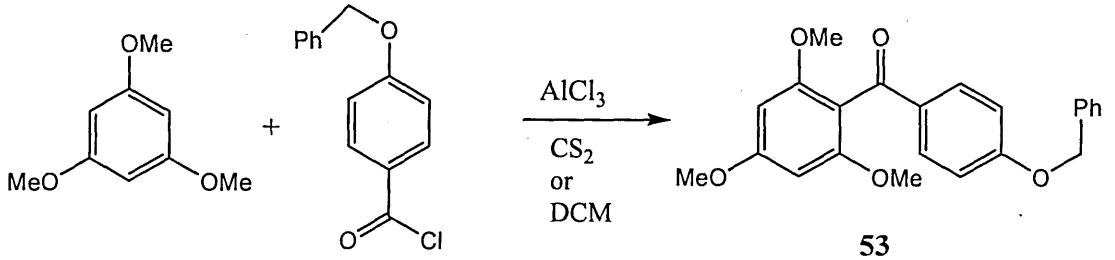


4

Nigellidine

During this research to develop methods for the synthesis of Nigellicine **1** the synthesis of Nigellidine **4** for which the ketone **53** was a key intermediate compound, was also addressed.

Scheme 20

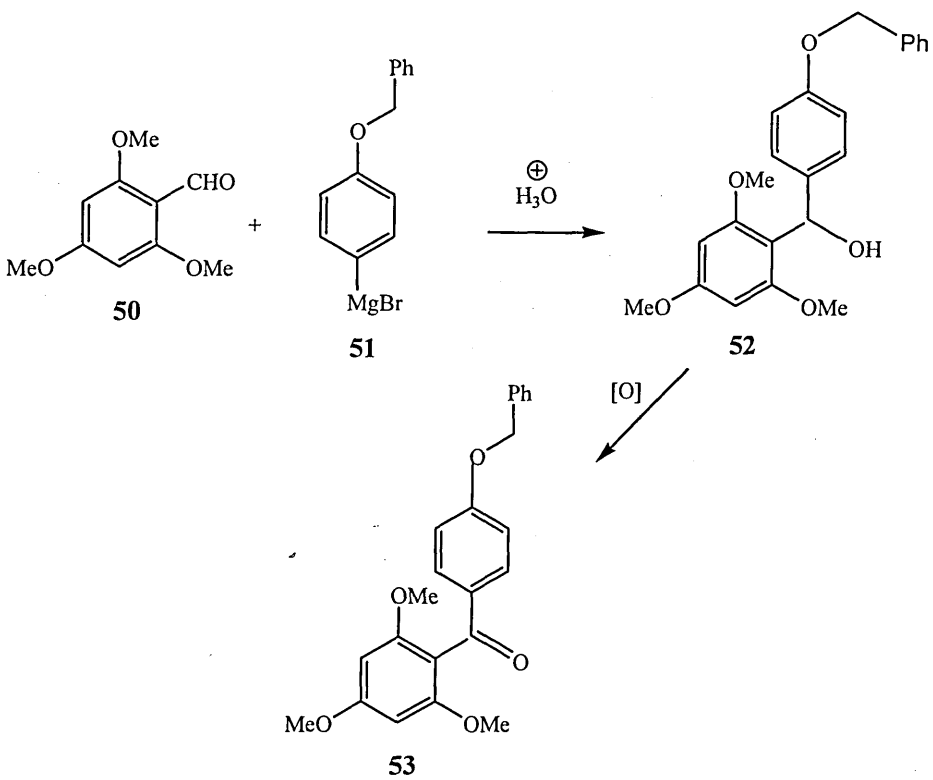


6a **53**

The initial synthetic strategy for the synthesis of ketone **53** involved the Friedel-Crafts acylation of 1,3,5-trimethoxybenzene with 4-benzyoxybenzoyl chloride **6a**. as shown in scheme 20. The acid chloride was freshly prepared as described in chapter one of this thesis and was reacted with 1,3,5-trimethoxybenzene with 4-benzyloxy-benzoyl chloride **6a** in both CS_2 and DCM as reaction solvents. In all the cases the reaction failed to give any desired product **53**. In fact the product isolated from the reactions was identified from its ^1H NMR spectrum as 4-benzyloxybenzoic acid **5a**. Literature survey suggests that one limitation for the Friedel-Crafts reaction is that it doesn't succeed on an aromatic ring that is substituted either by an amino group or by a strongly electron-withdrawing group[67]. In this case the steric factors as well as the large electron donating benzyloxy group must have contributed to the failure of the

reaction. The failure of the Friedel-Crafts reaction requires an alternative method for the synthesis of ketone **53** to be considered. The synthesis of the ketone **53** by oxidation of the alcohol **52** which could be made by a Grignard reaction between 2,4,6-trimethoxybenzaldehyde **50** and the Grignard reagent **51** derived from benzyl 4-bromophenyl ether as shown in scheme 21, appeared possible.

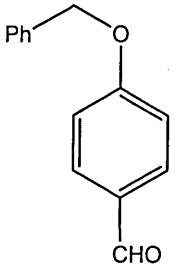
Scheme 21



The synthesis of the Grignard reagent was initially tried in dry diethyl ether but it failed to form. Even when dry THF was used as the reaction solvent, the formation of the Grignard reagent **51** required strong heating as well as a crystal of iodine as catalyst. The chiral alcohol **52** was obtained in 55% yield after purification by column chromatography as a racemic white crystalline solid. The compound **52** was characterised spectroscopically. Some characteristic features of its ^{1}H NMR spectrum, recorded in deuterochloroform, were the high field resonance of the OH group at δ 1.60 and the low field resonance in the aromatic region of the tertiary proton on the carbon bearing the OH group at δ 6.15-6.23. The 1,4-disubstituted aromatic ring

protons resonated as an AB system at δ 6.90 and δ 7.25. The electron ionisation mass spectrum of **52** showed a molecular ion at m/z 380 and a fragment ion at m/z 197 was a base peak indicative of the loss of benzyloxyphenyl group.

Oxidation of **52** to the ketone **53** was initially tried with commercially available activated manganese dioxide in DCM and chloroform at different concentrations of the oxidant. All the reactions were monitored by TLC and no product formation was observed. Oxidation of the alcohol **52** by Jones reagent in acetone 0°C for a short time did not give any product **53**. However, oxidation with Jones reagent for a prolonged time at RT yielded some product according to TLC [EtOAc:petrol, 1: 3 v/v] analysis. The crude reaction product was isolated and purified by flash chromatography to give firstly a pure product which was identified as the cleavage product **80**. Continued elution of the column yielded a second product which was identified from its ^{1}H NMR spectrum as recovered starting alcohol **53**. The ^{1}H NMR spectrum of **80** showed a two proton singlet at δ 5.20 for the benzylic protons and a one proton singlet at δ 9.90 for the aldehyde proton. The disubstituted aromatic ring resonated as an AB system at δ 7.10 and 7.90 whilst the phenyl ring protons resonated as a multiplet at δ 7.3-7.5.



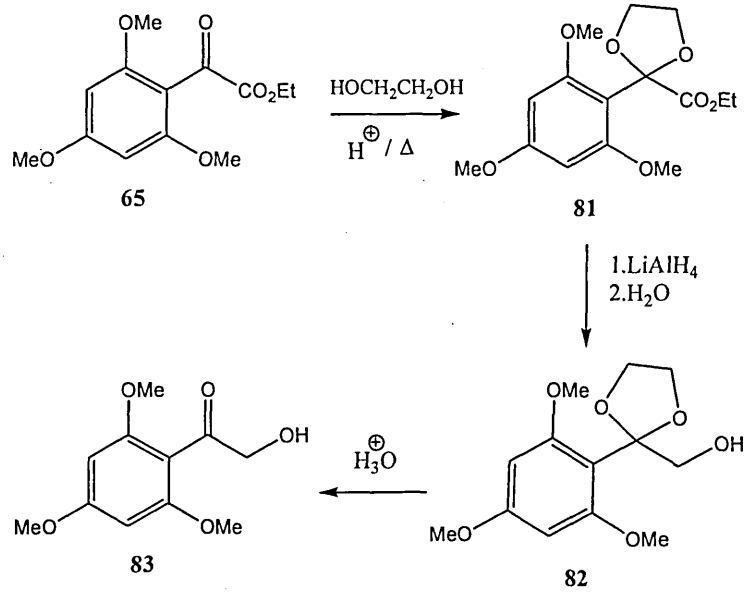
80

Oxidation of **52** with pyridinium chlorochromate in DCM also failed to give any ketone **53**. However, oxidation of the alcohol **52** with TEMPO and sodium hypochlorite in a mixture of DCM and a buffered aqueous solution gave the ketone **53** in 11.6% yield after purification by column chromatography. The IR spectrum of **53** showed a strong carbonyl absorption at1644 cm^{-1} whilst the ^{1}H NMR spectrum showed the absence of the tertiary proton at δ 6.15-6.23. The other most distinguishable feature in the ^{1}H NMR spectrum of **53** was the downfield shift of the doublet of the AB system adjacent to the carbonyl group. In the proton magnetic resonance spectrum of the alcohol **52** the aromatic protons adjacent to the methanol group resonated at δ 7.25 whilst in the ketone **53** the same aromatic protons resonated

at δ 7.88 as a result of deshielding by the newly formed carbonyl group. The quantity of ketone **53** obtained in this experiment was insufficient to do any more chemistry. The TEMPO/hypochlorite reaction for making ketone **53** is a poor yielding method and an alternative methodology is needed for producing ketone **53** in a satisfactory yield.

In one other strategy it was decided to make compound **83** from the α-ketoester **65** by the steps shown in scheme 22. When compound **65** was reacted with ethylene glycol and a catalytic amount of tosic acid in dry toluene using a Dean-Stark set up for 48 h, the product of the reaction isolated by column chromatography was identified as 1,3,5-trimethoxybenzene in 66.4% yield. Thus the acetal **81** could not be made by this procedure. The outcome of this reaction was surprised. This methodology was temporarily abandoned until a better method was found for protecting the keto group in the α-ketoester **65**.

Scheme 22



3.3 Experimental

For *general directions* see the Experimental section of chapter one.

Prepararion of Jones reagent (8N Chromic acid)

Conc. H_2SO_4 (23ml) was carefully added to a solution of chromium trioxide (26.72g) in H_2O (20ml) and the resulting solution was diluted with distilled water to 100ml. Jones reagent (1ml) oxidizes 4mmol of a monohydric alcohol.

General method for the synthesis of 4,6-dimethoxy-3-methyl-1H-indazole **47** *by cyclization of the hydrazone formed in situ from the reaction of 2,4,6-trimethoxyacetophenone with hydrazine hydrate in the presence of polyphosphoric acid (PPA)*

A mixture of 2,4,6-trimethoxyacetophenone (5 mmol), hydrazine hydrate (10 mmol) and glacial acetic acid (10 drops) was stirred under heating (see table 1) and the reaction progress was monitored by TLC. After a period of time (see table 1) when all the starting ketone had disappeared, PPA (10g) was added to the cooled mixture, which was then stirred under heating (see table 1) until all the intermediate **46** had disappeared. After cooling, ice-water was added to the mixture and the product was extracted with EtOAc (3 x 50 ml). The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated to give crude product **47** which was purified by flash chromatography using a mixture [1:4, EtOAc: petrol]; IR ν_{max} 3325 cm^{-1} (NH) ; ^1H NMR δ 2.68 (3H, s, Me), 3.85 (3H, s, OMe), 3.93 (3H, s, OMe), 6.10 (1H, s, H-5), 6.45 (1H, s, H-7), 8.02 (1H, broad s, NH); EIMS m/z 192 (M^+, 100%), 177 (M-Me, 22%), 161/163 (M-OMe, 15%).

Table 1

Entry	Wt(g),(mmols)	Temp. (^{0}C)	Time (h)	Product **47** (g) (% yield)
1	2.10 (10)	110-120	0.30	0.12[a] (6.3)
2	2.10 (10)	110120	0.60	0.13[a] (6.8)
3	2.10 (10)	110-120	2	0.30[a] (15.6)
4	2.10 (10)	110-120	1.5	0.25[a] (13)
5	2.10 (10)	110-120	2	0.31[a] (16.1)
6	0.50 (2.4)	110-120	1	0.07[a] (15.2)
7	1.05 (5)	80-85	1	0.91[b] (94.8)
8	4.20 (20)	110-120	Overnight	5.34[b] (139)
9	4.20 (20)	110-120	6	5.12[b] (133)
10	0.42 (2)	110-120	3	0.36[b] (93.7)

a – pure chromatographed product; b – crude product after work up.

Isolation of the hydrazone **46** *from the above reaction of 2,4,6-trimethoxyacetophenone with hydrazine hydrate using acetic acid as a catalyst.*
A mixture of 2,4,6-trimethoxyacetophenone (5 mmol), hydrazine hydrate (10 mmol) and glacial acetic acid (10 drops) was heated overnight under stirring. Ethyl acetate (50ml) was added and the resultant solution washed with saturated $NaHCO_3$ solution (30ml) and water (30ml). The solution after drying (Na_2SO_4) was evaporated evaporated to yield the hydrazone **46** (1.0g, 89%) as a brown gum which eventually solidified, m.p. 97-100°C; IR ν_{max} 3171 cm^{-1} (broad, NH_2); ^1H NMR δ 2.20 (3H, s, Me), 3.79 (9H, s, 3xOMe), 6.15 (2H, s, Ar), 8.10 (2H, broad s, NH_2); ESMS m/z 225 (M+1).

Synthesis of 4,6-Dimethoxy-1,3-dimethyl-1H-indazole **57** *by cyclization of 2, 4, 6-trimethoxyacetophenone with methyl hydrazine in the presence PPA*
A mixture of 2,4,6-trimethoxyacetophenone (2.1g, 10 mmol) , methyl hydrazine (0.92 g, 20 mmol) and glacial acetic acid (10 drops) was stirred under heating at 110-120 ^0C for 2 h. PPA (10g) was added to the cooled mixture which was then stirred under heating at 120-135 ^0C for two hours. After cooling, ice-water was added to the mixture and the product was extracted with EtOAc (3 x 50 ml). The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated to give crude product which was purified by flash chromatography using a mixture [1:4, EtOAc: petrol] to yield pure **57** (0.34g, 18%); ^1H NMR δ 2.65 (3H, s, Me), 3.78 (6H, s, 2 x OMe), 3.81 (3H, s, >NMe), 6.20 (1H, s, Ar), 6.40 (1H, s, Ar), EIMS m/z 206 (M^+, 100%), 191 (M-Me, 54%), 177 (M-NMe, 21%), 163 (M-MeN_2, 80%).

Synthesis of 1-Benzyl-3-methyl-4,6-dimethoxy-1H- indazole **59** *by cyclization of 2, 4, 6 -trimethoxyacetophenone with benzyl hydrazine in the presence of PPA.*
A mixture of 2,4,6- trimethoxyaceto phenone (4.2 g, 0.02 mol), benzylhydrazine dihydrochloride (4.3 g, 0.022 mol) and AcOH (4ml) in 95% EtOH (80ml) was heated under stirring at 110-120 ^0C with monitoring of the reaction progress by TLC [1:2, EtOAc:petrol]. After two hours the solvent was evaporated and PPA (10g) was added to the cooled residue which was then heated at 120-135 ^0C with stirring for 2 h. After cooling, ice-water (50ml) was added to the mixture and the product was extracted with EtOAc (3x40 ml). The combined extract was washed with water, dried (Na_2SO_4) and evaporated to give the crude product **59** as a brown gum (5.98g) which according to TLC consisted of a string of products. The product mixture was separated by flash chromatography and one of the fraction was identified as the product **59** (0.90g,

16%); ^{1}H NMR δ 2.75 (3H, s, Me), 3.80 (6H, s, 2 x OMe), 4.80 (2H, s, -CH_2-), 6.15 (1H, s, Ar), 6.60 (1H, s, Ar), 7.20-7.35 (5H, m, Ph); ESMS m/z 283 (M+1).

Attempted oxidation of 4,6-dimethoxy-3-methyl-1H-indazole **47** *by* SeO_2

The 3-methylindazole **47** (0.95g, 4.95mmol) was dissolved in dioxane (6ml) in a 50ml Rb flask. The solution was stirred and heated in an oil bath at 100 ^{0}C for few minutes and then selenium dioxide(0.63g, 0.01mol) and water (0.2 ml) were added. The mixture was heated with stirring for 3 hours and then the progress of reaction was checked by TLC [1:3, EtOAc:petrol]. Heating was continued overnight at 120 ^{0}C and the mixture was extracted with DCM (30 ml) and after drying ($MgSO_4$) the solvent was evaporated to give a crude product (1.5g) which showed two spots on TLC. Purification by flash chromatography gave pure product (0.43g, 45%) which was identified by its ^{1}H NMR spectrum as the starting indazole **47**.

Oxidation of 2,4,6-trimethoxyacetophenone with SeO_2 *using two methods*

Method 1: 2,4,6-Trimethoxyacetophenone (2.1g, 11mmol) was dissolved in dioxane (30ml) and water (30ml) in a 50ml round bottom flask. The solution was stirred and heated in an oil bath at 100 ^{0}C for few minutes then SeO_2 (2.6g, 23.4mmol) was added followed by water (0.2ml). The mixture was heated in an oil bath with stirring for 1-3 hours. The reaction progress by TLC [1:3, EtOAc:petrol] indicated the presence of starting material and the mixture was allowed to heat overnight at 120 ^{0}C. The dioxane was evaporated to give the crude product (3.42g) as a brown solid; ^{1}H NMR δ 2.15 (2 unit area, Me), 3.70 (27 unit area, OMe), 6.10 (2.6 unit area, Ar), 9.40 (1 unit area, -CHO). The crude product was dissolved in acetone (20ml) and Jones's (3.5ml) was added and the stirred mixture was heated at 60°C for 2 h. The cooled mixture was filtered and the acetone evaporated to leave a residue which was redissolved in ethyl acetate (50ml) and washed with $NaHCO_3$ solution, dried (Na_2SO_4), filtered and evaporated to give the crude product (1.5g); ^{1}H NMR δ 1.75 (broad peak, 7.9 unit area), 3.6-3.9 (32.2 unit area), 2.2-2.3 (3.5 unit area), 6.14 (3.5 unit area), 7.35 (1.2 unit area), 9.50 (1.0 unit area).

Method 2: A solution of 2,4,6-trimethoxyacetophenone (2.1g, 11mmol) in pyridine (5ml) containing water (8 drops) in a 50ml round bottom flask was stirred and heated in an oil bath at 100 ^{0}C for few minutes. Then SeO_2 (2.6g, 23.7mmol) was added followed by water (0.2ml) and the reaction mixture was heated in the oil bath with

stirring for 1-3 h. TLC indicated the presence of starting material and the reaction was allowed to heat overnight at 120 ^{0}C. After acidification with dilute hydrochloric acid (2M, 30ml) the mixture was extracted with DCM (50ml) which after drying ($MgSO_4$) was evaporated to give the crude product (2.2 g) as a pale brown solid; ^{1}H NMR δ 3.7 (9H, m, 3 x OMe), 5.95 (2H, s, Ar), 8.10 (1H, broad s, -OH).

Reaction of 1, 3, 5- trimethoxybenzene with ethyl oxalylchloride[66] *(Friedel-Craft reaction) to form the ketoester* **65**[65]

To a cooled and stirred solution (0 ^{0}C) of 1, 3, 5- trimethoxybenzene (TMB) in the solvent(see table 2) were added ethyl oxalyl chloride ($ClCOCO_2Et$) and anhydrous powdered $AlCl_3$ respectively with the formation of a reddish suspension that was subsequently stirred at room temperature for period of time shown in table 2. The reaction mixture was poured into ice (20ml) and extracted with DCM (4 x 30ml). The combined extract was washed with saturated $NaHCO_3$ (2 x 30ml), dried ($NaSO_4$), filtered and evaporated under reduced pressure to give a light brown solid which was purified by flash chromatography [petrol : EtOAc , 7:3] to give the α-ketoester **65** as a white crystalline solid; m.p. 68-70°C (Lit.[65] 70-71°C); IR ν_{max} 1725, 1678 (>C=O), 1603 cm^{-1} (>C=C<); ^{1}H NMR δ 1.31 (3H, t, J = 7.35 Hz, Me), 3.77 (6H, s, 2 x OMe), 3.81(3H, s, OMe), 4.27 (2H, q, J = 7.35 Hz, -OCH_2), 6.06 (2H, s, Ar); ^{13}C NMR δ 14.2, 56.9, 60.8, 100.6, 106.2, 163.6, 163.8, 167.0, 186.7; EIMS m/z 268 (M^+, 2%), 195 (M-CO_2Et, 100%), 168 (M-$COCO_2Et$).

Table 2

Expt No.	TMB (mmol)	$ClCOCO_2Et$	$AlCl_3$	Solvent	Time (h)	Crude product	Pure product (%)
1	4.00g (23.8)	15ml	2g	100ml CS_2	20	6.38g	6.03g (94)
2	4.00g (23.8)	15ml	2g	100ml CS_2	24	-	6.20g (97)
3	16.60g (98.8)	48ml	27g	300ml CS_2	24	-	25.70g (97)
4	14.81g (88)	45ml	6g	300ml DCM	24	-	23.00g (97)

Hydrolysis[66] *of the α- ketoester* **65** *to the α- ketoacid* **66**[65]

To a solution of the ketoester **65** in [ethanol: water, 7:3 v/v], powdered KOH was added and the resulting mixture was stirred at room temperature for 24 hours. Ethanol was evaporated under reduced pressure and the resulting mixture was extracted with ether (2 times). The alkaline aqueous phase was acidified with 5 N hydrochoric acid and extracted with CH_2Cl_2 (4 times). The combined extract was dried (Na_2SO_4), filtered and evaporated under reduced pressure to give the *α- ketoacid* **66** as a yellow solid which was recrystalized [6:4, hexane: EtOAc] (table 3); m.p. 143°C (Lit.[65] 154°C); IR ν_{max} 2789-3690 (OH), 1757, 1724 (>C=O), 1604 cm^{-1} (>C=C<) ; ^1H NMR (D_2O) δ 3.82 (9H, s, 3 x OMe), 4.78 (4H, broad s, 2 x H_2O), 6.15 (2H, s, Ar); ^{13}C NMR δ 56.3, 92.0, 107.4, 164.1, 167.7, 168.2, 187.5; EIMS m/z. 240 (4%, M^+), 195 (100%, M-CO_2H).

Table 3

Expt. no.	Keto ester (mmol)	KOH	EtOH/water 70:30	Time (h) at RT	Pure product (% yield)
1	2.30g (8.58)	1.05g	110ml	Overnight	0.79g (81)
2	5.70g (21.27)	2.3g	520ml	Overnight	4.71g (92)
3	1.09g (4.07)	0.44g	100ml	Overnight	0.53g (93)

Attempted synthesis of 3-carboxy-4,6-dimethoxy-1H-indazole **48** *by cyclization of the ketoacid* **66** *with hydrazine hydrate in the presence of PPA.*

A mixture of the ketoacid **66** (1.0 g, 4.2mmol), hydrazine hydrate (0.21g, 4.2mmol), distilled water (2.5 ml), and acetic acid (8 drops) was placed in a 50 ml round bottom flask and heated under stirring at 110-120^{0}C overnight. The water was removed on the rotavapor at 80°C. To the cooled reaction mixture was added PPA (10g) and then stirred at 120-135 ^{0}C for two hours. The cooled reaction mixture, after adding ice-water (100 ml) to it was extracted with EtOAc (3 x 40 ml) and the combined extract was washed with water, dried (Na_2SO_4) and evaporated to give the crude product **70** as a brown solid (0.5g); IR ν_{max} 2600-3700 (broad, OH), 3397 (OH), 1626 cm^{-1} (>C=O); ^1H NMR δ 1.90 (2H, broad s, NH_2), 3.77 (6H, s, 2 x OMe), 3.85 (3H, s, OMe), 4.78 (HDO), 6.35 (2H, s, Ar); ESMS m/z 255 (M+1).

Attempted synthesis of 3-carboxy-4,6-dimethoxy-1H-indazole **48** *by cyclization of the ketoacid* **66** *with hydrazine hydrate in the presence of PPA.*

A mixture of the ketoacid **66** (1.0 g, 4.2mmol), hydrazine hydrate (0.21g, 4.2mmol), distilled water (2.5 ml), and acetic acid (8 drops) was placed in a 50 ml round bottom flask and heated under stirring at 110-120^{0}C overnight. The water was removed on the rotavapor at 80°C. To the cooled reaction mixture was added PPA (10g) and then stirred at 120-135 ^{0}C for two hours. The cooled reaction mixture, after adding ice-water (100 ml) to it was extracted with EtOAc (3 x 40 ml) and the combined extract was washed with water, dried (Na_2SO_4) and evaporated to give the crude product **48** as a brown solid (0.5g); IR ν_{max} 2600-3700 (broad, OH), 3397 (OH), 1626 cm^{-1} (>C=O); ^{1}H NMR δ 1.90 (2H, broad s, NH_2), 3.77 (6H, s, 2 x OMe), 3.85 (3H, s, OMe), 4.78 (HDO), 6.35 (2H, s, Ar); ESMS m/z 223 (M+1).

Attempted synthesis of 1-benzyl-3-carboxy-4,6-dimethoxy-1H-indazole by cyclization of α-ketoacid **66** *with benzylhydrazine dihydrobromide by two methods.*

Table 4

Method	wt of keto acid **66** used (g)(moles)	wt of benzyl hydrazine (g)(moles)	wt of acetate used (g)	Solvent used (ml)	wt of crude product (g)	wt of pure product (g)
1	2.40 (10mmol)	3.9 (20mmol)	NH_4OAc 2	DMF 16	3.09	0.40
2	1.0 (4.2mmol)	0.85 (4.2mmol)	NaOAc 0.7	o-xylene (20ml)	1.55	0.25

Method 1: A mixture of the ketoacid **66**, benzylhydrazine dihydrobromide and ammonium acetate in dry DMF was heated under stirring at 100-120 ^{0}C for 72 h (see table 4 below) and the reaction progress was monitored by TLC [EtOAc: petrol, 1:2]. The solvent was removed by reduced pressure distillation and the residue obtained was purified by flash chromatography [EtOAc: petrol, 1:2] to yield 5 fractions which had the following proton magnetic spectra: *fraction 1* (0.35g yield), δ 1.27 (s, 3.68 unit area), 3.77-3.82 (m, 5.63 unit area), 6.11 (s, 1.7 unit area), 6.15 (s, .08 unit area), 7.17-7.40 (m, 3.59 unit area), 7.43-7.50 (m, 1.60 unit area), 7.83-7.90 (m, 1.00 unit area), 8.70 (s, 0.44 unit area); *fraction 2* (0.30g yield), δ 2.60 (s, 1.36 unit area), 3.70-

3.80 (m, 5.45 unit area), 5.18 (s, 0.90 unit area), 5.27 (s, 0.90 unit area), 6.10 (s, 0.97 unit area), 7.10-7.40 (m, 9.98 unit area), 7.50-7.63 (m, 2.30 unit area), 9.04 (s, 0.42 unit area); *fraction 3* (0.06g yield), δ 1.26 (s, 9.96 unit area), 2.60 (s, 1.54 unit area), 3.7-3.85 (m, 28.23 unit area), 4.71 (s, 1.00 unit area), 5.18 (s, 0.87 unit area), 5.27 (s, 1.03 unit area), 6.09 (s, 4.93 unit area), 7.15-7.50 (m, 22.15 unit area), 7.55-7.65 (m, 3.33 unit area), 7.65-7.75 (m, 1.72 unit area); *fraction 4* (0.53g yield), δ 3.87 (9H, s),6.07 (2H, s); *fraction 5* (0.42g yield), δ 2.01 (s, 2.15 unit area), 3.62-3.84 (s, 4.64 unit area), 4.45 (dd, J = 14.65 and 6.10 Hz, 2.18 unit area), 5.34 (s, 1.00 unit area), 6.08-6.15 (m, 1.60 unit area), 7.18-7.40 (m, 10.32 unit area).

Method 2: A mixture of the keto acid **66**, benzylhydrazine dihydrobromide and sodium acetate in o-xylene was heated under stirring at 150 ^{0}C for 72 h (see table 4 below) and the reaction progress was monitored by TLC [EtOAc: petrol, 1:2]. The solvent was removed by reduced pressure distillation and the residue obtained was purified by flash chromatography [EtOAc: petrol, 1:2] to yield 6 fractions which had the following proton magnetic spectra: fraction 1 (0.34g), δ 1.26 (s, 7.31 unit area), 1.67 (broad s, 1.82 unit area), 3.82 (s, 2.00 unit area), 6.14 (s, 0.50 unit area), 7.15-7.40 (m, 2.24 unit area), 7.46-7.49 (m, 1.59 unit area), 7.85-7.90 (m, 1.00 unit area), 8.70 (s, 0.43 unit area); *fraction 2* (0.10g), 1.26 (s, 5.79 unit area), 1.70 (broad s, 1.89 unit area), 2.61 (s, 1.55 unit area), 3.70-3.83 (m, 5.20 unit area), 5.27 (s, 1.00 unit area), 6.10 (s, 0.53 unit area), 7.16-7.40 (m, 7.59 unit area), 7.53-7.63 (m, 2.35); *fraction 3* (0.27g), δ 1.26 (s, 3.83 unit area), 2.55 (s, 3.69 unit area), 3.70-3.80 (m, 13.85 unit area), 5.23 (s, 2.47 unit area), 6.01 (s, 2.98 unit area), 7.20-7.35 (m, 9.97 unit area), 8.03 (s, 1.21 unit area); *fraction 4* (0.10g), δ 2.43 (2H, s), 3.86 (s, 6H), 3.83 (3H, s), 5.23 (2H, s), 6.08 (2H, s), 7.20-7.45 (m, 5H); *fraction 5* (0.25g), δ 1.26 (s, 3.57 unit area), 1.81 (broad s, 2.23 unit area), 3.89 (s, 1.30 unit area), 6.09 (s, 0.20 unit area), 7.27 (m, 0.90 unit area), 7.45-7.65 (0.36 unit area), 8.09 (m, 0.15 unit area), 10.36 (s, 0.07 unit area).

Attempted synthesis of 3-ethoxycarbonyl-4,6-dimethoxy-1H-indazole **72** *by cyclization of keto ester* **65** *with hydrazine hydrate in (a) DMF and (b) o-xylene.*

(a) A mixture of the ketoester **65** (0.53g, 1.97 mmol) and hydrazine hydrate (0.24g, 4.8 mmol) in DMF (10ml) containing 10 drops of glacial acetic acid was heated under stirring at 120-135 ^{0}C for 5 h. The DMF was removed by reduced pressure

distillation to give a crude product which was identified as 1,3,5-trimethoxybenzene from its proton magnetic resonance spectrum.

(b) A mixture of the ketoester **65** (0.53g, 1.97mmol) and hydrazinehydrate (0.24g, 4.8mmol) in o-xylene (10ml) containing a catalytic amount of tosic acid was refluxed for 5 h. The xylene was removed by distillation *in vacuo* and the residue was extracted into DCM (50ml). After washing with $NaHCO_3$ solution and drying over $MgSO_4$ the DCM was evaporated to give a residue (0.28g) which according to its ^1H NMR spectrum was 1,3,5-trimethoxybenzene.

Preparation of 3,5 dimethoxytoluen **73**

Into a 500ml three-necked flask , equipped with an addition funnel, a sealed mechanical stirrer and a reflux condenser, was placed commercially available 3,5-di hydroxytoluene (37.2g, 0.30mol) and a solution of sodium hydroxide (26.0g, 0.65mol) in distilled water (200ml). To the vigorously stirred solution maintained below 10 ^0C was added dimethyl sulphate (75.6g, 0.6mol) dropwise via the dropping funnel over a period of 1h. Next the vigorously stirred mixture was heated for 2.5h at 70- 80 ^0C. The precipitated product was filtered on a Buchner funnel and washed with sodium hydroxide solution (2M) first and then with plenty of water to give, after drying ($MgSO_4$), 3,5-dimethoxytoluene (27.4g, 60%); ^1H NMR δ 2.35 (3H, s, Me), 3.75 (6H, s, 2 x OMe), 6.10 (1H, s, Ar), 6.15 (2H, s, Ar); ESMS m/z 153 (M+1).

Reaction of 3,5 dimethoxytoluene with ethyl oxalylchloride[66] *to form the keto ester* **74**

To a cooled solution (0°C) of the dimethoxytoluene (9.0g, 59.2 mmol) in CS_2 (200 ml) was added ethyl oxalylchloride (30ml, a large excess) followed by powdered anhydrous $AlCl_3$ (16g, 121 mmol) and the reddish suspension formed was stirred at room temperature overnight. The resulting mixture was poured into ice (40ml) and extracted with DCM (4 x 30 ml). The combined organic extract was washed with saturated $NaHCO_3$ solution (2 x 30ml), dried ($NaSO_4$), filtered and evaporated to give the product as a brown solid (17.25g, >100%) which was purified by flash chromatography [petrol : EtOAC, 7:3] to give the pure product **60** (8.80g, 59%) as a light cream coloured solid m.p. 66-67°C; IR ν_{max} 1747, 1663 cm^{-1}; ^1H NMR δ 1.40 (3H, t, J = 7.35 Hz, CH_3), 2.45 (3H, s, Me), 3.80 (3H, s, OMe), 3.85 (3H, s, OMe), 4.35 (2H, q, J = 7.35 Hz, -OCH_2-), 6.30 (1H, s, H-4), 6.38 (1H, s, H-6); ^{13}C NMR δ 14.0 (Me), 21.3 (Me), 55.7 (OMe), 55.8 (OMe), 61.4 (OCH_2), 95.8 109.3, 115.9,

144.5, 162.5 (Ar), 164.1 (CO_2H), 165.0 (Ar), 187.0 (>C=O); EIMS m/z 252 (M^+, 8%), 193 (30%), 180 (M-CO_2Et, 61%), 179 (100%).

Hydrolysis[62] *of toluene keto ester* **74** *to toluene keto acid* **75**[65]

To a solution of toluene keto ester (8.22g, 32.6mmol) in 450ml (7:3) ethanol/ water, potassium hydroxide (3.65g, 0.0365mol) was added and the resulting mixture was stirred at room temperature for overnight. Ethanol was evaporated under reduced pressure and the resulting mixture was extracted with ether (2x200ml). The alkaline a quos phase was acidified with 5 N hydrochoric acid and extracted with DCM (4x100ml). The extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a solid which was recrystalized from hexane/ EtOAc (6:4) to give pure product **75** (6.57g, 90%); m.p. 114-115°C; IR ν_{max} 2800-3700 (OH), 1754 cm^{-1} (>C=O) ; ^1H NMR δ 2.35 (3H, s, Me), 3.78 (6H, s, OMe), 6.10 (1H, s, H-3), 6.15 (1H, s, H-5); ^{13}C NMR δ 21.3 (Me), 55.9 (OMe), 56.0 (OMe), 96.0, 109.5, 115.3, 144.7 (Ar), 162.1 (CO_2H), 162.9, 167.2 (Ar), 186.5 (>C=O); EIMS m/z 224 (8%, M^+), 193 (52%, M-OMe), 179 (M-CO_2H), 151 (7%, M-$COCO_2H$); ^{13}C NMR δ 21.2, 55.9, 96.0, 109.5, 115.3, 144.7, 162.1, 162.9, 167.2, 186.5.

Reaction of indazole with dibromobutane to form the iminium salt **78**

A solution of indazole (236mg, 2 mmol) and dibromobutene (432mg, 2 mmol) in 1,4-dioxane (5 ml) in a 50 ml round bottom flask was stirred and heated in an oil bath at 150 ^0C for 48h. The dioxane was evaporated and to the residue was added dry ether (10ml). The mixture was filtered on a Buchner funnel to yield the crude product **78** (430mg, 64%) as a cream coloured solid; m.p. 164-169°C; ^1H NMR δ 1.90 (broad s, H_2O), 2.3-2.4 (4H, m, -CH_2CH_2-), 4.64 (2H, t, J = 6.3 Hz, >N-CH_2-), 5.25 (2H, t, J = 6.1 Hz, =N^+-CH_2-), 7.45 (1H, t, J = 8.1 Hz, H-5), 7.65 (1H, d, J = 8.1 Hz, H-4), 7.80 (1H, t, J = 8.1 Hz, H-6), 8.25 (1H, d, J = 8.1 Hz, H-7), 10.02 (1H, s, H-3);
EIMS m/z 252/254 (M^+, 18%), 173 (M-Br, 53%), 91 (100%).

Reaction of 4,6-dimethoxy-3-methyl-1H-indazole **47** *with 1,4-dibromobutane to form the iminium salt* **79**

4,6-dimethoxy-3-methyl-1H-indazole **47** (192mg, 1mmol) and 1,4-dibromobutane (220mg, 1mmol) in 1,4- dioxane (5ml) in a 50 round bottom flask was stirred and heated in an oil bath at 150 ^{0}C for 48h. The dioxane was removed *in vacuo* and dry ether (10ml) was added to the residue. Buchner filtration of the mixture yielded the crude product **79** (320mg, 78%); m.p.200-202°C; ^{1}H NMR δ 2.10 (4H, m, -CH_2CH_2-), 2.85 (3H, s, Me), 3.75 (6H, m, 2 x OMe), 4.28 (2H, m, >N-CH_2-), 4.40 (2H, m, =$^+$N-CH_2-), 6.16 (1H, s, H-5), 6.48 (1H, s, H-7); ^{13}C NMR δ 11.9, 19.6, 20.1, 34.5, 47.2, 47.6, 56.0, 56.8, 82.9, 96.8, 142.4, 156.0, 166.2; ESMS m/z 327/329 (M+1).

Reaction of 1, 3, 5- trimethoxybenzene with 4-benzyloxybenzoyl chloride **6a**

To a cooled solution (0 ^{0}C) of 1, 3, 5- trimethoxybenzene (TMB) in the solvent, 4-benzyloxy benzoyl chloride **6a** (BBC) was added followed by powdered anhydrous $AlCl_3$ and the reddish suspension obtained was stirred at room temperature for period of time (table 5). The resulting mixture was poured into ice (20g) and extracted with DCM (4 x 30ml). The combined organic extract was washed with saturated $NaHCO_3$ solution (2 x 30ml), dried ($NaSO_4$), filtered and evaporated to give the crude product as a brown solid which was purified by flash chromatography [petrol : EtOAc, 7:3] and identified as 4-benzyloxybenzoic acid **5a** from its 1H NMR spectrum.

Table 5

Expt. No.	TMB	BBC	$AlCl_3$	Solvent	Time	Crude product	Pure product
1	1.0g	8.04g	0.5g	30ml CS_2	Overnight	-	2.5g
2	1.04g	8.04g	1.8g	60 ml CS_2	Overnight	-	2.8g
3	1.68g	2.5g	2.7g	50ml DCM	Overnight	-	2.7g

Synthesis of 4-(1-Benzyoxylphenyl)-1- (2,3,5-trimethoxyphenyl)methanol **52**

A dry 500ml 3-necked flask was equipped on a magnetic hotplate stirrer with a condenser carrying a $CaCl_2$ guard tube, a dropping funnel and a stopper. Magnesium powder (2.70g, 0.11 mol) along with a crystal of iodine was placed in the flask and a solution of benzyl 4-bromophenyl ether (28.80g, 0.11 mol) in dry THF (70ml) was placed in the dropping funnel. A small amount (10ml) of the bromo compound was introduced and the flask subsequently heated using the hotplate. Once the exothermic reaction had started, the remainder of the bromo solution was added dropwise at a rate that maintained a constant reflux of the mixture. After all the bromo solution had been added the reaction mixture was refluxed for 1 h and then it was cooled in an ice bath. A solution of 2,4,6-trimethoxybenzaldehyde (20.60g, 0.10 mol) in dry THF (200ml) was added dropwise with constant shaking of the mixture until all had been added. The reaction mixture was magnetically stirred under reflux overnight. The THF was removed by rotary evaporation and to the residue was added a solution of ammonium chloride (36%, 50ml). The mixture was extracted with DCM (3 x 100ml) and the combined organic extract was dried (Na_2SO_4) and the solvent evaporated to give the crude product **52** which was chromatographed. Elution with [EtOAc:petrol, 1: 3 v/v] gave the pure product **52** as a crystalline white solid (22.0g, 55%), m.p. 88.5°C; IR ν_{max} 3506 (s, OH); ^{1}H NMR δ 1.60 (1H, s, OH), 3.78 (6H, s, 2 x OMe), 3.84 (3H, s, OMe), 5.05 (2H, s, -OCH_2), 6.15-6.23 (3H, m, Ar and >CH-O-), 6.90 (2H, d, AB system J = 8.4 Hz, Ar ortho to benzyl group), 7.25 (2H, d, AB system J = 8.4 Hz, ortho to >CH-O-), 7.30-7.47 (5H, m, Ph); ^{13}C NMR δ 55.7, 56.1, 68.4, 70.4, 91.6, 114.2, 114.6, 127.3, 127.8, 128.1, 128.2, 128.9, 137.6, 138.11, 157.9, 158.8, 161.0; EIMS m/z 380 (M^+, 5%), 348 (M-MeOH, 11%), 197 (M -$C_6H_4OCH_2Ph$, 100%), 91 ($PhCH_2$, 60%).

Oxidation of of 4-(1-Benzylphenyl)-1- (2,3,5-trimethoxyphenyl)methanol **52** *by several methods.*

Method 1: Using Jones' reagent.

a) To a stirred solution of the secondary alcohol **52**(1.00g, 2.6mmol) in of acetone (6ml) at 0^0C was slowly added Jones's reagent (1.00ml). After 15 min. the reaction mixture was filtered on a sinter funnel, containing some silica gel, at the pump and isopropyl alcohol (2ml) was added to the filtrate. Rotary evaporation of

the solvent at RT gave a residue to which was added H_2O (20ml) and extract with EtOAc (3 x 50ml). After drying ($MgSO_4$) the organic solution was filtered and evaporated to give a crude product (0.90g) which was identified by TLC [EtOAc:petrol, 1:3 v/v] and its ^1H NMR spectrum as the starting alcohol **52**.

b) To a stirred solution of the secondary alcohol **52** (1.00g, 2.6mmol) in of acetone (6ml) at 0^0C was slowly added Jones's reagent (1.00ml). The ice bath was removed and the reaction mixture was stirred at RT for 1 h. Work up similar to above gave a crude product (0.85g) which according to TLC [EtOAc:petrol, 1:3] consisted of the starting material and a product. The mixture was separated by flash chromatography to give first the product (260mg, 47%) which was identified as 4-benzyoxybenzaldehyde ( ); ^1H NMR δ 5.20 (2H, s, OCH_2), 7.10 (2H, d AB system, J 8 Hz, Ar ortho to OCH_2Ph), 7.30-7.50 (5H, m, Ph), 7.90 (2H, d AB system, J 8 Hz, ortho to CHO), 9.90 (1H, s, CHO); followed by the starting alcohol **52** (300mg, 30%).

Method 2: Using activated MnO_2

a) To a solution of the secondary alcohol **52** (1.0g, 2.63 mmol) in DCM (60ml) was added activated MnO_2 (4g) and the mixture was heated with stirring for 2 h. TLC [EtOAc:petrol, 1:3] showed only starting material. More MnO_2 (4g) was added and refluxing under stirring was continued for a further 2 h. Analysis by TLC [EtOAc:petrol, 1:3] showed only one spot corresponding to starting material. The mixture was filtered on a sinter funnel and the solvent was evaporated to give recovered starting material **52** (0.8g).

b) A mixture of the secondary alcohol **52** (1.0g, 2.63 mmol) in $CHCl_3$ (60ml) containing MnO_2 (4g) was stirred and heated under reflux 2 h. After analysis by TLC another aliquot of MnO_2 (4g) was added and heating under reflux continued for 2 h and 12 h. TLC analysis revealed the presence of starting material **52** only.

c) A mixture of the secondary alcohol **52** (1.0g, 2.63 mmol) in $CHCl_3$ (60ml) containing MnO_2 (7.5g) was stirred and heated under reflux 2 h. After analysis by TLC another aliquot of MnO_2 (7.5g) was added and heating under reflux continued for further 2 h and 12 h. TLC analysis revealed the presence of starting material **52** only.

Method 3: Using Pyridinium Chlorochromate (PCC)

To a stirred solution of the secondary alcohol **52** (1.14g, 3 mmol) in dry DCM (20ml) containing anhydrous sodium acetate(0.063g, 0.77 mmol) and powdered dry molecular sieves (0.7g) was added PCC (0.97g, 4.46 mmol). The mixture was stirred for 12 h with TLC analysis being done at 2 h intervals. The mixture was filtered through a layer of silica gel in a sinter funnel to give crude a product (1.0g) which was recovered starting alcohol **52**.

Method 4: Using TEMPO

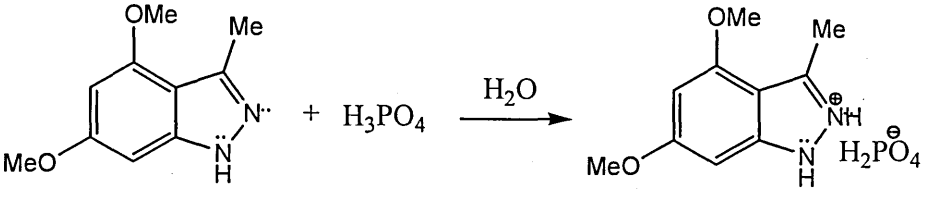
A 100ml 3-necked round bottom flask was equipped on a magnetic stirrer hotplate with a nitrogen bubbler in the central neck , an addition funnel and a stopper. To a stirred solution of sodium bicarbonate (1.36g, 40 mmol) and sodium bromide (65mg, 1 mmol) in water (20ml) at 0 ^{0}C was added a solution of TEMPO (5mg, 3.2 mmol) in DCM (8 ml) followed by the secondary alcohol **52** (3.04g, 8 mmol). Aqueous sodium hypochlorite solution (14%, 3.6ml, 18.4 mmol) was added dropwise via the addition funnel, making sure the temperate does not exceed 15^{0}C. The reaction was complete when the colour remained permanently red. More DCM (30ml) was added and the bottom organic layer was separated and dried ($MgSO_4$). TLC [EtOAc:petrol, 1:3] showed formation of product. The DCM solution was filtered and evaporated to give the crude product which was purified by flash chromatography to give the keto product **53** (0.35g, %); m.p.148-151^{0}C; IR ν_{max} 1693, 1644 (>C=O), 1595 (C=C); ^{1}H NMR δ 3.85 (3H, s, OMe), 3.90 (6H, s, 2 x OMe), 5.18 (2H, s, OCH_2), 6.20 (2H, s, Ar), 7.10 (2H, d, AB system J = 8.4 Hz, ortho to benzyl group), 7.33-7.50 (5H, m, Ph), 7.88 (2H, d, AB system J = 8.4 Hz, ortho to >C=O); ESMS m/z 378 (M+1).

Protection of carbonyl group in keto ester compound **65** *as acetal* **81**

A solution of the keto ester (15g, 0.056mol) and dry 1,2 dihydroxyethane (3.6g, 0.056mol) in dry toluene (50ml) containing a catalytic amount of 4-toluene sulphonic acid was refluxed for 48 h using a Dean-Stark water separator. The solvent was evaporated and the residue was chromatographed [EtOAc:petrol, 1:3] to yield firstly the product (6,25g, 66.4%) identified as 1,3,5-trimethoxy bezene from its ^{1}H NMR spectrum and secondly elution with EtOAc yielded the starting keto ester (4.16g).

3.4 Conclusions

In this chapter the literature approach[58] involving the use of the route II strategy enabled 4,6-dimethoxy-3-methyl-*1H*-indazole **47** to be made, albeit in very low isolated yield (6-16%), by heating 2,4,6-trimethoxyacetophenone and hydrazine hydrate in PPA at 110-120°C. Product isolation from PPA reactions was considered a major problem. The results of Zhenqi *et.al.*[59] could not be reproduced. who made 4,6-dimethoxy-3-methyl-*1H*-indazole **47** in a remarkable 65% yield could not be reproduced. Furthermore, the work-up procdure of Zhenqi *et.al.*[59] does not involved extraction of the indazole product directly from the aqueous reaction mixture of PPA without basification or neutralisation. This is remarkable since indazole would be expected to form a salt with phosphoric acid through its most basic iminium nitrogen atom as shown below. The crude yields achieved in the study, described in this thesis were high because of



47 salt form soluble in water

inorganic matter but the purified yields after column chromatography were very low. This reaction using the method of Zhenqi *et.al.*[59] has previously been carried out in the laboratories at Sheffield Hallam University and the same observations were reported[18].

Oxidation of the 3-methyl group in **47** to the corresponding carboxylic acid was achieved in low yield but this reaction could be improved upon by fine tuning of reaction conditions and exploring other oxidising agents.

The α-ketoester **65**[65] and α-ketoacid **66**[65] reacted with hydrazine hydrate and benzylhydrazine dihydrobromide could not be cyclised to give the desired indazole products. These reactions produced mixtures of undesired products amongst which was 1,3,5-trimethoxybenze as a cleavage product.

Attempts to make the starting ketone **53** by Friedel-Crafts reaction in CS_2 failed and the product was eventually made in low yield from the oxidation of alcohol **52** by TEMPO.

Chapter 4

General Discussion and Conclusions

4.1 Summary of Research Work Reported in This Thesis

The major outcomes from this programme of synthetic work were:

1. 4-Benzyloxybenzoyl chloride **6a**[30] was prepared in two steps from 4-hydroxybenzoic acid[28] by protection with benzyl bromide and acylation using thionyl chloride. Phenyhydrazine and 2-ethylphenylhydrazine were reacted with benzoyl chloride **6b**, 4-benzyloxybenzoyl chloride **6a**[30] and 4-nitrobenzoyl chloride **6c** in pyridine to give the desired β-monoacylated acid hydrazides **7a-d** that were contaminated with a minor amount of α-monoacylated derivatives. The β-monoacylated acid hydrazides **7a-d** were purified by passing HCl gas through their solution in chloroform at 0°C. Cyclisation of the acid hydrazides **7a-d** in polyphosphoric acid (PPA), polyphosphate ester (PPE) and concentrated sulphuric acid gave recovered starting material and no cyclised products **8a-c**. Cyclisation of 4-nitrobenzoyl 2-ethylphenylhydrazide **7d** in xylenes containing an equivalent amount of tosic acid at 150-160°C produced ethylhydrazine as a cleavage product and the desired indazole **8c** in 37.5 % yield which was spectroscopically characterised.

2. In an alternative synthesis of indazoles 2,4,6-trimethoxyacetophenone was heated with hydrazine hydrate with catalytic amount of glacial acetic acid to yield the desired 4,6-dimethoxy-3-methyl-1H-indazole **47** albeit in low isolated yield (6-16%) . Reaction of 2,4,6-trimethoxyacetophenone with methylhydrazine and acetic acid under similar conditions produced the 4,6-dimethoxy-1,3-dimethy-1H-indazole **57** in 18% yield only. Heating 2,4,6-trimethoxyacetophenone with benzylhydrazine dihydrobromide in PPA gave a complex mixture of products from which 1-benzyl-4,6-dimethoxy-3-methyl-1H-indazole **59** was isolated in 16% yield. Oxidation of indazole **47** and 2,4,6-trimethoxyacetophenone with selenium dioxide in dioxane gave recovered starting materials and no products. However, oxidation of 2,4,6-trimethoxyacetophenone with selenium dioxide in pyridine produced the α-ketoacid **66**[65] according to its ^{1}H NMR spectrum.

3 Friedel-Crafts acylation of 1,3,5-trimethoxybenzene with ethyl oxalylchloride in carbon disulphide and dichloromethane gave the α-ketoester **65**[65] in excellent yield. Similarly, alkylation of 3,5-dimethoxytoluene, which was prepared from 3,5-dihydroxytoluene and dimethyl sulphate, underwent Friedel-Crafts acylation with

ethyl oxalylchloride to give the corresponding α-ketoester **74** in excellent yield. Hydrolysis of both the α-ketoesters **65** and **74** in dilute aqueous alcoholic potassium hydroxide yielded the corresponding α-ketoacids **66**[65] and **75**[65] in high yields. Attempted synthesis of 3-carboxy-4,6-dimethoxy-1H-indazole by reacting the α-ketoacid **66** with hydrazine hydrate in PPA at elevated temperature only gave the hydrazone as the reaction product in low yield. Reaction of the α-ketoacid **66** with benzylhydrazine dihydrobromide in DMF containing ammonium acetate and in xylene containing sodium acetate at high temperature produced mixtures of reaction products which were separated but did not contain any of the desired 1-benzyl-3-carboxy-4,6-dimethoxy-1H-indazole. When the α-ketoester **65** was heated with hydrazine hydrate in two different solvents (DMF and xylene) it produced 1,3,5-trimethoxybenzene as the sole reaction product.

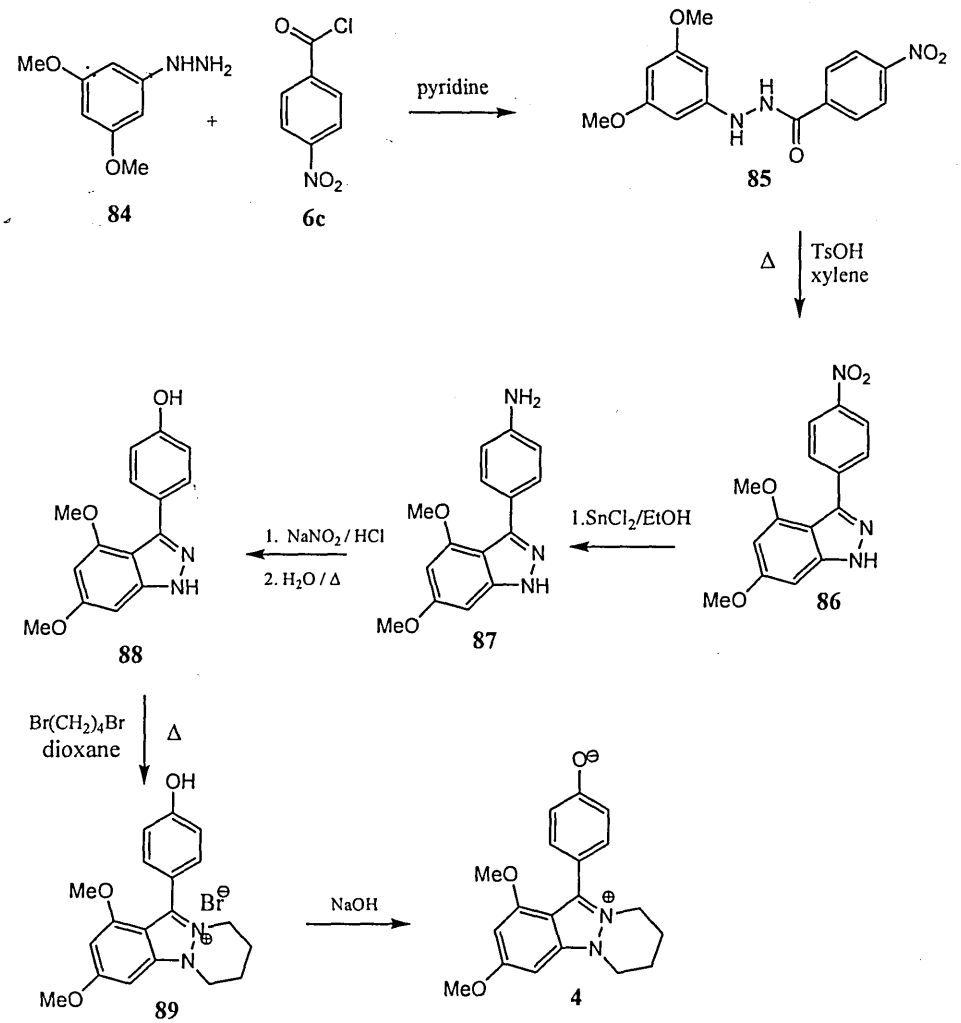
4 Indazole was chosen as a model system for the formation of the six membered heterocyclic ring in Nigellicine **1**. When a solution of indazole and 1,4-dibromobutane in 1,4-dioxane was refluxed for 48 h, the cyclised iminium bromide **78** was formed in 64% yield. Similarly a refluxing solution of 4,6-dimethoxy-3-methyl-1H-indazole **47** and 1,4-dibromobutane in 1,4-dioxane yielded the corresponding heterocyclic system **79** in 78% yield.

5 Friedel-Crafts acylation of 1,3,5-trimethoxy benzene with 4-benzyloxybenzoyl chloride **6a** in carbon disulphide and dichloromethane did not give the anticipated ketone **53**. Reaction of 2,4,6-trimethoxybenzaldehyde with 4-benzyloxyphenyl magnesium bromide in tetrahydrofuran produced the alcohol **52** in good yield. Oxidation of the alcohol **52** with activated manganese dioxide, pyridinium chlorochromate and Jones's reagent failed to produce the expected ketone **53**. Prolonged treatment with Jones's reagent at room temperature caused cleavage of the alcohol **52** to yield 4-benzyloxybenzaldehyde **80** as the reaction product. Oxidation of alcohol **52** in dichloromethane with TEMPO and buffered aqueous hypochlorite solution gave the ketone **53** in 11.6% isolated yield. Attempted protection of the keto group in α-ketoester **65** with ethyleneglycol in refluxing toluene containing a catalytic amount of tosic acid did not give the acetal **81** but produced the cleavage product 1,3,5-trimethoxybenzene instead.

4.2 Suggestions for future work

It was established by the work reported in chapter 2 of this thesis that acid hydrazides will only undergo ring closure reactions if the aryhydrazine portion had electron releasing groups and the benzoyl ring had electron withdrawing groups. An example was the cyclisation of acid hydrazide **7d** to 1H-indazole **8c**. In accordance with this finding the acid hydrazide **85** derived from 3,5-dimethoxyphenylhydrazine **84** and 4-nitro benzoyl chloride **6c** would be expected to cyclise into the 1H-indazole **86**. Selective reduction of **86** with stannous chloride[68] in ethanol would give the corresponding amino derivative **87** which can be transformed into the phenol **88** by diazotization and hydrolysis. Reaction of the phenolic *1H*-indazole **88** with 1,4-dibromobutane in refluxing dioxane would then generate the iminium salt **89** which on brief exposure with sodium hydroxide solution would give the desired natural product Nigellidine 4 (Scheme 23).

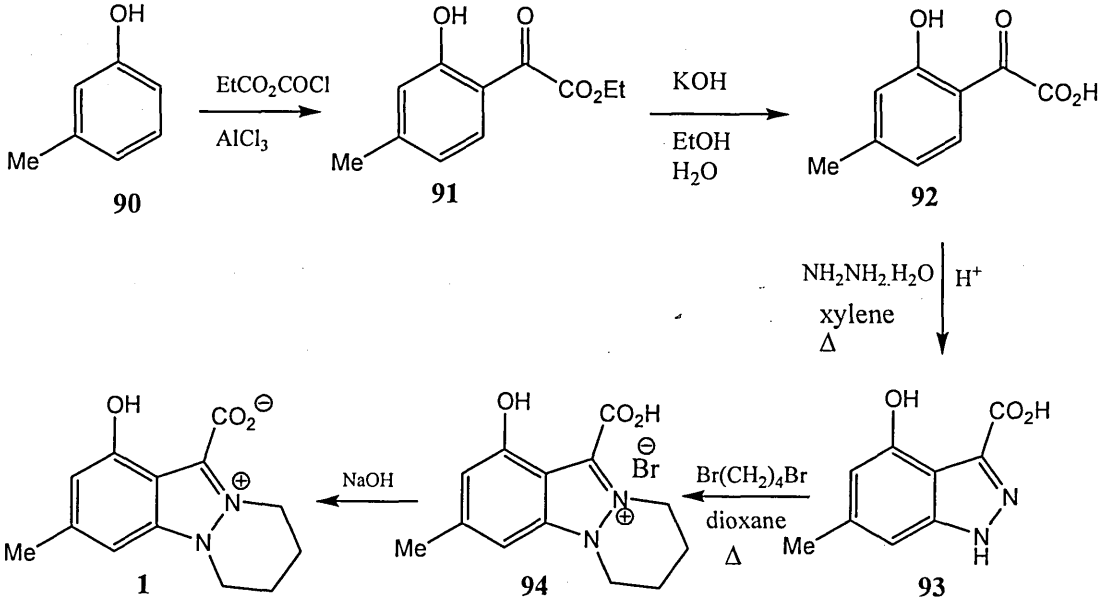
Scheme 23



The 3,5-dimethoxyphenylhydrazine is unavailable commercially and can be made from 3,5-dimethoxyaniline via the sydnone route[23-24] or by diazotization and reduction[21].

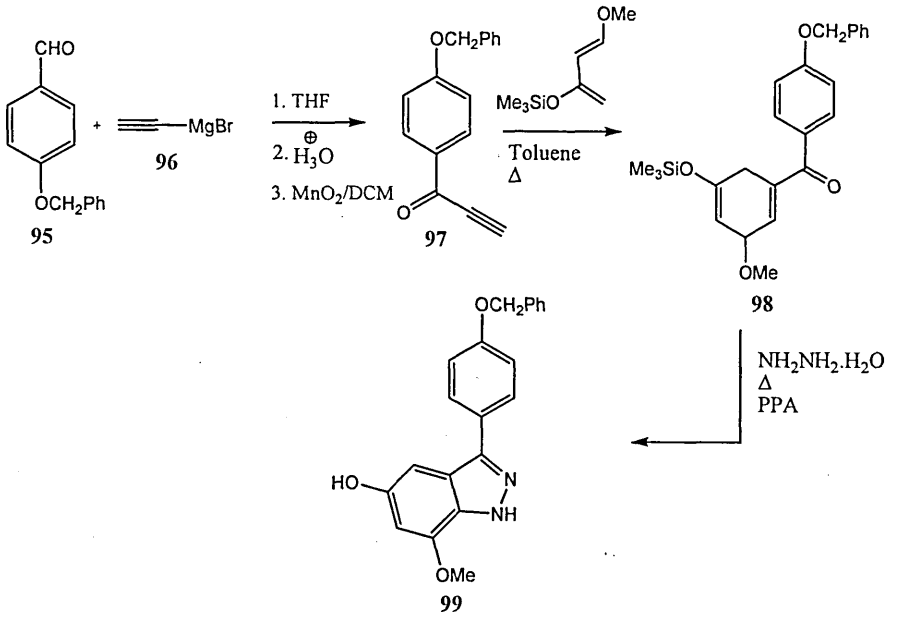
From the work reported in chapter 3 at this thesis the α-ketoester **91** can be made from m-cresol **90** by the Friedel-Crafts acylation with ethyl oxalylchloride and then hydrolysed with KOH/EtOH/H_2O to the α-ketoacid **92.** Reaction of **92** with hydrazine hydrate in xylene should work, after some experimentation with reaction conditions to give the *1H*-indazole **93** which on treatment with 1,4-dibromobutane in refluxing dioxane would yield **94**. Careful basification with sodium hydroxide should then provide the alkaloid Nigellicine **1** (scheme 24).

Scheme 24



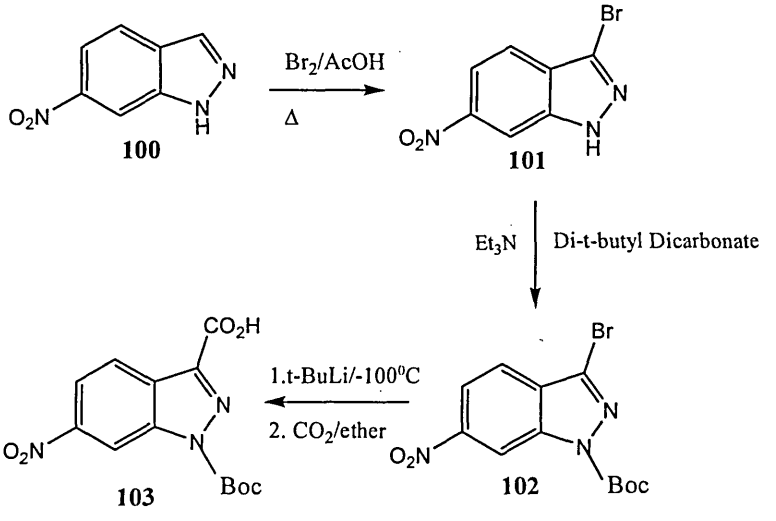
Another strategy can be used which has been developed in these laboratories[69]. This involves the acetylenic ketone **97** made in two steps from 4-benzyloxybenzaldehyde **95** and acetylenemagnesium bromide **96** (scheme 25). The acetylenic ketone **97** underwent cycloaddition reaction with Danishefsky's diene to yield the cycloadduct **98**. The indazole product **99** was obtained in low yield but was not fully characterised. However, this route offers scope as a new synthetic strategy. By making a 1,3-dimethoxybuta-1,3-diene and reacting it with dienophile **97** another possible route could be sought for the synthesis of Nigellidine **4**.

Scheme 25



In these laboratories exploratory experiments have been carried out[70] to carboxylate 6-nitro-*1H*-indazole **100** at the most reactive 3-position *via* the bromointermediate **101** and Boc-protected bromo intermediate **102** to obtain the product **103** (scheme 26). This methodology could be further explored by synthesising 4-hydroxy-6-methoxy-*1H*-indazole for Nigellicine synthesis as shown.

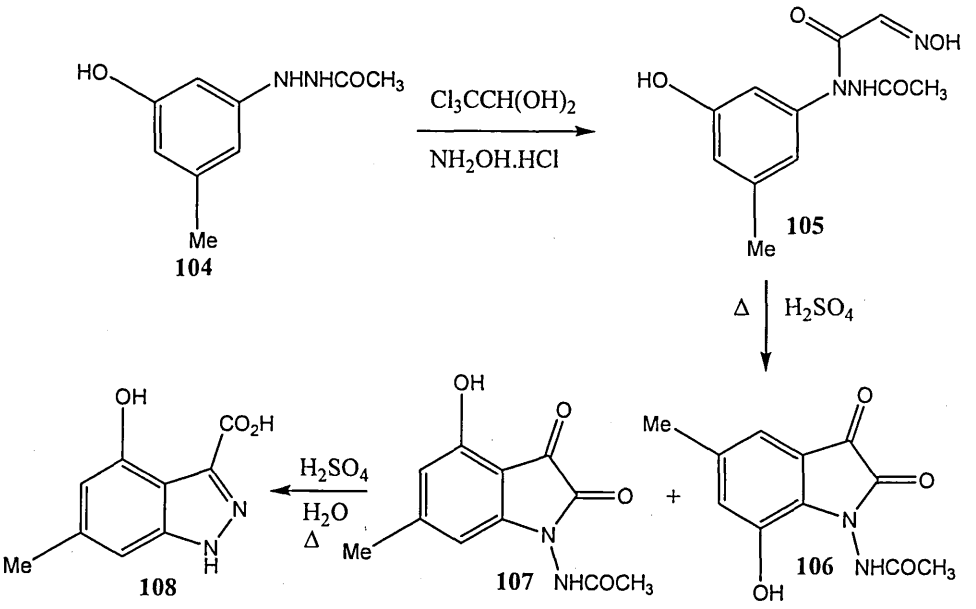
Scheme 26



Boc = $-CO_2CMe_3$

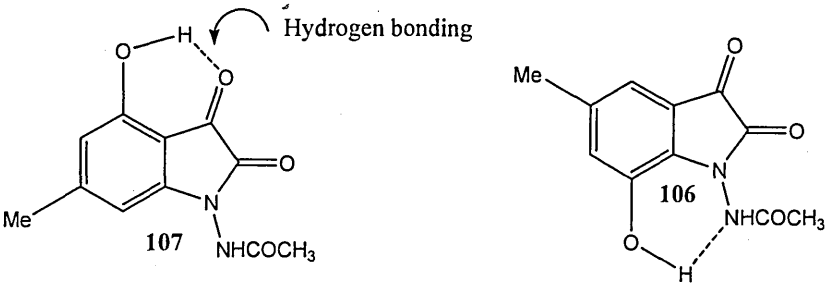
The improved synthesis of indazole-3-carboxylic acid[64] by Sisti *et al.* can be extended to the synthesis of Nigellicine from the appropriate arylhydrazine **104** as outlined in scheme 27.

Scheme 27



mixture of these two products is expected which can then be separated by column chromatography

Compounds **106** and **107**will both hydrogen bond but the strength of each will be different and it should be possible to separate them



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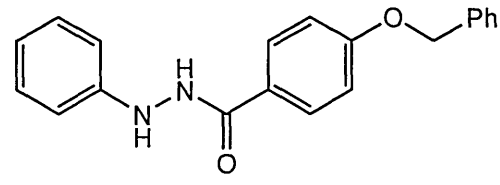
List of structures of new compounds synthesised



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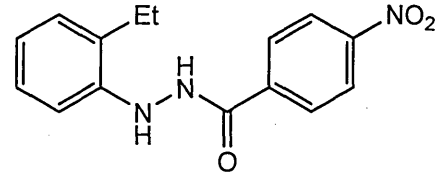

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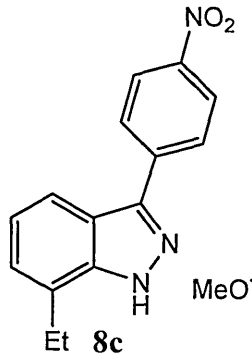

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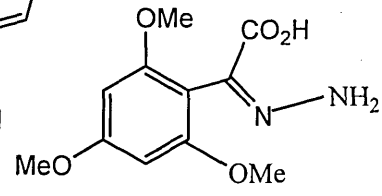

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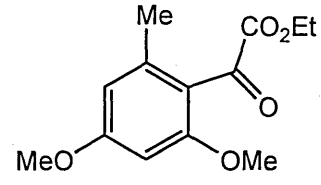

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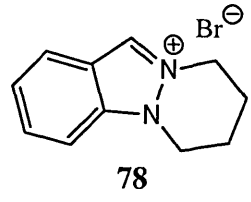

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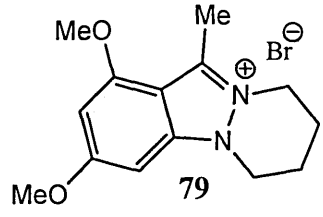

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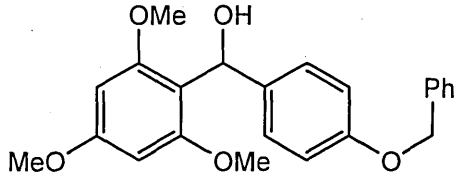

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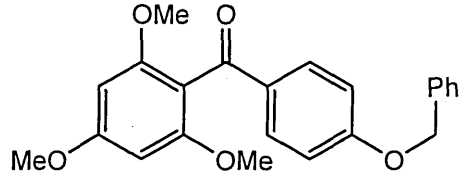

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Appendix

Table 1: Nutritional Components of Black Seed

S.No.	Component Percent
1- Protein	21%
2- Carbohydrates	35%
3- Fats	35-38%

Table 2: Nutritional Value including Vitamins and Minerals of Black Seed

S.No.	Component Quantity
1- Protein	208 µg/g
2- Thiamin	15 µg/g
3- Riboflavin	1 µg/g
4- Pyridoxine	5 µg/g
5- Niacin	57 µg/g
6- Folacin	610 IU/g
7- Calcium	1.859 mg/g
8- Iron	105 µg/g
9- Copper	18 µg/g
10- Zinc	60 µg/g
11- Phosphorus	5.265 mg/g

Table 3: Fatty Acids Contents of Nigella sativa Linn

S.No.	Fatty Acids Quantity (%)
1- Saturated Acid	18.1
2- Monounsaturated Acid	23.8
3- Polyunsaturated Acid	58.1

Table 4: Fatty Acid Profile of Nigella sativa

S.No.	Fatty Acids Quantity (%)
1- Myristic Acid	(C14:0) 0.5
2- Plamitic Acid	(C16:0) 13.7
3- Plamitoleic Acid	(C16:1) 0.1
4- Stearic Acid	(C18:0) 2.6
5- Oleic Acid	(C18:1) 23.7
6- Linoleic Acid	(C18.:2) (□-6) 57.9
7- Linolenic Acid	(18:3n-3) (□-3) 0.2
8- Arachidic Acid	(C20:0) 1.3

Table 5: Essential Oil Profile of Nigella sativa

S.No.	Essential Oil Composition (1.4 %) Quantity (%)
1- Carvon	21.1
2- □-Pinene	7.4
3- Sabinene	5.5
4- □-Pinene	7.7
5- P-cymene	46.8
6- Others	11.5