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1,4-DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS AS CARDIOVASCULAR AGENTS

BY

RAYMOND DOMINIC ANANA 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY.**

IN

PHARMACEUTICAL SCIENCES

(MEDICINAL CHEMISTRY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

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The undersigned certify that they have read, and recommend to the Faculty of Graduate studies and Research for acceptance, a thesis entitled **1,4-DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS AS CARDIOVASCULAR AGENTS** submitted by **RAYMOND DOMINIC ANANA** in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **PHARMACEUTICAL SCIENCES (MEDICINAL CHEMISTRY).**

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Dated JANUARY 30, 1996

Dedicated to my wife, Francisca and children, Akan, Odu, Ini, Nsukidem, Eti, and Angela-Unwana.

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ABSTRACT

The design and synthesis of a series of 1,4-dihydropyridine (1,4-DHP) calcium channel modulators have been carried out.

The first class of compounds investigated was alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates (**38a-38f**). These compounds were generally synthesized using a modified Hantzsch reaction, comprising the condensation of an oxazolinyl enamine (**32**) with appropriately functionalized Knoevanegal derived adduct (**37**). **38c,** was prepared by a different method, involving the cylization of 5-{N-1-(1,1-dimethyl-2-hydroxyethyl)aminocarbonyl moiety of an intermediate 1,4-DHP (**41**), using thionyl chloride, to form the 5-(4,5-dihydro-4,4-dimethyloxazolin-2-yl) group.

The second class of compounds investigated was the dialkyl 1,4-dihydro-2,6-dimethyl-4-[(*Z*)-N-oxo-N-(aryl/vinyl methylene)-λ^5-azanyl]phenyl}-3,5-pyridinedicarboxylates (**53a-53i**). Two general synthetic methods were used for their preparation. The first method involved the reaction of nitrophenyl 1,4-DHPs (**52b-52e**) with benzylmagnesium chloride to afford the corresponding nitrones (**53a-53d**). The second method involved reduction of nitrophenyl 1,4-DHPs (**52f** and **52g**) to the corresponding hydroxylamines (**54a** and **54b**), which on condensation with an apppropriate aldehyde, afforded the corresponding nitrones (**53e-53i**).

The third class of compounds studied was dialkyl 1,4-dihydro-2,6-dimethyl-4-(quinolinyl)-3,5-pyridinedicarboxylates (**61a-6e**), which were also synthesized using a three component modified Hantzsch reaction.

Lastly, diethyl 1,4-dihydro-2,6-dimethyl-4-(1-oxido-4-pyridyl)-3,5-pyridinedicarboxylate (**62**) was synthesized using a modified three component Hantzsch reaction.

Molecular mechanics calculations (MM^{+}) were performed using the Hyperchem Version 3 for Windows minimizer program from Autodesk (IBM PC Version) to obtain information pertaining to the most stable conformation for compounds **38a-38f** and stereochemistry of the nitrone moiety in compounds **53d** and **53i**.

All of the compounds synthesized as described above, were evaluated as calcium channel antagonists, using a guinea pig ileal longitudinal smooth muscle (GPILSM) assay. These compounds were less active antagonists than the reference drug, nifedipine (**1**). Also, all the compounds, except **61b-61e**, were evaluated as calcium channel agonists (positive inotropes) using the guinea pig left atria (GPLA) assay. Most of the compounds exhibited dual smooth muscle calcium channel antagonist/cardioselective calcium channel agonist effects at their calcium channel antagonist IC_{50} values, or at lower concentrations. Some compounds described in these studies may prove to be good leads for the development of potential drugs for the treatment of congestive heart failure.

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LIST OF ABBREVIATIONS AND SYMBOLS

α	Alpha
AOPA	Aryloxypropanolamine
ap	Antiperiplanar
ATPase	Adenosine triphosphatase
AV	Atrioventricular
β	Beta
bp	Boiling point
CC	Calcium channel
CCags	Calcium channel agonists
CCAs	Calcium channel antagonists
^{13}C	Carbon-13
$CDCl_3$	Deutero-chloroform
CHF	Congestive heart failure
DAG	Diacylglycerol
δ	Delta
DHP	Dihydropyridine
DMAP	Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
DTZ	Diltiazem
γ	Gamma
GPILSM	Guinea pig ileal longitudinal smooth muscle
GPLA	Guinea pig left atria
GPRA	Guinea pig right atria
HPSS	HEPES buffered physiological saline solution
HRMS	High resolution mass spectrometry
^{1}H	Proton

IC_{50}	The molar concentration of the test compound required to produce a 50% decrease in the slow component or tonic contractile response in GPILSM by the muscarinic agonist carbachol (1.64×10^{-7} M)
IP_3	Inositol triphosphate
IR	Infrared
ISA	Intrinsic sympathomimetic activity
LDA	Lithium diisopropylamine
lit	Literature
mp	Melting point
mm Hg	Millimetre of mercury
ml	Millilitre
mM/L	Millimolar per litre
mmol	Millimole
MEPs	Molecular electrostatic potentials
MOPAC	Molecular orbital program calculations
N	Neuronal
nmr	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
P	Passive
ROESY	Rotating-Frame Overhauser effect spectroscopy
SA	Sinoatrial
SARs	Structure-activity relationships
SEM	Standard error of the mean
sp	Synperiplanar
THF	Tetrahydrofuran
tlc	Thin layer chromatography
TMS	Tetramethylsilane
µm	Micrometre

1.0.0.0 INTRODUCTION

Cardiovascular disease is one of the leading causes of death today.[1] The cardiovascular, or circulatory system, encompasses the heart and blood vessels. There are two separate parts to the circulatory system that operate simultaneously. Thus, the pulmonary circulation effects the exchange of oxygen and carbon dioxide between blood and lungs, whereas the systemic circulation provides oxygen and nutrients to cells.

The cardiac cycle is initiated by nerve impulses that originate within the heart, particularly the sinoatrial (SA) node. These impulses travel through the atria, or upper chambers of the heart, *via* a specialized conducting system to the ventricles, or lower chambers. Blood is pumped in succession from atria to ventricles, arteries, arterioles, capillaries, venules, veins and then back to the heart. The heart rate is primarily controlled by the autonomic nervous system, the activity of which is mediated by the release of neurotransmitter substances. Parasympathetic, or vagal activation, slows the rate of contraction, whereas sympathetic activation increases the heart beat and strengthens the force of contraction. Cardiac output (the volume of blood pumped by the heart each minute) depends on the heart rate, stroke volume (the amount of blood ejected by each heart beat), and peripheral resistance in the arterioles.

1.1.0.0 ETIOLOGY OF CARDIOVASCULAR DISEASE

There are several diseases of the cardiovascular system, and some of these will be discussed in the following sections. Generally cardiovascular diseases involve malfunctions that affect one or more of the following: myocardio contraction, magnitude of heart beat, heart rate and peripheral blood pressure.

1.1.1.0 Hypertension

Arterial pressure fluctuates with each contraction of the heart. The maximum pressure resulting from ventricular contractions, or systole, is defined as the systolic pressure, and the lower pressure resulting from ventricular relaxation is referred to as diastolic pressure. Blood pressure is usually recorded as systolic pressure divided by the diastolic pressure where the average is about 120/80. When the blood pressure exceeds 145/90, the individual is said to have high blood pressure or hypertension. Acute, or severe, blood pressure elevation may precipitate a medical emergency requiring prompt reduction of blood pressure to prevent death or progressive injury to vital organs. A marked elevation of blood pressure may constitute severe hypertension alone, a hypertensive urgency or a true hypentensive emergency, depending on the pressure or absence of target-organ damage.[2-4]

Essential hypertension is by far the most common underlying cause of hypertensive emergencies.[5] The etiology of essential hypertension is not completely understood. It has

been demonstrated that essential hypertension is a heterogeneous disorder and that the mechanism of pressure elevation may vary from one patient to another. Severe uncontrolled hypertension due to any cause may progress to an accelerated or malignant phase.[6] Untreated malignant hypertension is fatal in 80-90% of patients within one year.[7] Aggressive treatment with potent antihypertensive agents, and dialysis or renal transplantation when required, has resulted more recently in 5-year survival rates of 60 to 75%.[8,9]

1.1.2.0 Cardiac Arrhythmias

The normal rhythm of the heart may be altered by disturbances in the pacemaker activity of the sinoatrial (SA) node or any part of the heart usurping pacemaker function. For example, the atrioventricular (AV) node, or Purkinje fibers, may begin to generate impulses more rapidly than the SA node and thereby set a new pace for the heart. As a result, the atria and ventricles contract independently and at different rates, disrupting normal cardiac rhythm that causes the heart to become a less efficient or even an inefficient pump. Some of these arrhythmias merely prove to be annoying to the patient, while others could cause immediate death. Prevention of atrial fibrillation and atrial flutter requires administration of drugs, that prolong the atrial refractory period or slow atrial conduction, such as antiarrhythmic agents exhibiting class I or III properties.

1.1.3.0 Congestive Heart Failure

Congestive heart failure (CHF) has been estimated to affect 4 million Americans for which the two-year survival statistic is less than 30%.[10] A patient suffering from CHF has a feeble, but rapid heart-beat, a below-normal cardiac output, and impaired systemic circulation. Incomplete emptying of blocd from the heart during ventricular contractions eventually cause an enlargement of the heart and an increase in venous pressure. Impairment of blood flow to kidneys may lead to a reduction in urine output, thereby causing edema or swelling. Substantial progress has been made over the past two decades in the understanding and therapy of CHF. One of the most important advances has been a growing understanding of the pathophysiology of CHF, particularly the role of hormonal factors and the peripheral circulation. As systolic function decreases, a number of important reflex mechanisms are activated, including increases in serum catecholamines[11] and arginine vasopressin,[12] and activation of the renin-angiotensin system.[13] These changes result in an increase in heart rate, contractility and systemic vascular resistance.

Despite the overall progress in our understanding and management of chronic heart failure, many problems remain. The prognosis for CHF is grim since the one-year mortality rate in severely ill patients (class III and IV) ranges from 34-48%.[14] The most important cause of CHF is coronary artery disease and a decline in coronary disease through preventive measures will impact on the number of people who develop CHF.

1.1.4.0 Coronary Artery Disease (Angina Pectoris)

Coronary artery disease, the major cause of death in Western nations, results from an inadequate blood supply to heart muscle. Angina pectoris is one type of coronary artery disease that occurs when the oxygen requirements of the myocardium temporarily exceed the supply, such as that during vigorous exercise. This oxygen deficiency produces an extreme crushing pain in the left arm and chest. Current evidence strongly suggests that coronary atherosclerosis is a common denominator in most patients with ischemic heart disease. Furthermore, the turbulance and stasis of blood flow caused by atherosclerotic plaque can be responsible for intermittent platelet aggregation in diseased vessels,[15] as well as intermittent coronary artery thrombosis.[16-19] Plaque that develops slowly over decades may result in total coronary artery occlusion. Coronary atherosclerosis usually proceeds to a myocardial infarction or, in some cases, to sudden cardiac death.[20]

1.2.0.0 DRUGS USED FOR TREATMENT OF HEART DISEASE

Many drugs have been developed to treat diseases of the cardiovascular system. Some of these drugs exert their action on the heart by modifying the rate and force of its contraction or correcting the abnormal heart beat. Other drugs act on peripheral vascular blood vessels to maintain blood

flow to and from the heart. Some representative examples of these drugs are discussed in the following sections.

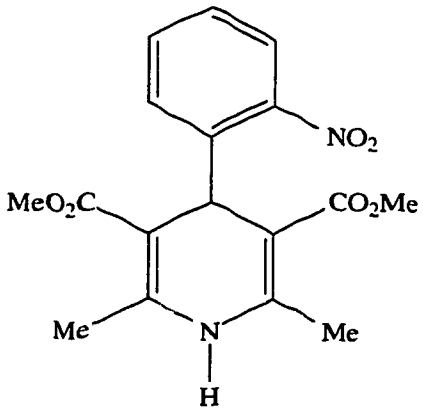
1.2.1.0 Calcium Channel Modulators

The calcium channel modulators are a class of drugs that control or modulate the movement of calcium ions (Ca^{2+}) across extracellular membranes. Those compounds that block or prevent the entry of Ca^{2+} into cells are called calcium channel antagonists (CCAs) or calcium entry blockers, whereas those that enhance the entry of calcium into cells are called calcium channel agonists (CCags). There are three major classes of calcium channel modulators which include the 1,4-dihydropyridines, the phenylalkylamines and the benzothiazepines.

1.2.1.1 1,4-Dihydropyridine Calcium Channel Modulators

The 1,4-dihydropyridine (1,4-DHP) class of compounds has been widely investigated as cardiovascular agents in recent years.[21] Nifedipine (**1**) was the first 1,4-DHP to be used clinically. Nifedipine (3,5-dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate) is a potent vasodilator that selectively dilates arterial resistance vessels with little effect on venous pooling.[22] Nifedipine is a calcium channel antagonist that blocks the entry of calcium ions (Ca^{2+}) through L-type potential-dependent calcium channels. Nifedipine, like most other calcium-

ion blocking agents, exhibits an *in vitro* negative inotropic effect.[23] In contrast, vascular smooth muscle is relaxed by nifedipine *in vivo* at a lower concentration than that needed to induce a direct depressant effect on the myocardium.[24,25] Therefore, following a dose of nifedipine, the overall effects are lowering of blood pressure, enhancement of cardiac contractility and segmental ventricular function, and a modest increase in both heart rate and cardiac output.[26] Nifedipine has also been shown to have antiplatelet actions,[27] that is, it prevents blood from clotting; and this might enhance its overall effect during acute myocardial ischemia.



1

Hugenholtz et al.,[28] and Moses and co-workers,[29] have demonstrated the effectiveness of nifedipine for the treatment of unstable angina pectoris. Nifedipine has also been proposed for the treatment of congestive heart failure, since it decreases cardiac afterload.[30] Despite its initial success and continued clinical use, there are a number of side effects associated with nifedipine administration. Most

of these side effects are caused primarily by excessive peripheral vasodilation,[31] which causes dizziness, hypotension, flushing, headache and palpitations. Ankle edema caused by the overall effect of precapillary dilation without accompanying venodilation may also occur. There have been reports of myocardial ischemia, or even infarction when nifedipine was used to treat hypertensive emergencies.[32] The usual cause of this myocardial ischemia is exaggerated or excessive hypotension. Although nifedipine appeared to be well tolerated in a group of patients with dilated cardiomyopathy,[33] other investigators have reported dramatic falls in blood pressure[34] and even the precipitation of pulmonary edema.[35] Adverse effects including systemic hypotension, cardiogenic shock, pulmonary edema and even death have also been reported.[36-39]

The clinical success of nifedipine stimulated an array of structure-activity studies in an effort to obtain analogs with higher potencies, a longer duration of action and less side effects.[40] Some of these second-generation calcium channel antagonists include nicardipine(**2a**) nitrendipine (**2b**), nimodipine(**2c**), isradipine(**2d**) and felodipine (**2e**).[40-43]

Nicardipine, 3-methyl 5-[2-(N-benzyl-N-methylamino)ethyl -1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate hydrochlo -ride,[44] was synthesized in Japan, in the early 1970s and introduced as a cerebral vasodilator in 1976.[45] Nicardipine, like nifedipine, is also effective for the treatment of cardiovascular disease. Nicardipine has been found to be effec-

tive in the management of patients with myocardial ischemia and systemic hypertension. Nicardipine, which is the most potent of the dihydropyridines studied for their effect on vascular smooth muscle, also exhibits the highest degree of selectivity for vascular smooth muscle relaxation.[46] Nicardipine shows a higher selectivity for vascular smooth muscle than nifedipine[47] and it exhibits fewer side effects compared to nifedipine.

Nitrendipine (**2b**) exhibits a longer duration of action than nifedipine, has a high selectivity for vascular smooth muscle relative to myocardium, and it does not exhibit a negative inotropic effect.[48] Nitrendipine can therefore be given safely to patients with congestive heart failure and those with hypertension complicated by left ventricular failure or in combination with β-blockers. Side effects associated with nitrendipine are usually mild and transient.

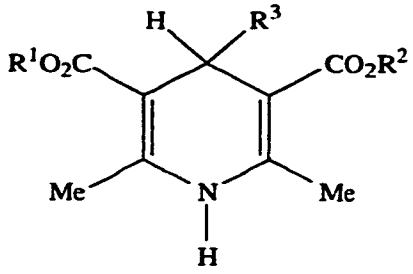
Nimodipine (**2c**) possesses a high lipid solubility which enhances its ability to cross the blood-brain barrier more readily. This property accounts for its potent and beneficial cerebrovascular effects, which include a reduction in cerebral spasm[49] and the treatment of migraine.[50-52]

Isradipine (**2d**), like other calcium-channel blockers, decreases blood pressure by reducing total peripheral resistance.[53] In contrast to many other calcium channel antagonists that exert a negative inotropic effect[54], isradipine possesses potent vasodilatory properties at doses much lower than those associated with a negative inotropic effect.

Isradipine appears to be an ideal drug for the treatment of hypertension, angina pectoris and even congestive heart failure.[55] To date, few side effects have been reported for isradipine, and when present they usually abate during longer term treatment. For example isradipine tends to increase glucose and cholesterol levels, but to a minor extent.[56,57]

Felodipine (**2e**) exerts its potent antihypertensive effect by relaxing vascular smooth muscle predominantly in arteriolar resistant vessels.[58] In contrast to most CCAs, felodipine has no simultaneous direct effect on cardiac function.[59,60] Since felodipine is an extremely lipophilic agent that is capable of crossing the blood-brain barrier readily, a number of effects on the cerebral vasculature have been reported.[61] Felodipine exhibits no negative inotropic effect which enhances its usefulness in hypertensive patients who also suffer from congestive heart failure, angina pectoris and renal impairment. The most commonly observed side effects after acute felodipine administration are headache, flushing, dizziness, fatigue, palpitations and peripheral edema[62], all of which are dose dependent.

Structural modification of nifedipine also gave rise to related compounds having effects diametrically opposed to the calcium channel antagonists. Compounds which enhanced cardiac contractility as well as the contraction of vascular smooth muscle were discovered. The prototype of this class of compounds is BAY K8644 (**3a**), which is a calcium channel activator or calcium channel agonist.[63-65] Bay K8644 is a



2

2a: Nicardipine: $R^1 = Me$

$R^2 = -CH_2CH_2N(CH_3)CH_2Ph$

$R^3 = 3\text{–}O_2N\text{–}C_6H_4\text{–}$

2b: Nitrendipine: $R^1 = Me$

$R^2 = Et$

$R^3 = 3\text{–}O_2N\text{–}C_6H_4\text{–}$

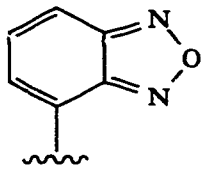
2c: Nimodipine: $R^1 = -CHMe_2$

$R^2 = -CH_2CH_2OMe$

$R^3 = 3\text{–}O_2N\text{–}C_6H_4\text{–}$

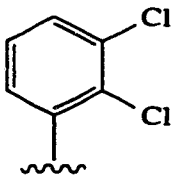
2d: Isradipine: $R^1 = Me$

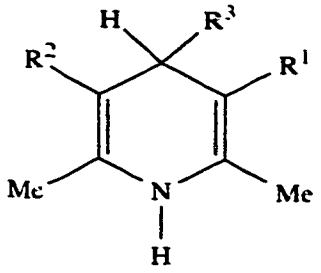
$R^2 = -CHMe_2$

$R^3 =$ 

2e: Felodipine: $R^1 = Me$

$R^2 = Et$

$R^3 =$ 



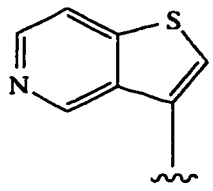
3

3a: Bay K844: R^1 = $-NO_2$; R^2 = $-CO_2Me$; R^3 = $2\text{-}CF_3\text{-}C_6H_4$

3b: PN 202-791: R^1 = $-NO_2$; R^2 = $-CO_2Pr\text{-}i$; R^3 = 4-(2,1,3-benzodiazol-4-yl)-

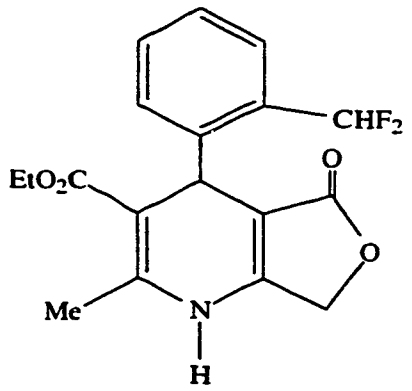
3c: YC-170: R^1 = PhNHCO-; R^2 = 2-(2-pyridylethyl-CO_2)-; R^3 = $2\text{-}Cl\text{-}C_6H_4$-

3d: LY 249933: R^1 = $-NO_2$; R^2 = $-CO_2CH(Me)Ph$; R3 =



3e: AK-2-38: R^1 = $4\text{-}Me\text{-}C_6H_4(CH_2)_2CO_2$-; R^2 = $i\text{-}PrCO_2$-; R^3 = 2-pyridyl-

racemate with calcium channel agonist [(-)-(S)-enantiomer] and antagonist [(+)-(R)-enantiomer] properties.[66,67] The racemic mixture displays agonist activity at low concentrations and antagonist properties at high concentrations.[63,68] Other CCags that exhibit similar properties to Bay K8644 include PN 202-791 (**3b**)[69], CGP 28392 (**4**)[70], YC-170 (**3c**)[71] and LC 249933 (**3d**).[72-73] However these CCags are not useful clinically due to their vasoconstrictive effects on smooth muscle. To quote a recent review, "No CC activator has been reported that is cardioselective, that is able to increase myocardial contractility without also increasing vascular resistance and blood pressure".[74] In contrast, AK-2-38 (**3e**),[75] was the first 1,4-DHP reported to exhibit dual smooth muscle selective CC antagonist and cardioselective CC partial agonist effects. The future clinical use of calcium agonists to treat congestive heart failure will therefore be dependent upon separating their vasoconstricting effects from their cardiostimulant properties.[76]



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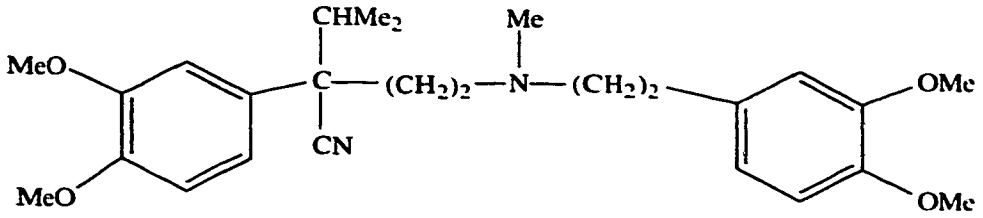
1.2.1.2 Phenylalkylamines

The best known phenylalkylamine used as a cardiovascular agent is verapamil (**5**).



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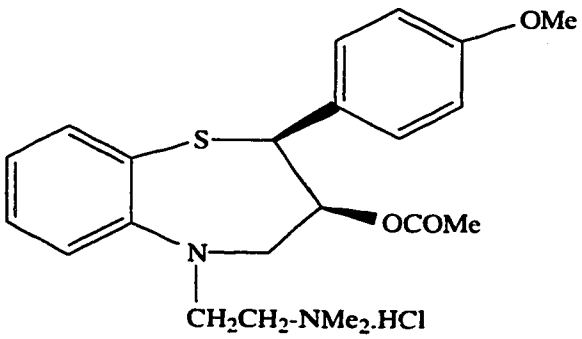
Verapamil was the first slow-channel calcium channel antagonist described with the property of selectively inhibiting the transmembrane flux of calcium ions into excitable tissues. Verapamil, which was introduced in Europe in 1963, is structurally different from the 1,4-dihydropyridines and benzothiazepines. The two enantiomers of racemic verapamil exhibit different electrophysiologic properties. The (+)-isomer depresses the maximal rate of rise of the action potential and has other electrophysiologic properties, including an influence on the overall shape of the action potential.[77] Verapamil has a pronounced effect on the SA and AV nodes, causing a decrease in heart rate. Important therapeutic applications of verapamil include the control of supraventricular tachyarrhythmias, where it is a drug of choice for use as a class IV antiarrhythmic, antihypertensive, and anti-angina pectoris agent.[78-81] The major side effects of verapamil, when administered orally, include headache, dizziness, nausea and ankle edema.[81]

Intravenous administration produces the expected transient decrease in blood pressure. However, serious side effects such as persistent hypotension, bradycardia, and occasional ventricular asystole have also been reported.[83]

1.2.1.3 Benzothiazepine Calcium Channel Antagonists

This class of calcium channel antagonists is represented by diltiazem (**6**). Diltiazem (DTZ, *d-cis*-3-acetoxy-2,3,dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(*5H*)-one hydrochloride), has two asymmetric centers and is capable of *cis-trans* isomerism. The *l-cis* enantiomer has a ten-fold longer duration of action than *d-cis* (DTZ), but it has low potency.[84]

Diltiazem induces a weaker effect on peripheral resistance and myocardial contractility than verapamil and nifedipine, but it is a more potent vasodilator.[85] DTZ slows



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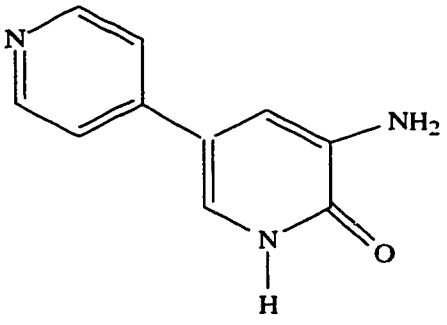
conduction in the AV node, but to a lower degree than verapamil. In general, DTZ is used for the same spectrum of dis-

eases as verapamil, *viz*, supraventricular tachycardias, hypertension and angina pectoris. The major advantages of diltiazem are its relatively few side effects and a modest negative inotropic effect. DTZ is increasingly being observed to have hemodynamic advantages in the treatment of angina pectoris, due to its peripheral vasodilation and mild negative inotropic effect properties.

1.2.2.0 Phosphodiesterase III Inhibitors

Several phosphodiesterase inhibitors have been investigated as agents for the treatment of congestive heart failure.[86] The first of these compounds to be thoroughly evaluated, for the the treatment of patients with severe congestive heart failure (CHF), was amrinone (**7**).



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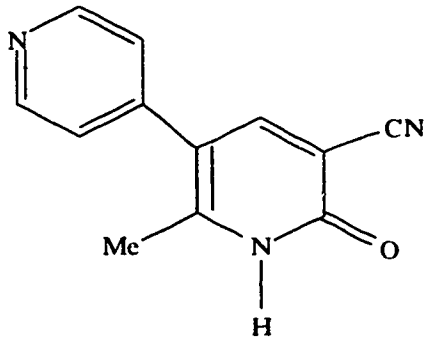

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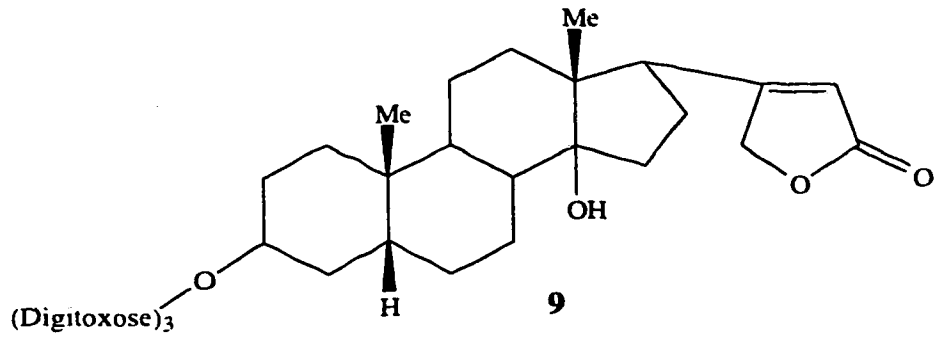
Amrinone inhibition of phosphodiesterase III produces a combined inotropic and vasodilator effect. In patients with severe CHF, the vasodilator effect dominates with a variable direct positive inotropic contribution.[87-90] Amrinone is rapidly distributed in the circulation with a half-life of 5 minutes and an elimination half-life of 4 hours.

Several second-generation phosphodiestrase inhibitors have been described. One of these is milrinone (**8**), which has been approved for intravenous use, and is under investigation as an oral agent.[91-92] Milrinone is 20-fold more potent than amrinone, and it has the same mechanism of action. Milrinone produces a greater vasodilatory effect than amrinone and it is better tolerated.[93,94]

These drugs exhibit serious side-effects. For amrinone, these include thrombocytopenia, ventricular arrhythmias, hepatoxicity, hypotension, nausea and vomiting, which may be severe at times. However, during acute use, side effects may be slight.[95] Chronic use of milrinone may lead to some patients developing a risk of arrhythmias. Milrinone may also lead to an increased incidence of heart failure that limits survival despite its hemodynamic benefits.[96]

1.2.3.0 Other Drugs for the Treatment of Cardiovascular Diseases

Other drugs such as the cardiac glycoside, digitoxin (**9**), and β-agonists such as dobutamine (**10**) have been the drugs of choice for the treatment of CHF. Unfortunately, cardiac glycosides possess many adverse side effects and limitations that include a low therapeutic index of 2-3 (narrow margin of safety), erratic absorption, harmful drug-drug interactions and often limited efficacy. β-Agonists may cause tachyphylaxis resulting from β-receptor down-regulation,[97] and can also induce a negative inotropic effect (decreases the force of contraction of the heart).



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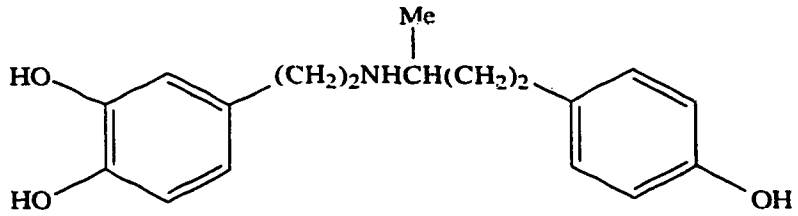

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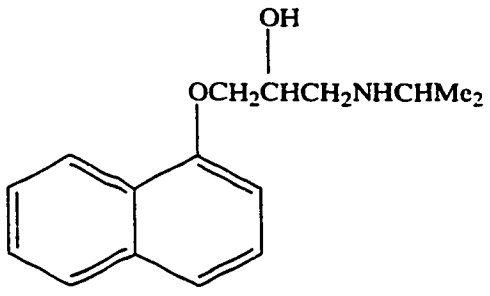
β-Blockers such as propranolol (**11**) are also used for the treatment of cardiovascular diseases such as angina pectoris, hypertension and acute myocardial infarction. Major side effects of β-blockers include bronchospasm, and an excessive negative inotropic effect which may precipitate heart failure.



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1.3.0.0 MODE OF ACTION OF 1,4-DIHYDROPYRIDINES

The 1,4-dihydropyridine class of compounds modulate the entry of Ca^{2+} across extracellular membranes. This property constitutes the basis for many therapeutic applications in the treatment of cardiovascular diseases.

1.3.1.0 Role of Calcium in Cells and the Cardiovascular System

Calcium plays an important role in the physiology of cells. Ca^{2+} mediates a number of effects such as smooth muscle contraction, cardiac contractility, pacemaker activity, neuroendocrine, endocrine and exocrine secretion.[98-100] The concentration of intracellular Ca^{2+} determines many cell activities in a variety of cell types. The Ca^{2+} concentration rises in response to many extracellular stimuli such as membrane depolarisation, neurotransmitters and hormone binding to receptors in the cell membrane. Stimulation can trigger opening of calcium channels in the cell membrane, or it may generate intracellular messengers such as diacylglycerol (DAG) and inositol triphosphate (IP_3). These effects cause calcium channels to open, or the release of intracellularly stored Ca^{2+}. The open channels then allow Ca^{2+} to move down its electrochemical gradient into the cell. Ca^{2+} may also be released from the sarcoplasmic reticulum storage sites by the action of second messengers such as IP_3.[101,102] The resulting elevation of Ca^{2+} concentration initiates a chain of reactions (phosphorylation-

dephosphorylation), culminating in a physiological response. Termination of the signal occurs, at least in part, when the Ca^{2+} concentration returns to homeostatic (resting) levels. A variety of mechanisms, including calcium resequestration in sarcoplasmic reticulum and extrusion to the extracellular space are also operative.

At rest, the intracellular concentration of free Ca^{2+} is about 10^{-8} M, compared to an extracellular concentration of approximately 10^{-5} to 10^{-4} M, a 1,000 to 10,000 fold difference.[98] The resting state is thus a dynamic equilibrium in which the tendency of Ca^{2+} to move into cells is held in check by mechanisms which extrude Ca^{2+} from the cells (*eg* Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchange). Therefore, Ca^{2+} acts as a final common messenger for a number of diverse signals, and is accurately controlled to ensure proper interpretation of the signal in the cell. Failure of the regulatory processes lead to pathological conditions involving altered Ca^{2+} homeostasis.

In cardiac muscle, the Ca^{2+} concentration fluctuates on a beat-to-beat basis. The cycle lasts from several milliseconds to greater than a second depending on heart rate and the animal species. The actual mechanism has not been fully elucidated. However, both Ca^{2+} influx from extracellular space, as a result of membrane depolarisation, and release from sarcoplasmic reticulum are involved. Ca^{2+} released from the sarcoplasmic reticulum may play a greater role in mediating contraction on a beat-to-beat basis.

In pathological conditions involving smooth muscle contractility, impairment of transmembrane Ca^{2+} movements result in the tissues' inability to maintain the Ca^{2+} gradient at rest, or to return the concentration to resting levels after cell activation. The consequence of this is increased intracellular Ca^{2+}, resulting in increased tone and/or spasm. Abnormalities in Ca^{2+} homeostasis have been postulated as primary pathogenic factors in hypertension. The strongest arguments for the role of altered Ca^{2+} regulation as the central pathophysiological mechanism underlying cardiovascular disease have been proposed by Hermsmeyer.[103] Hermsmeyer stated that calcium channel antagonists decrease total peripheral resistance and also normalize blood pressure. The fact that hypertension is not always associated with an elevated intracellular Na^{+} concentration provides strong evidence for the role of Ca^{2+} as an important cation in hypertension.

Similar disturbances of calcium homeostasis are observed in pathological conditions of the heart and other organs and tissues where Ca^{2+} is required to mediate intracellular activities. Uncontrolled hypertension has a devastating effect on heart and kidney, and its etiology is associated with Ca^{2+} regulation.[104-106]

1.3.2.0 Nature and Types of Calcium Channels

The existence of at least four different types of calcium channels has been proposed to explain transmembrane cal-

cium movements in smooth muscle cells.[107] These channels have been designated as "Leak", "Stretch sensitive", "Receptor-operated" and "Voltage-gated" calcium channels. Of these, the voltage-gated calcium channels are the most important in controlling transmembrane movement of Ca^{2+}.

1.3.2.1 Voltage-Gated Calcium Channels

To date, at least four distinct sub-types of voltage-gated, or voltage-dependent, Ca^{2+} channels have been described that are defined as N, P, T and L calcium channels.[108] These channel sub-types are differentiated on the basis of their anatomical location, and their biophysical and pharmacological properties.[109] The N and P type channels are present in neurons, where they play an important part in controlling neurotransmitter release. T-type channels are widely distributed in many tissues and are thought to modulate pacemaker activity in areas such as the SA node.[110] L-type channels are present in a variety of excitable cells, but they are dominant in the cardiovascular system and certain endocrine cells. L-type channels mediate excitation-contraction coupling and hormone release, respectively.[111]

The L-type calcium channel is the best characterized in terms of its physiology and biochemistry. This calcium channel receptor has been found to consist of a heteromeric assembly of five proteins: alpha$_1$(α_1), alpha$_2$(α_2), beta(β), gamma(γ) and delta(δ), as shown in Fig. 1[112] on the following page.

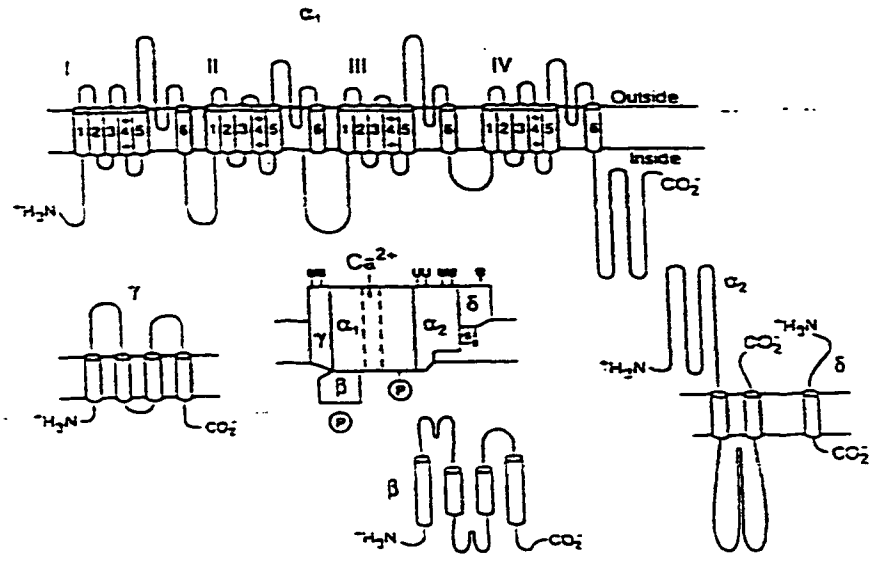


Figure 1. Subunit Compostion of the L-type Ca2+ Channel. From Rampe and Triggle, Progress in Drug Research, 1993, vol. 40, p. 197.

The δ-subunit is disulphide bonded to the α_2-subunit, while the γ-subunit may only be found in skeletal muscle. The α_1, δ and β-subunits likely play a role in mediating channel expression and kinetics.[113] The α_1-subunit is the major pore-forming protein of the channel, and it alone is capable of forming a functional Ca^{2+} channel. The α_1-subunit consists of four domains, each containing six putative membrane spanning regions S1-S6. The S4 region is thought to be the voltage sensor for the channel, while the loop between S5 and S6 forms at least a portion of the channel pore. The α_1-subunit of the L-type Ca^{2+} channel is found to contain high affinity binding sites for calcium channel modulators.[114] It has been confirmed, using photolabelling antibody mapping techniques, that separate receptor sites for the 1,4-dihy-

dropyridine,[115,116] phenylalkylamine[117], and benzothiazepine[118] classes of compounds reside on the α_1-subunit.

1.3.3.0 STRUCTURE-ACTIVITY RELATIONSHIPS OF 1,4-DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS

Loev and co-workers[119] were the first to report the systematic pharmacological characterisation of a variety of "Hantzsch-type" dihydropyridine compounds as hypotensive agents. Later, Rodenkirchen *et al*[120] identified structure-activity relationships of nifedipine analogues in isolated canine cardiac muscle. Both studies described in detail the effects which various functional groups attached to the dihydropyridine nucleus had on pharmacological activity. Further studies have provided information regarding which structural features confer agonist and antagonist activity.[121-123]

The X-ray crystal structures for a number of 1,4-dihydropyridine Ca^{2+} agonists and antagonists have been determined.[122] For antagonists, the dihydropyridine ring generally assumes a flat boat conformation, which places the aryl substituent at C(4) in a pseudoaxial orientation with respect to the 1,4-DHP ring system. The plane of the aromatic ring is coplanar with N(1)-C(4) symmetry plane of the dihydropyridine ring. In nautical terms, the pseudoaxial aryl substituent is often referred to as the *bowsprit* with the other substituents being located on either the *port* (left) or *starboard* (right) side of the dihydropyridine ring system (see

Figure 2). The carboxylic acid ester substituents present on the *port* and *starboard* sides of the dihydropyridine ring are in an equatorial-like orientation with respect to the C(4) substituents.

The ester substituents normally adopt an *s-cis/s-trans* or *s-cis/s-cis* arrangement, relative to the double bonds of the dihydropyridine ring. Molecular Orbital Program Calculations (MOPAC) suggest that both carboxylic esters present in calcium channel agonists are oriented in a plane intersecting the plane of the dihydropyridine ring at an angle between 30-60^0.[122] For good antagonist activity, it has been suggested that the carboxylic ester on the *port* side should be in the *s-cis* orientation.[122-124] In most cases,

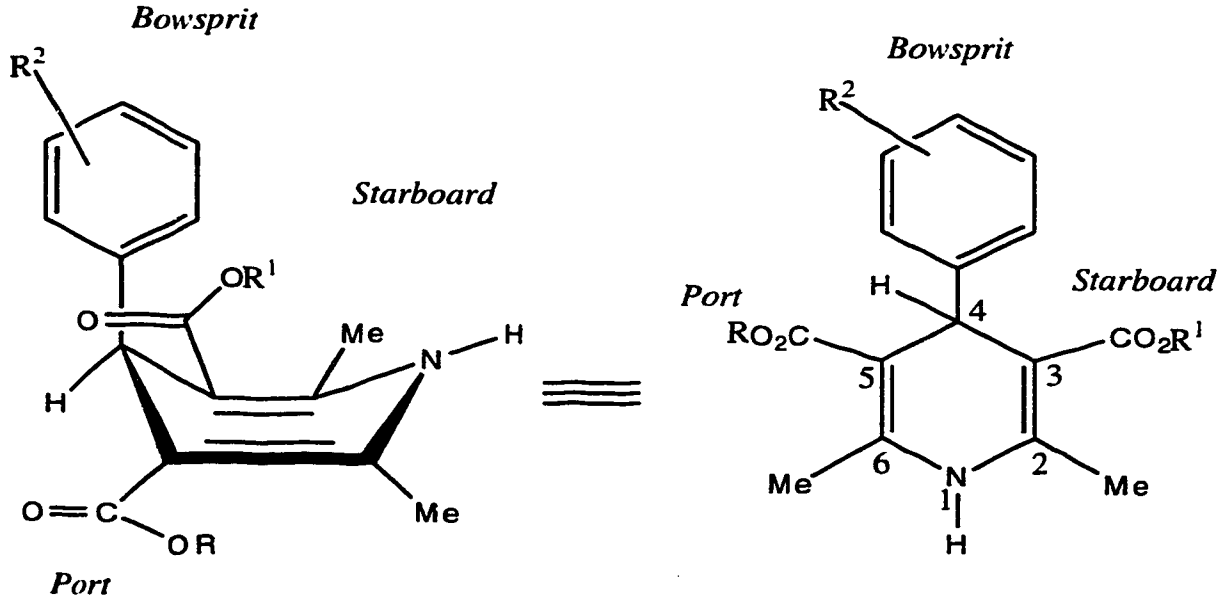


Figure 2. General Conformation of 1,4-Dihydropyridine Calcium Channel Antagonists.

the substituents located at either the *ortho*- or *meta*-position on the C-4 aryl ring are oriented synperiplanar (sp) to the hydrogen atom at C(4) of the dihydropyridine ring system.

Studies have also shown that the nature and size of the substituents on the 1,4-dihydropyridine ring influence potency. These structure-activity relationships (SARs) are summarized as follows:

a) The boat-shaped 1,4-dihydropyridine (1,4-DHP) ring system in which the N(1) nitrogen atom is a secondary amine is an important requirement since it likely hydrogen bonds to the calcium channel receptor. Oxidation of the 1,4-DHP ring to a planar pyridyl ring results in reduced pharmacological activity as a calcium channel antagonist.

b) Increasing the size of substituents at the C(4)-position of the 1,4-DHP ring in the order hydrogen, methyl, ethyl, butyl, cycloalkyl, heterocyclic and phenyl resulted in an increase in calcium channel antagonist (CCA) activity.

c) For substituted C(4) phenyl analogs of nifedipine, *ortho*-substituents generally provided a greater potency than the corresponding *meta*-substituents. The *para*-substituted analogs were the least active. This is most likely because substituents at the *ortho*- and *meta*-positions are involved in greater non-bonded steric interactions with groups at the C(3)- and C(5)-positions. This contributes to maintaining the flat boat-shaped 1,4-DHP ring, for effective binding to

the receptor. The *para*-substituent is too far removed to participate in these non-bonded interactions.

d) Ester substituents at C(3) and C(5) of the 1,4-DHP ring system conferred optimal CCA activity. The absence of a substituent, or the presence of an electron-withdrawing group such as cyano or carbonyl at these positions decreased activity. The ester group possesses a partial negative charge on the oxygen atom, that is likely involved in hydrogen bonding to the receptor site.

e) A chiral C(4) carbon, due to unsymmetric C(3) and C(5) ester substituents, usually increases CCA activity and the resultant enantiomers often exhibited stereoselective antagonist effects.

Several studies by Knaus *et al*,[125-131] involving C(4) pyridinyl groups have observed similar conformational preferences. A C(4)-pyridinyl substituent is bioisosteric with a 4-(nitrophenyl) substituent on a 1,4-DHP ring, where *ortho*-, *meta*-, and *para*-nitrophenyl are bicisosteric with 2-, 3-, and 4-pyridyl, respectively.[129] These authors also observed similar correlations between the nature and size of ester groups at C(3) and C(5) positions of the 1,4-DHP ring with respect to CCA properties.

Conformational preferences are also observed for the 1,4-DHP calcium channel agonist Bay K8644 (Figure 3). The dihydropyridine ring again adopts a flat boat conformation, with the aryl or heteroaryl ring at the C(4) position assuming a pseudoaxial position which is coplanar with the

N(1)-C(4) plane. Generally, *ortho* and *meta* substituents on the C(4) aryl or heteroaryl ring are also synperiplanar to the hydrogen atom at C(4). The carboxylic ester at C(3) may be in either a s-*cis* or s-*trans* orientation. An ester group, or a nitro group at the C(5) position as in Bay K8644, is coplanar with the dihydropyridine ring. That is, the nitro group intersects the plane of the DHP ring at an angle of 0°.

All CC agonists that have been reported are chiral at C(4), due to unsymmetrical substitution at the C(3) and C(5) positions of the DHP ring. In instances where the enantiomers have been separated (Bay K8644), the enantiomers exhibit different pharmacological profiles.[67] Most CCags that have been reported have one of the ester groups at the C(3) or C(5) position on the 1,4-DHP replaced by an electron-

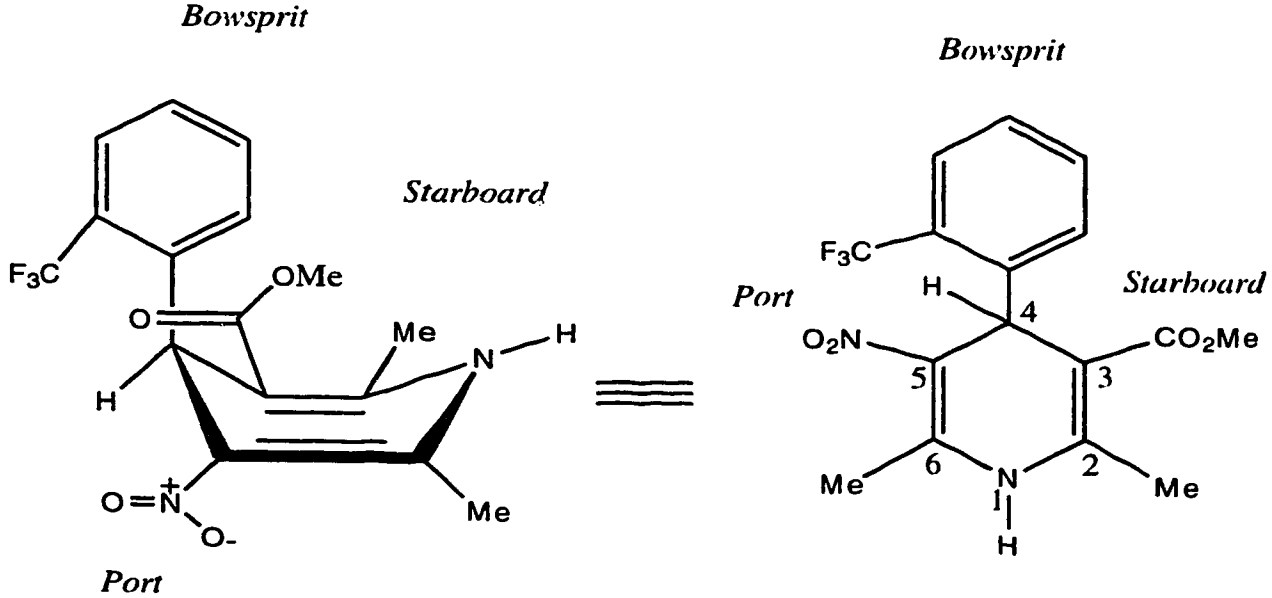


Figure 3. Approximate Conformation of Bay K8644.

withdrawing group such as NO_2, lactone, thiolactone or simply a hydrogen substituent. It has been observed that the enantiomer having the agonist activity has the electron withdrawing group on the *port* side of the DHP ring. In contrast, enantiomers having these electron-withdrawing groups on the *starboard* side of the DHP ring are generally weak antagonists. These observations have led to the speculation that dihydropyridine CCags place a negative charge on the *port* side of the DHP ring in the vicinity of the aromatic ring.[121] This negative charge may influence binding to the receptor site and hence the pharmacological effect. This postulate accommodates agonists containing nitro, lactone and thiolactone functionalities, but does not account for agonists possessing a hydrogen at C(5) of the DHP ring. For compounds possessing hydrogen at C(5) of the DHP ring, potential energy calculations suggest that a negative potential is created on the *port* side of the DHP ring, due to the unshielded electrons of the aromatic ring. However, weak agonists such as YC-170 (**3c**) and AK-2-38 (**3e**) are not explained by these postulates.

A recent study by Rovnyak *et al*[132] proposes a new model to explain the structural and conformational requirements for modulation of calcium channels by 1,4-DHPs. They prepared dihydropyrimidine DHP mimics, with only one ester group, (the second ester or electron-withdrawing group or hydrogen, being replaced by a ring nitrogen) and compared their biological activities with analogous 1,4-DHPs. From the results obtained, they proposed a normal versus capsized DHP boat

model to explain the structural and conformational requirements for modulation of calcium channel function. An obligatory left-hand side alkoxy *cis*-carbonyl interaction is required for maximal DHP receptor affinity. The effect on channel function is determined by the orientation of the 4-aryl group. Enantiomers that have an *up-oriented* pseudoaxial aryl group (normal DHP boat) elicit calcium antagonist activity, whereas enantiomers having a *down-oriented* pseudoaxial aryl group (capsized DHP boat) exhibit calcium agonist activity (Figure 4). This model is consistent with and provides a rational explanation of previously reported data in this area, particularly the observation of drastic decrease in potency upon replacement of an ester group by hydrogen in the Bay K8644 series.

The mechanism whereby 1,4-DHP agonists enhance L-channel activity have been studied in detail using the single cell patch clamp electrophysiology technique. The most obvious stimulatory effect observed using whole cells include increases in current amplitude and prolongation of tail current decay.[133] These effects are due in part to the ability of these drugs to dramatically prolong the mean open time of single Ca^{2+} channels. Under control conditions, open time *tau* values approximate 1 msec, but following saturating doses of DHP activators, prolonged open time of 10-20 msec were observed.[133,135]

Radiolabelled 1,4-DHP antagonists have been used extensively to probe the dihydropyridine binding site on L-type

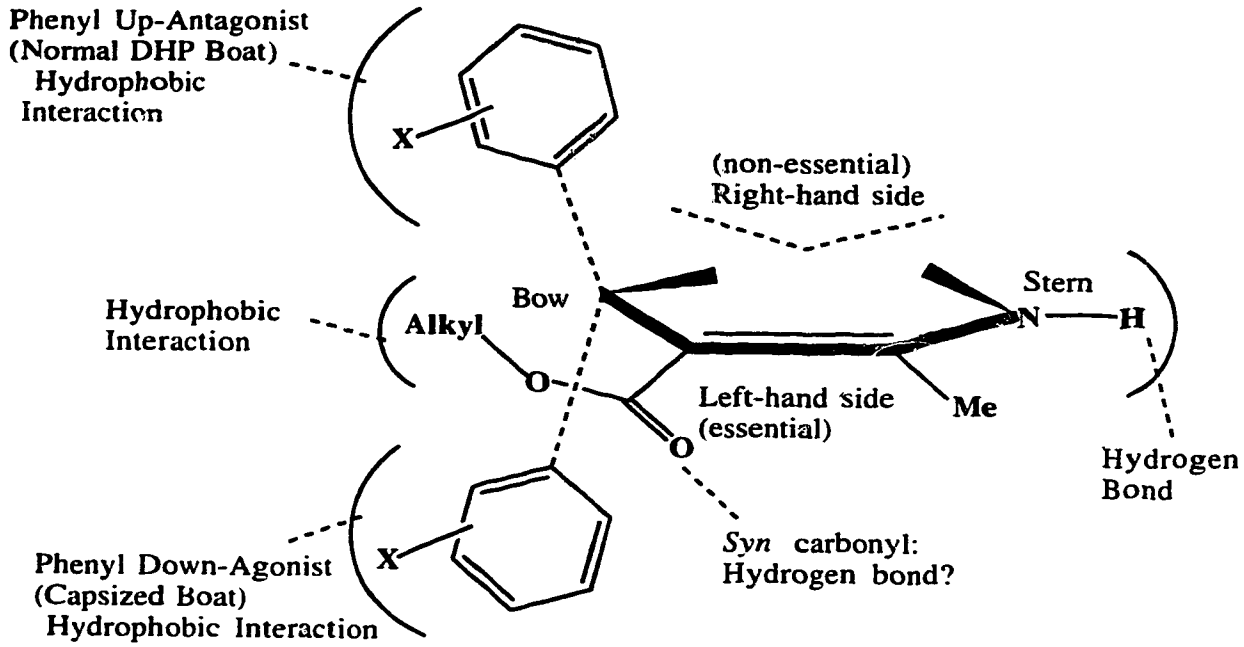


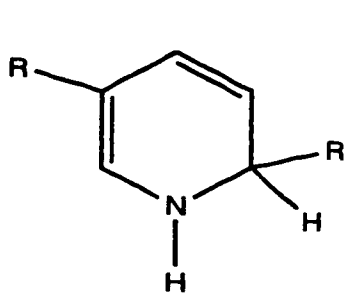
Figure 4. Schematic diagram of essential left-hand side alkoxycarbonyl for both calcium antagonists (phenyl up in normal DHP boat) and calcium agonists (phenyl down in capsized boat). Right-hand side interactions are nonessential. Right and left are defined by viewing the DHP ring from N1 (stern) toward C4 (bow).

Ca^{2+} channels. These compounds are found to bind to a single high affinity site on the L-channel near the pore region.[115,136] The close structural similarity between DHP antagonists and agonists led to an earlier suggestion that they share a common binding site on the L-channel.[137,138] However, radioligand binding studies in intact cells have suggested the existence of separate binding sites for activator and antagonist molecules.[134] Most recently, Tang *et al*[139]

obtained evidence for a distinct agonist binding site at the level of the channel protein by constructing chimeric calcium channels in which portions of a DHP insensitive calcium channel were introduced into the cardiac L-type channel. One of these chimeras (termed CB C7) responded normally to DHP antagonists but virtually lost its ability to be stimulated by various activators. This led to the conclusion that the two classes of drugs recognize separate binding sites. Further studies are necessary to define the site of action for both 1,4-DHP activators and antagonists.

1.4.0.0 THE CHEMISTRY OF DIHYDROPYDINES

A number of reviews have been published describing the synthesis, structural elucidation, physical and chemical properties of 1,4-dihydropyridines.[140-144] The most common dihydropyridines are the 1,6-(**12**), 1,2-(**13**) and 1,4-(**14**) isomers. Of these three isomers, 1,4-dihydropyridyl isomers are thermodynamically the most stable.[145] Molecular orbital calculations indicate that the nitrogen lone electron pair and the electrons of the two olefinic bonds for 1,4-dihydropyridines are delocalized. Thus, electron-withdrawing substituents at the C(3) and/or C(5) positions, that are conjugated to the enamine moiety, enhance stability due to electron delocalisation. In contrast electron-donating groups destabilize the dihydropyridine ring system.[148]



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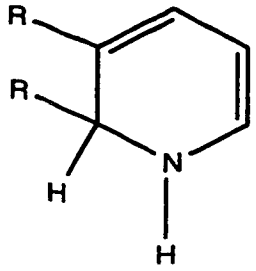

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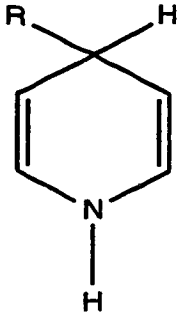

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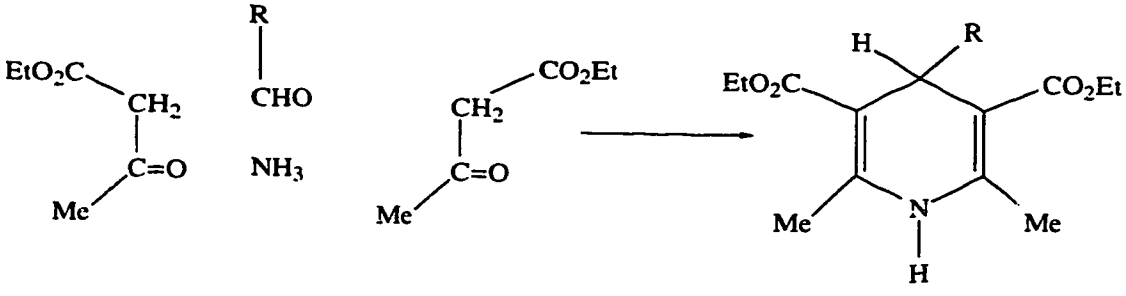
1.4.1.0 Synthesis Of 1,4-Dihydropyridines Using Hantzsch and Modified Hantzsch Syntheses

Derivatives of 1,4-dihydropyridines are most often prepared by cyclocondensation reactions, particularly by various modifications of the Hantzsch synthesis. Some of the synthetic approaches to monocyclic 1,4-dihydropyridines are discussed below.

1.4.1.1 Hantzsch Synthesis

The original Hantzsch dihydropyridine synthesis involved the reaction of ethyl acetoacetate with an aldehyde and ammonia to give product (**15**), as shown below.



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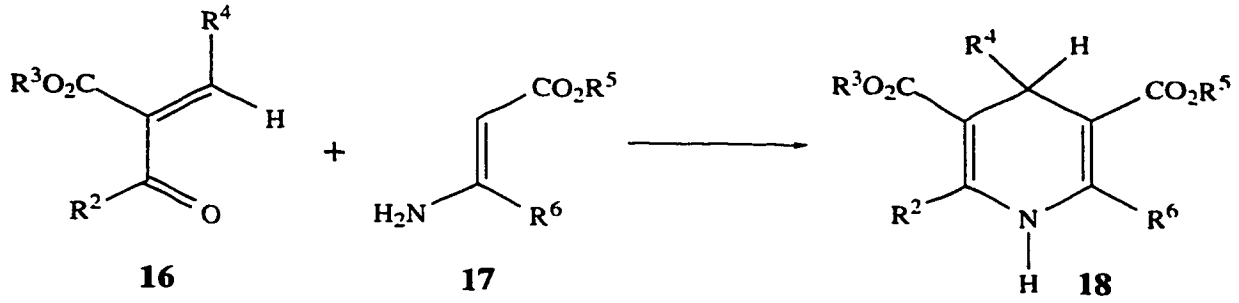
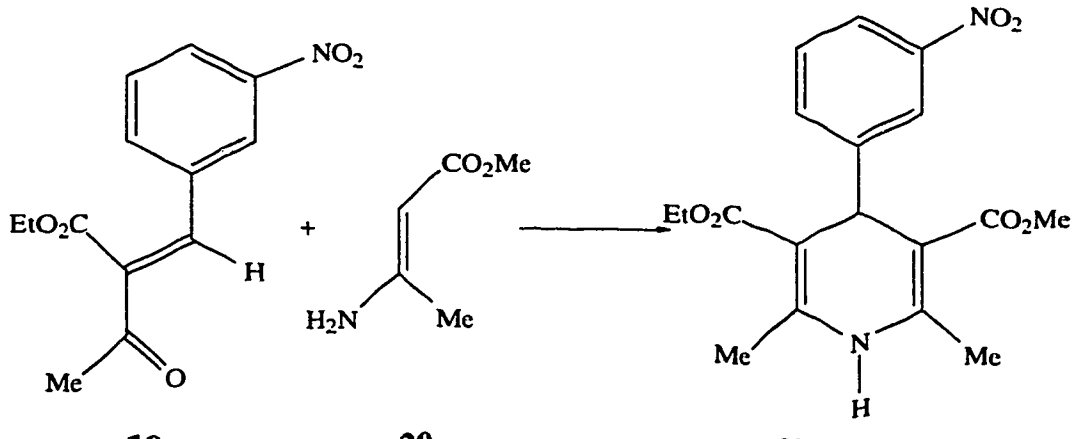
1.4.1.2 Synthesis of 1,4-Dihydropyridines using Enamines

Modification of the classical Hantzsch synthesis enables a variety of 1,4-dihydropyridines to be synthesized. A typical method involves the condensation of an appropriately functionalized Knoevenagel adduct (**16**) with an enamine (**17**)[143] to give the 1,4-dihydropyridine (**18**) as illustrated in the scheme below.



16 **17** **18**

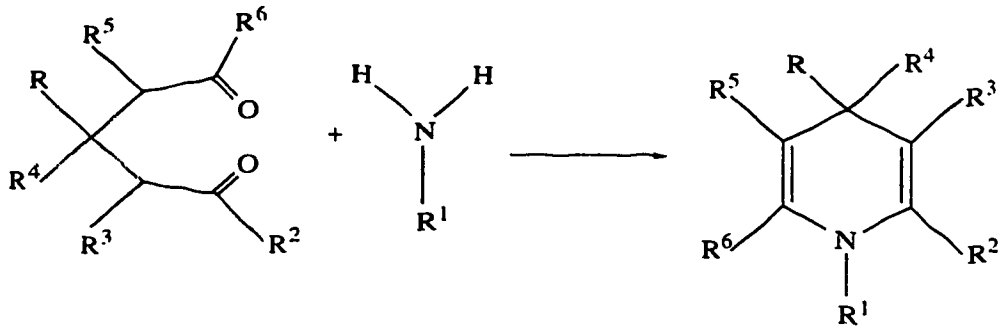
For example, the synthesis of nitrendipine (**2b**) can be accomplished by the condensation of the Knoevanagel adduct (**19**) with methyl 3-aminocrotanate (**20**).



19 **20** **2b**

1.4.1.3 N-Substituted-1,4-Dihydropyridine Synthesis

The synthesis of 1-substituted-1,4-dihydropyridines(**23**) can be accomplished by cyclization of 1,5-dicarbonyl compounds (**21**) using ammonium acetate, amides, hydrazines or amines (**22**) as illustrated below.

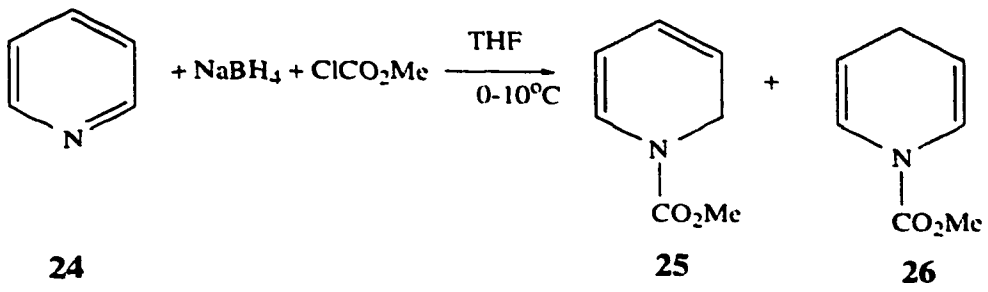


21 **22** **23**

Various other Hantzsch modifications are well documented in a review by Sausins and Duburs.[143]

1.4.2.0 Synthesis of Dihydropyridines by the Sodium Borohydride Reduction of Pyridinium Salts

A variety of dihydropyridine compounds have been employed as precursors for the synthesis of nitrogen-containing compounds and natural products.[146] One of the most useful approaches to prepare these synthons is a reaction developed by Fowler.[147] Thus, the sodium borohydride reduction of a N-alkoxycarbonylpyridinium salt, that is formed *in situ* by reaction of a pyridine with a chloroformate ester, provides a useful one-pot reaction to prepare 1,2- and 1,4-dihydropyridyl isomers.



24 25 26

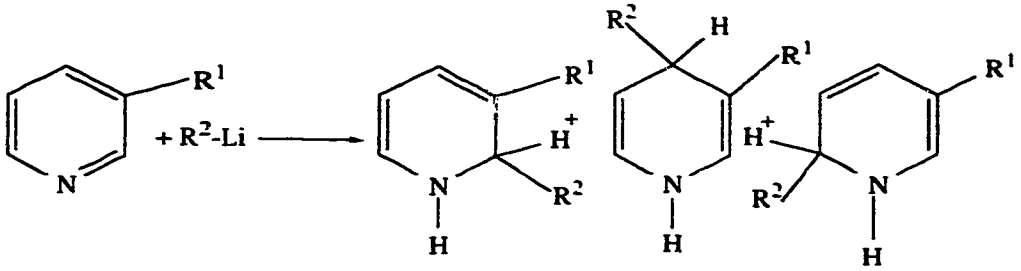
Substituent effects, and the nature of the reducing agent, on the regioselective reduction of 3-substituted-N-alkoxycarbonylpyridinium salts has been studied by Sundberg *et al*[148] They observed that electron-donating substituents exhibited regiochemical control to the extent of 90% or more, favoring hydride addition at the 2-position. More bulky substituents such as trimethylsilyl also favored 1,2-reduction, but to a lesser degree. Acceptor substituents such as carbomethoxy and cyano, were not regioselective under similar reaction conditions. Generally, steric effects due to the reducing agent had little or no regio-directive effect. Abramovitch and co-workers[149] have used London (attractive) forces to explain an *ortho* directing effect observed upon addition of organolithium reagents to 3-alkylpyridines. Knaus and Redda[150] studied the effect of temperature on the sodium borohydride reduction of pyridinium salts. It was found that lowering the temperature of the reaction to -65°C afforded predominantly the 1,2-dihydropyridine isomer with the ratio 1,2:1,4-isomers being 8:1. Reaction at 25°C gave a 1,4-:1,2-DHP ratio of 5:4. Reduction of pyridinium salts

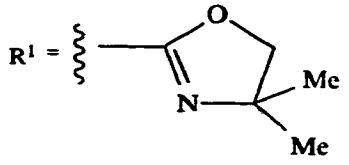
with sodium dithionite,[151] or a copper hydride complex reagent,[152] yields the 1,4-dihydropyridine product.

1.4.3.0 Synthesis of Dihydropyridines Using Nucleophilic Organometallic Reagents

The use of organometallic reagents to introduce substituents directly onto the pyridine ring have been studied extensively.[140,141,146] Several groups such as those of Knaus[153-156] Akiba[157], Piers[158], and Comins[159] have reported the successful regioselective introduction of alkyl or aryl substituents at the 4-position of the pyridine ring. The regioselectivity of these reactions has been found to be dependent on the nature of the organometallic reagent.

The effect of solvent, temperature and the nature of the organolithium reagent on the regioselective nucleophilic addition of organolithium reagents such as phenyl-, n-butyl- and methyllithium to 3-(4,4-dimethyloxazolin-2-yl)pyridine (**27**) has been reported by Knaus and Dubey,[156] and Hauck and Giam.[160] It was noted that low temperatures (-78 to 0°C) increased the yield of the 1,4-isomer, and that less polar solvents such as ether gave higher yields of C(2) (**28**) and C(6) (**30**) substituted products. However, the use of tetrahydrofuran as solvent provided higher yields of 1,4-dihydropyridine products (**29**). Phenyllithium was more regioselective than n-butyl- or methyllithium since the former reagent afforded only 1,2- (**28**) and 1,4-(**29**) dihydropyridines, whereas the latter reagents afforded all three isomeric products (**28-30**).


R1
+ R2-Li
N
H+
R2
H
27
28
29
30


R1 =
O
N
Me
Me

2.0.0.0 OBJECTIVES OF RESEARCH

Cardiovascular disease is one of the leading causes of death in the world today.[1] Several classes of drugs have been developed to treat cardiovascular disorders, which include the 1,4-dihydropyridine (1,4-DHP) calcium channel modulators.

One type of cardiovascular disease that affects about 4 million Americans, and for which the two-year survival statistic is less than 30%, is congestive heart failure (CHF).[10] Most drugs currently used to treat CHF, such as β-agonists, cardiac glycosides, and phosphodiesterase III inhibitors have serious side-effects and limitations. Also, calcium channel agonists which could be effective in treating CHF are not used clinically because of their vasoconstrictive effects on smooth muscle. To quote a recent review, "No CC activator has been reported that is cardioselective, that is able to increase myocardial contractility without also increasing vascular resistance and blood pressure".[74] The future clinical use of calcium agonists to treat CHF will require the separation of their vasoconstricting effects from their cardiostimulant properties.[76]

The major objective of my research was to design, synthesize and pharmacologically evaluate 1,4-DHPs that could exhibit dual CC cardioselective agonist/smooth muscle selective antagonist properties (that is, positive inotropes that decrease afterload). Compounds possessing these dual properties would be very effective for the treatment of

congestive heart failure. A novel class of compounds, alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates was selected for investigation where the 5-nitro group of Bay K8644, or the ester group of nifedipine at the 5-position of the 1,4-DHP ring, was replaced by a 4,5-dihydro-4,4-dimethyloxazolin-2-yl group. It was envisioned that the oxazolinyl group could act as an isostere of the ester and/or nitro groups to conceivably exhibit the dual agonist-antagonist tissue selectivity described.

A second class of compounds investigated was dialkyl 1,4-dihydro-2,6-dimethyl-4-[(*Z*)-N-oxo-N-(aryl/vinylphenylmethylene)-λ^5-azanyl]phenyl-3,5-pyridinedicarboxylates. In an earlier study, Knaus and co-workers[75] synthesized the diesters, (+) and (-) AK-2-38 [3-isopropyl 5-(4-methylphenethyl) 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylates] that exhibited dual cardioselective CC partial agonist/smooth muscle selective CC antagonist properties. This was a significant discovery, since AK-2-38 was the first and the only 1,4-DHP diester reported to exhibit a CC agonist effect on heart and CC antagonist effect on vascular smooth muscle. This provided the rationale for investigating other diesters. In another study, Knaus et al.[161] found that the position of the heteroaryl nitrogen atom and / or electron density at the 1-position of 1-(5-quinolyloxy or isoquinolyloxy)-alkylamino-2-propanols was a determinant of β_1/β_2 selectivity. The quinolyl compounds were found to be

ten-fold more active as β_1- adrenergic antagonists than the corresponding isoquinolyl isomers. This result was consistent with the postulate put forward by Testa *et al*[162] that, β_1 selectivity was enhanced by the presence of a negative minimum located at the *meta*-position or somewhat beyond, and at the *para*-position of the phenyl ring of phenoxypropanolamines. A series of isopropyl 5-nitro-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates were also investigated by Knaus and co-workers.[163] The 2-pyridinyl isomer acted as a dual cardioselective CC agonist/smooth muscle selective CC antagonist, while the 3-pyridinyl and 4-pyridinyl isomers acted as CC agonists on both heart and vascular smooth muscle. 4-Arylnitrone 1,4-DHPs were therefore selected for investigation based on the electronic properties of the nitrone group. The nitrone group, which is highly charged could provide the negative minimum necessary for cardioselectivity.[162] The 4-arylnitrone class of 1,4-DHP may therefore exhibit the desired dual CC cardioselective agonist/smooth muscle antagonist properties needed for ideal drugs for the treatment of CHF. These studies would also provide useful structure-activity relationships (SARs). Compounds exhibiting dual CC cardioselective/smooth muscle selective CC antagonist activities would be ideal candidates to gain information pertinent to the nature of the drug-CC receptor interaction and the nature of the CC receptor binding site.

Other 1,4-DHPs possessing 4-(quinolinyl) and 4-(1-oxido-4-pyridyl) substituents were also investigated to obtain further SAR correlations for the 1,4-DHP calcium channel antagonist class of compounds.

3.0.0.0. RESULTS AND DISCUSSION

3.1.0.0. SYNTHESES

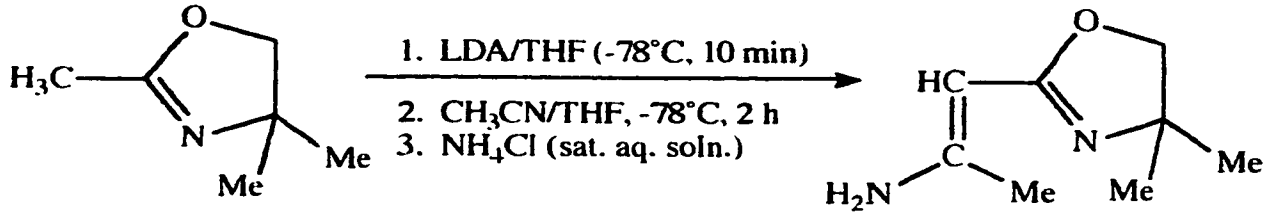
3.1.1.0. Synthesis of Alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates (38a-38f)

The clinical success of the calcium channel antagonist nifedipine (**1**) as an antihypertensive agent generated interest in the synthesis of other second generation unsymmetrical 1,4-dihydropyridines. The subsequent discovery that Bay K8644 (**3a**) exhibited calcium channel agonist activity prompted the synthesis of many nitro analogs (Section 1.2.1.1). However, none of these calcium channel agonists is useful clinically, due to their vasoconstrictive effects on vascular smooth muscle. In the alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates, the 5-nitro group of Bay K8644, or the 5-methoxycarbonyl group of nifedipine on the 1,4-DHP ring, has been replaced by a 4,5-dihydro-4,4-dimethyloxazolin-2-yl ring system. The C(4)-2-(trifluoromethylphenyl) substituent of Bay K8644 and the C(4)-2-(nitrophenyl) substituent of nifedipine were also replaced by a C(4)-pyridinyl substituent. The oxazolinyl group was expected to act as an isostere of these ester and/or nitro groups, with the hope that these new 1,4-DHP analogs would exhibit dual CC cardioselective agonist/smooth muscle selective antagonist properties. Such compounds would be ideal drugs for the

treatment of congestive heart failure. There is precedence for this dual activity since Knaus *et al*[163] have shown that isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(pyridinyl)-pyridine-5-carboxylate racemates and enantiomers had such pharmacological profiles. Determination of their *in vitro* calcium channel-modulating activities using guinea pig ileal longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA) assays showed that the (±)-2-pyridinyl isomer acted as a dual cardioselective calcium channel agonist (GPLA)/smooth muscle selective calcium channel antagonist (GPILSM). In contrast, the (±)-3-pyridinyl and (±)-4-pyridinyl isomers acted as calcium channel agonists on both GPLA and GPILSM. *In vitro* studies also showed that the (+)-2-pyridinyl enantiomer exhibited agonist activity on both GPILSM and GPLA, but the (-)-2-pyridinyl enantiomer exhibited agonist activity on GPLA and antagonist activity on GPILSM.

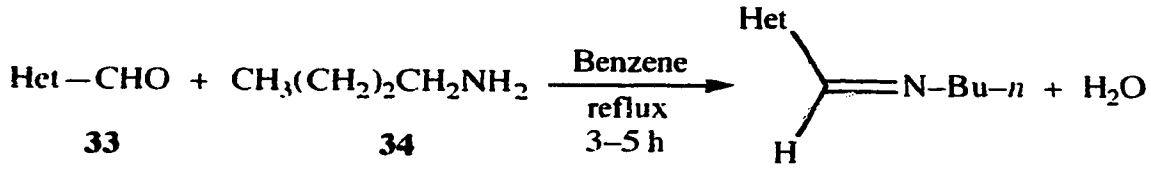
The syntheses of compounds **38a-38f** were accomplished using a modified Hantzsch reaction that involved the reaction of an enamine (**32**) with an appropriately functionalized Knoevanegal adduct (**37**).[164] Treatment of 2,4,4-trimethyl-4,5-dihydro-2-oxazoline (**31**) with freshly prepared lithium diisopropylamine (LDA) at -78°C, followed by the addition of acetonitrile at -78°C and quenching with a saturated solution of aqueous ammonium chloride afforded the enamine, 1-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)-1-propene-2-amine (**32**). The crude enamine (**32**) was purified by distillation under reduced pressure to afford a 12% yield (lit.[164],9%). The low yield

is presumably due to the presence of acidic α-protons in acetonitrile which can take part in transmetalation chemistry with the intermediate lithio base.[165]

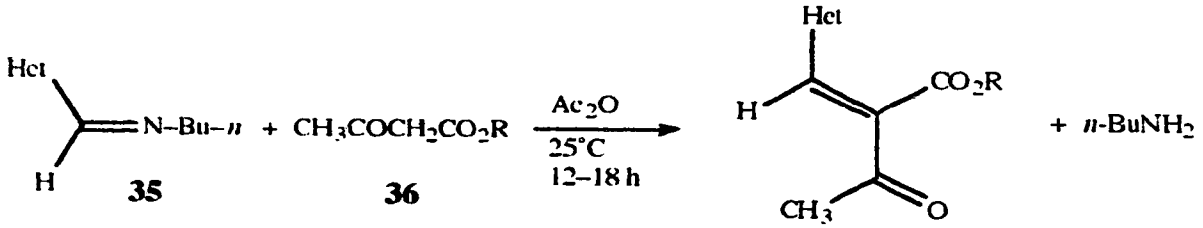


31 **32**

Azeotropic reflux of a mixture of a pyridinecarboxaldehyde and *n*-butylamine in benzene using a Dean-Stark apparatus gave quantitative yields of the imines (**35**). Reaction of these imines with acetoacetates in acetic anhydride as solvent at room temperature afforded the corresponding Knoevanegal adducts (**37**) in 65 to 85% yields.

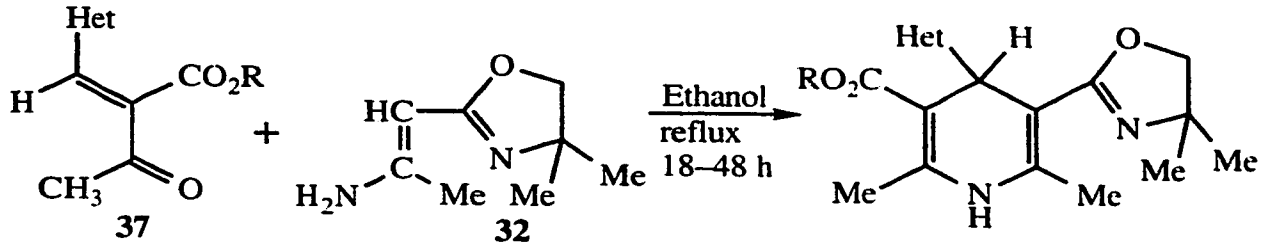


33 **34** Het = 2-pyridyl, 3-pyridyl, 4-pyridyl **35**



35 **36** **37**

The 1,4-dihydropyridines, **38a-38f** (except **38c**) were prepared by condensing a 2-alkoxycarbonyl-1-(pyridinyl)-but-1-en-3-one(**37**) with the enamino oxazolinc (**32**) in ethanol. The crude products (**38a-38f**) were purified using silica gel column and preparative silica gel thin layer chromatography providing yields in the 51-80% range.



38

38a Het = 3-pyr
R = Me

38b Het = 4-pyr
R = Me

38c Het = 2-pyr
R = *i*-Pr

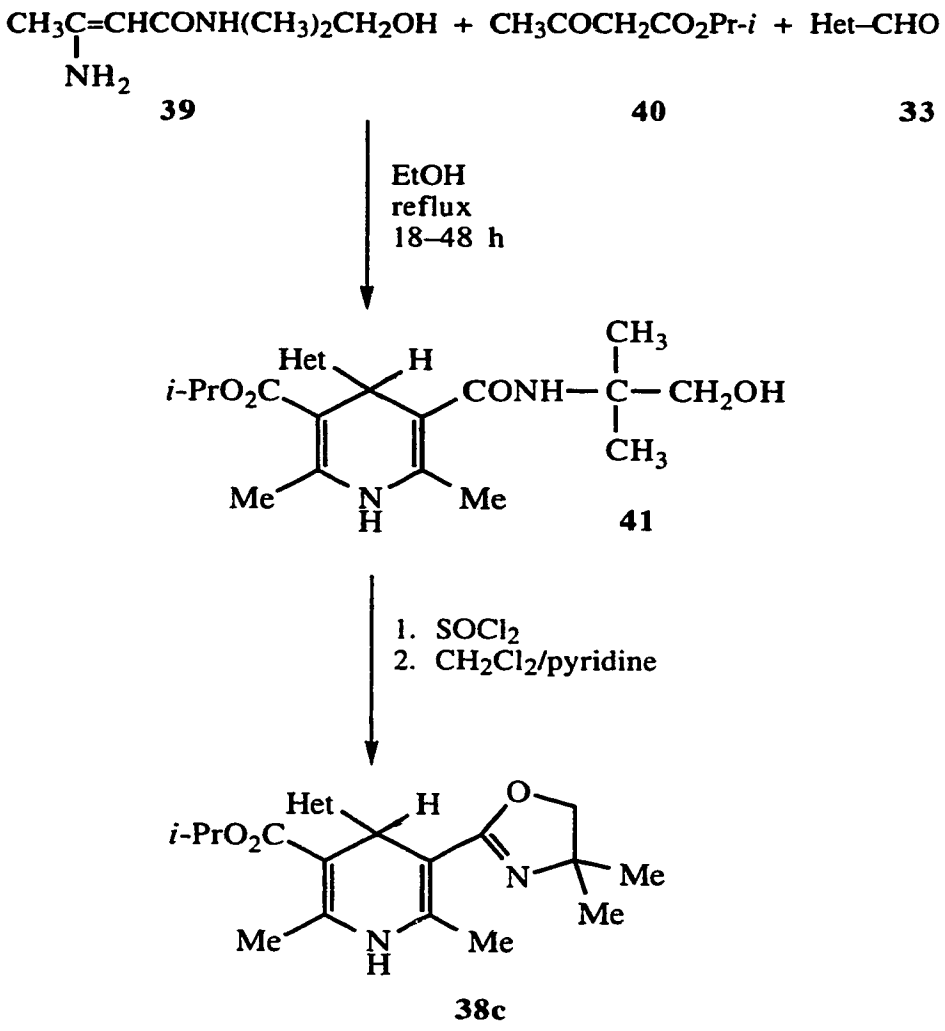
38d Het = 3-pyr
R = *i*-Pr

38e Het = 4-pyr
R = *i*-Pr

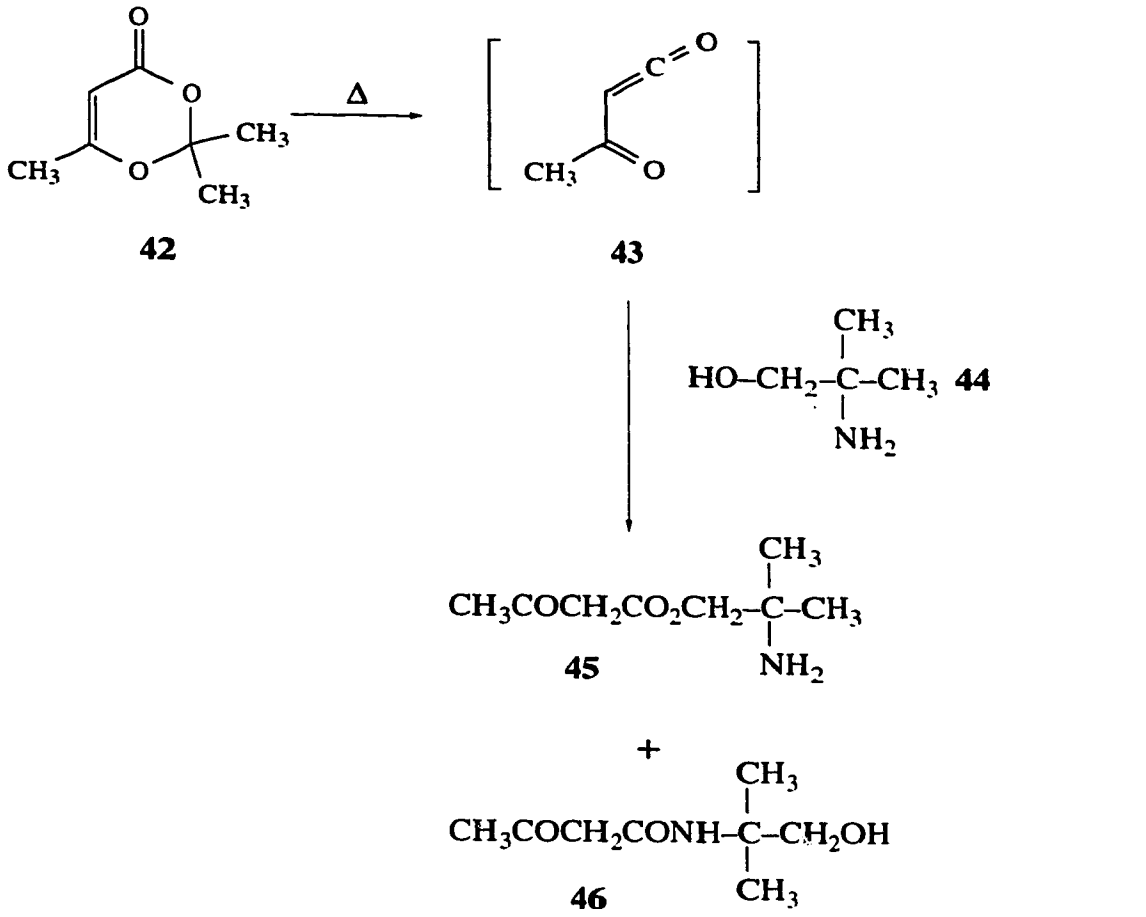
38f Het = 3-pyr
R = *i*-Bu

Earlier attempts were made to prepare this class of compounds (**38c-38e**) by the method illustrated in the following reaction scheme.

Compound **46** was prepared by heating 2,2,6-trimethyl-4H-1,3-dioxin-4-one (**42**) in the presence of 2-amino-2-methyl-1-propanol (**44**) in xylene using an oil bath maintained at about

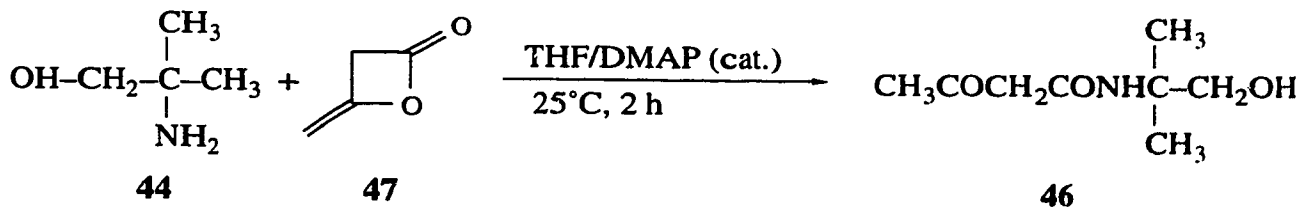


150°C. It has been reported that, at a temperature of about 100°C, 2,2,6-timethyl-4H-1,3-dioxin-3-one undergoes pyrolysis to give acetylketene (**43**), which in the presence of nucleophiles reacts to give the corresponding acetoacetylated adducts.[166-169] The major side-product was acetone which evaporated readily during the reaction. In this way, compound **46** was prepared in 60% yield. In addition, **45** (corresponding ester) was formed in about 15% yield. The crude product was purified by silica gel column chromato-



graphy and recrystallisation from ethyl acetate/hexane to obtain pure **46**.

Alternatively, the reaction of 2-amino-2-methyl-1-propanol (**44**) with diketene (**47**) in THF at room temperature in the presence of dimethylaminopyridine (DMAP)[170,171] as catalyst, afforded **46** exclusively in about 90% yield.



The Hantzsch reaction of the enamine (**39**), (obtained by passing ammonia gas through a solution of **45** in methanol), isopropyl acetoacetate (**40**) and pyridinecarboxaldehyde (**33**) in refluxing ethanol afforded 3-isopropyl 5-{N-[(1,1-dimethyl-2-hydroxyethyl)aminocarbonyl]}1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3-pyridinecarboxylate (**41**) in 30% yield; after purification by silica gel column chromatography and recrystallisation from ethyl acetate/hexane. The major side-product was the corresponding symmetrical 1,4-DHP diester which was formed in a yield of about 65%. The symmetrical isopropyl 1,4-DHP diester made up about 90% of the side products.

Several attempts were made to convert **41** to the final product (**38**) using various reagents to cyclize the 5-{[N-(1,1-dimethyl-2-hydroxyethyl)aminocarbonyl]} moiety to form the oxazolinyl ring system. The reaction did not proceed in the presence of tosyl chloride (ratio **41**:tosyl chloride = 1:1) for 1 hour, using pyridine as a solvent. Increasing the reaction time and/or the molar ratio of tosyl chloride resulted in decomposition products. In contrast, reaction of **41** with 1.5 equivalents of thionyl chloride with pyridine as solvent, a reaction time of between 1 to 2 hour(s) and purification by silica gel column and preparative thin layer chromatography, afforded **38c** in 54% yield. Only **38c** was prepared using this latter method, since the superior method described earlier, using the oxazolinyl enamine (**32**) and 2-alkoxycarbonyl-1-(pyridinyl)-but-1-ene-3-one (**37**) was found

to be more convenient, the products were easier to purify and the yields were generally higher.

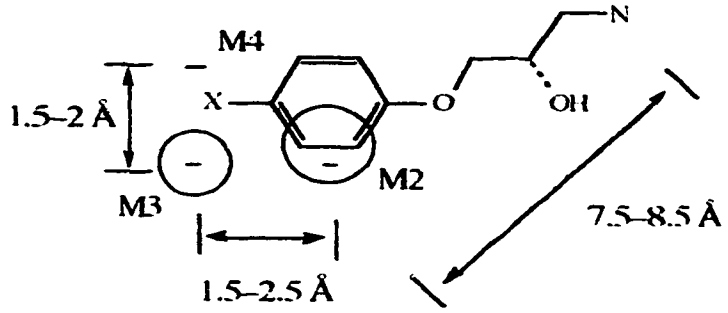
The spectral data (^{1}H nmr, IR and ^{13}C nmr) were consistent with the assigned structures. For example, the ^{1}H nmr spectrum for **38b** exhibited the following resonances : δ 8.42 (d, $J_{2,3} = J_{5,6}$ = 5 Hz, 2H, pyridyl H-2 and H-6), 7.23 (d, 2H, $J_{2,3} = J_{5,6}$ = 5 Hz, pyridyl H-3 and H-5), 5.84 (s, 1H, NH), 5.07 (s, 1H, 1,DHP H-4), 3.85 (q, J = 8 Hz, 2H, oxazolinyl H-5), 3.61 (s, 3H, CO_2Me), 2.23 and 2.37 (two s, 3H each, 1,4-DHP C-2 and C-6 Me), 1.20 and 1.23 (two s, 3H each, oxazolinyl C-4 Me). ^{1}H nmr, IR and ^{13}C nmr spectral data for **38a-38f** are given in the experimental section (Section 4). Compounds **38a–38f** are very stable at room temperature. The ^{1}H nmr of these compounds, taken after storage at room temperature for times up to one year showed no significant decomposition. Light had little or no effect on these oxazolinyl compounds. In contrast, some 1,4-DHPs such as nifedipine are sensitive to light, undergoing extensive decomposition and aromatization. However, these compounds were generally stored in the refigerator at about 0 °C. Compounds **38a-38f**, which possess a chiral centre at the C-4 position of the 1,4-dihydropyridine ring system, were tested as racemic mixtures. The pharmacological results (discussed in detail later) support the initial prediction that the oxazolinyl group acts as an isostere of both the methoxycarbonyl ester group of nifedipine and nitro group of Bay K8644.

3.1.2.0 Synthesis of Dialkyl 1,4-dihydro-2,6-dimethyl-4-{[(*Z*)-N-oxo-(aryl/vinylmethylene)-λ^5-azanyl]phenyl}-3,5-pyridinedicarboxylates (53a–i)

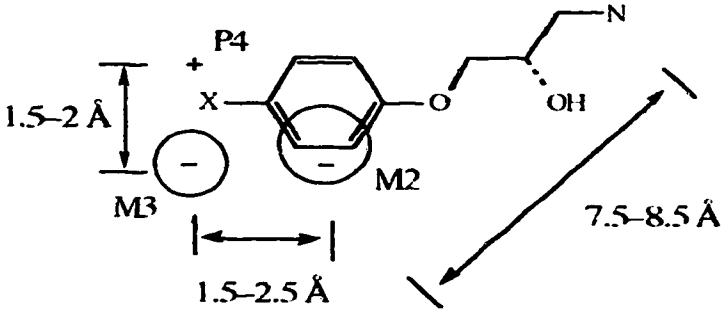
As mentioned previously (2.0.0.0), the discovery by Knaus and co-workers,[75] that the racemic diester and (+)- and (–)-enantiomers of AK-2-38 [3-isopropyl 5-(4-methylphenethyl) 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylate] exhibited dual cardioselective CC partial agonist/smooth muscle selective CC antagonist selectivities provided the rationale to synthesize and investigate other diesters. The (-)-enantiomer of AK-2-38 was 26-fold more potent than the (+)-enantiomer as a CC antagonist, while the (+)-enantiomer was a more potent cardiospecific partial CC agonist than the (-)-enantiomer. Studies by Testa *et al*,[162] involving the measurement of molecular electrostatic potentials (MEPs), determined the charge distribution necessary for β-adrenergic antagonist selectivity (β_1/β_2). In an aryloxypropanolamine (AOPA) class of β-adrenoceptor antagonists, a negative minimum (designated M2) was located between the *ortho*- and *meta*-positions of the aromatic ring in all compounds. A second negative minimum (designated M3) was located beyond the *meta*-position in all β_1-selective antagonists and in some nonselective and β_2-selective antagonists. An additional zone was present at the *para*-position of β_1-antagonists, which was positive (designated

P4) in full antagonists and negative (designated M4) in the antagonists with intrinsic sympathomimetic activity (ISA).

AOPAs
β_1-selective with ISA

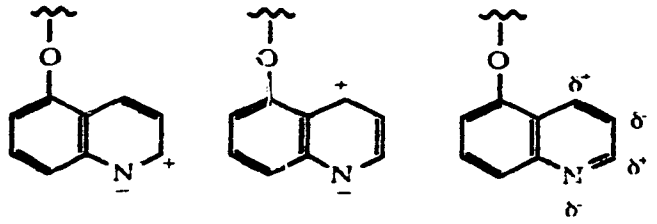


AOPAs
β_1-selective without ISA

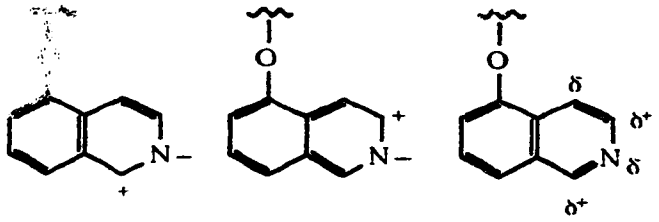


Further studies by Knaus *et al.*[161] on 1-(5-quinolyloxy)-3-alkylamino-2-propanols and 1-(5-isoquinolyloxy)-3-alkylamino-2-propanols provided further evidence on how charge distribution affects β-adrenergic antagonist activities. The quinolyl compounds were ten-fold more potent than the corresponding isoquinolyl analogs with respect to β_1-adrenergic antagonist activity. These results indicated that the position of the heteroaryl nitrogen atom and/or the electron-density at the 1-position of the heteroaryl ring, might be important determinants of β_1-adrenergic antagonist activity. The 1-quinolyl nitrogen atom is located at the same position

as the negative minimum (M3) at the *meta*-position of the aryloxypropanolamines.[162]



5-Quinolyloxy-

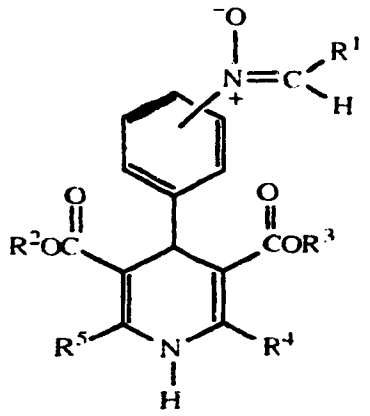


5-Isoquinolyloxy-

Furthermore, in the phosphodiesterase III inhibitor class of compounds that include amrinone (7) and milrinone (8), which exhibit positive inotropic and vasodilator effects, the nitrogen atom is located at the *para*-position of the heteroaryl ring (corresponding to the negative minimum, M3 in the aryloxypropanolamines). On the basis of these observations, it was expected that a highly charged nitrone group could provide a negative electrostatic potential or minimum that appears to be necessary for cardioselectivity. It was anticipated that a nitrone substituent attached at the 4-position of a C-4 phenyl ring in dialkyl 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylates may exhibit the desired dual CC cardioselective agonist/smooth muscle

selective antagonist properties required for calcium channel moulators that would be of value for the treatment of CHF.

In a U.S. Patent[172], nitrone-substituted-1,4-dihydropyridines of the general structure **48** were reported. These compounds were prepared by reducing the corresponding 4-(nitrophenyl)-1,4-DHP to the hydroxylamine using a zinc/ammonium chloride mixture in aqueous ethanol. The hydroxylamines were then treated with an appropriate aldehyde to afford the 4-(aryl-nitrone) products (**48**). These compounds (**48**), which were evaluated as antimetastatic and antithrombic agents, exhibited superior pharmacological profiles than the test drug (nimodipine, **2c**). It should be noted that all the compounds **48** reported in this Patent possessed a chiral centre at the C-4 position of the 1,4-DHP ring system and that they were tested as racemates. In this study, the *Z*-nitrone-isomer was reported to be preferentially formed.

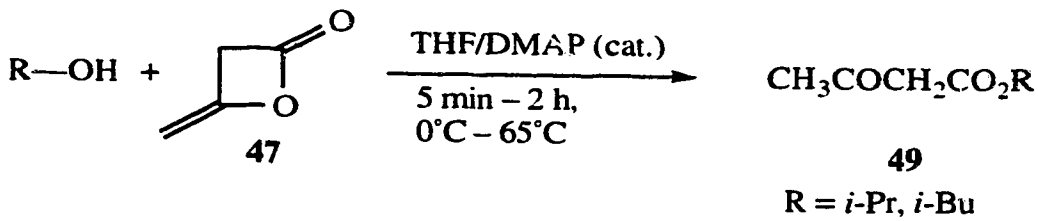


48

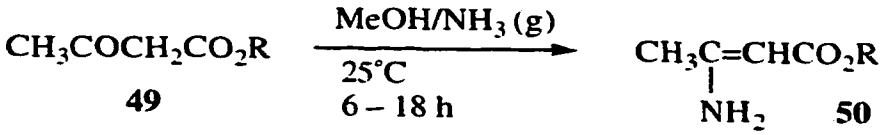
R^1 = substituted or unsubstituted aryl or heteroaryl groups
R^2, R^3 = lower alkyl
R^4, R^5 = alkyl

Two synthetic methods, to be described later, were employed for the synthesis of the target 4-arylnitrone class of 1,4-DHPs investigated in this study, which used the nitrophenyl compounds **52a-52g** as starting materials. The 1,4-DHP nitro compounds were synthesized using a modified three component Hantzsch reaction involving the condensation of an acetoacetate, enamine and an aldehyde.[143]

The alkyl acetoacetates (**49**) were prepared by refluxing the selected alcohol with diketene (**47**) in THF, in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP).[170] These reactions proceeded under mild conditions to provide the product **49** in greater than 80%. The use of other bases such as triethylamine or pyridine as a catalyst gave lower yields and required more drastic reaction conditions (higher temperatures that caused extensive decompositions).[167] Although, 2,2,6-trimethyl-4H-dioxin-4-one could be used, in place of diketene without the necessity for a catalyst since only volatile by-products are formed, the latter requires a high reaction temperature and it affords lower yields of the alkyl acetoacetates.[168] The reaction using DMAP as catalyst is shown below.



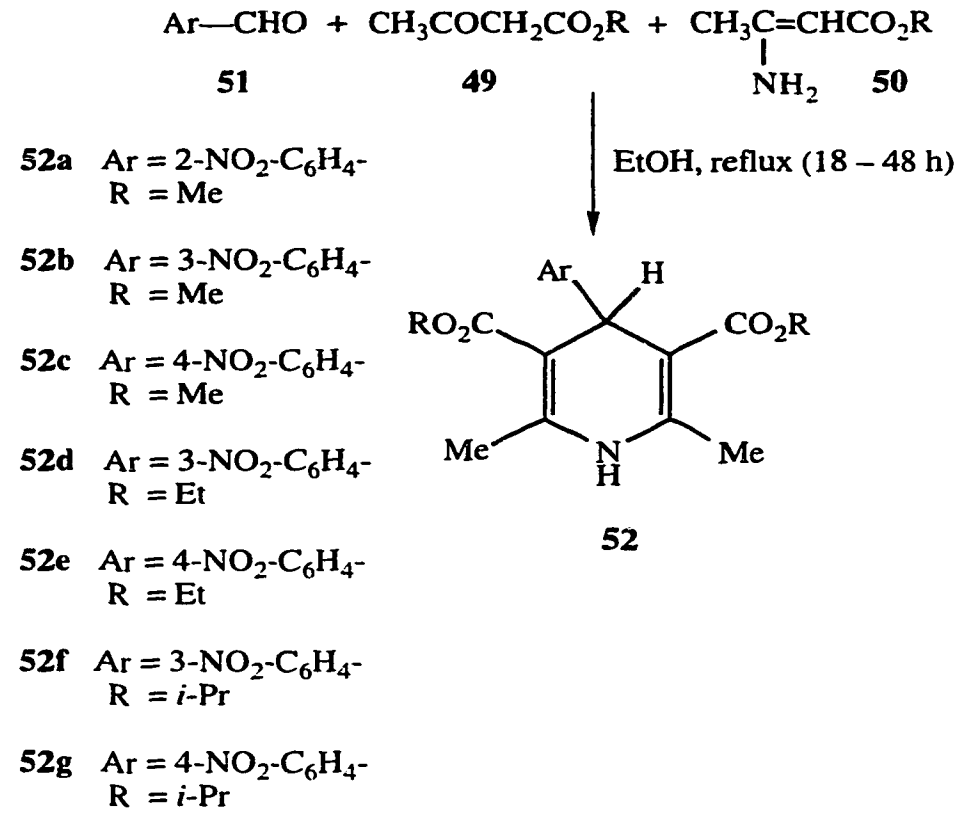
The alkyl 3-aminocrotonates (**50**) were prepared by passing ammonia gas through a solution of the alkyl acetoacetate (**49**) in methanol at room temperature (85-90% yield).



R = Me, Et, *i*-Pr, *i*-Bu

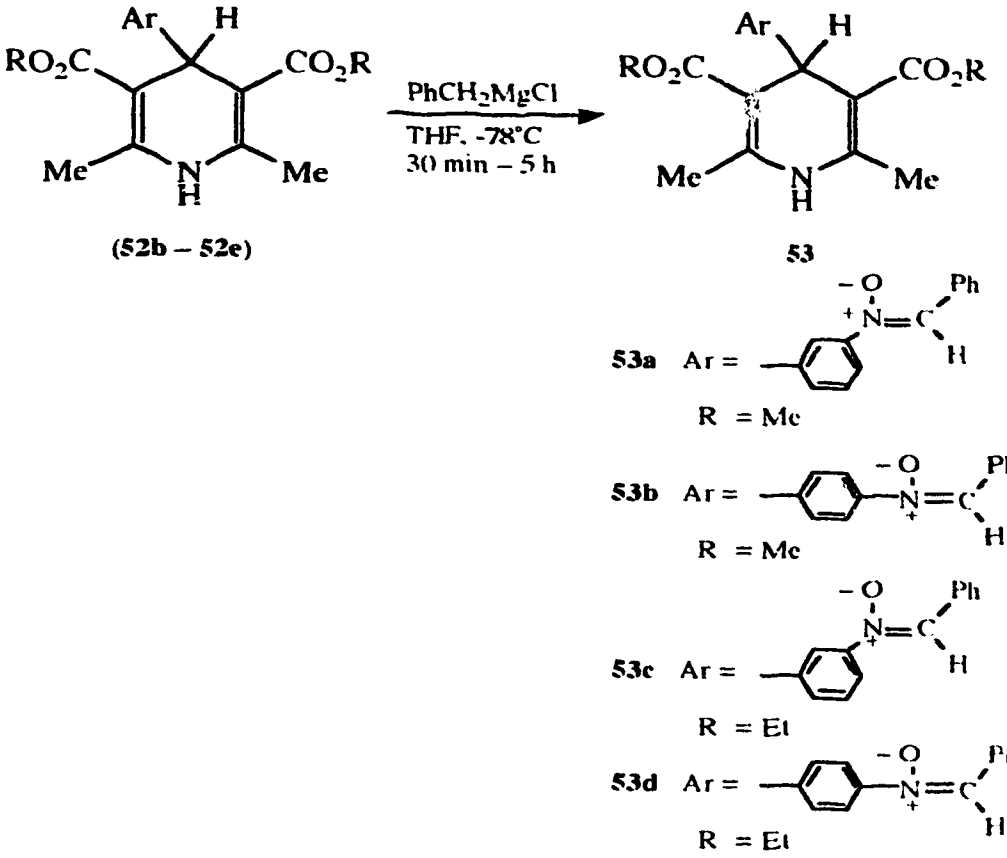
The modified Hantzsch condensation of the alkyl acetoacetate (**49**), with an alkyl 3-aminocrotonate(**50**) and either 2-, 3-, or 4-nitrobenzaldehyde (**51**) (obtained from Aldrich) afforded the corresponding C-4 2-, 3-, or 4-nitrophenyl products (**52a-52g**) which were required as starting materials for the synthesis of the target nitrones (**53a-i**). The crude products were purified by silica gel column chromatography and then recrystallization from ethyl acetate/hexane to provide isolated product yields in the 70-90% range. A general reaction scheme for the synthesis of **52a-52g** is shown on the following page.

The arylnitrone compounds (**53a-53d**), were prepared by reaction of the 3-nitrophenyl (**52b,52d**) and 4-nitrophenyl (**52c,52e**) 1,4-dihydropyridine compounds with benzylmagnesium chloride using a dry ice/acetone bath according to the method described by Bartoli *et al*[173] At a reaction temperature of about -78°C, it has been reported that Grignard reagents react chemo-selectively with a nitro group, even in the presence of the carbonyl moiety present in esters and ketones. In contrast, the reactivity of an aldehyde substituent with



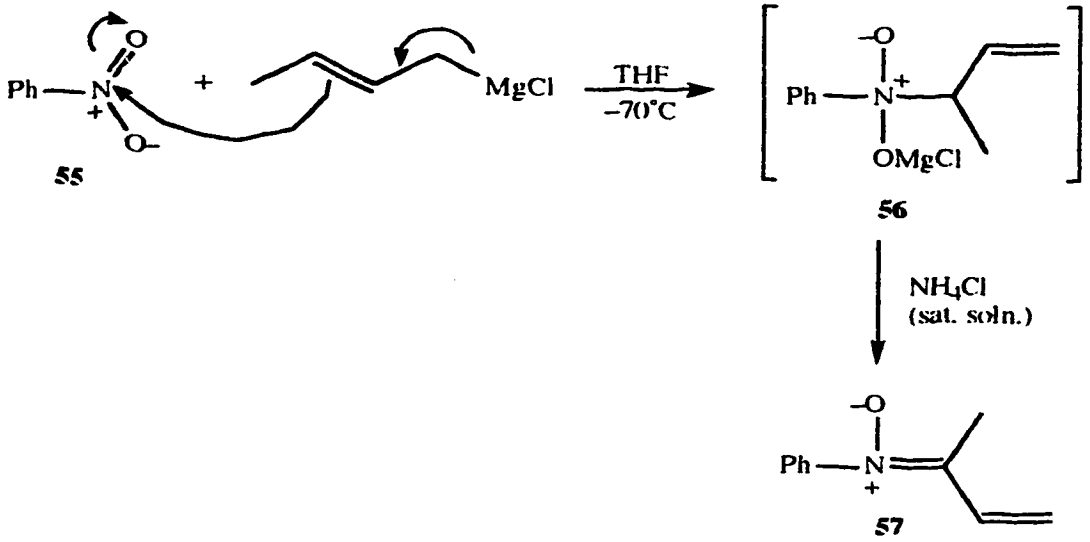
Grignard reagents under these conditions is comparable to that of the nitro group.[173-176] The crude products (**53a-d**) were purified by silica gel column chromatography and subsequent recrystallization from ethyl acetate/hexane to give the target compounds in 30-45% isolated yields. The reaction scheme employed is as shown on the following page.

Attempts to prepare the 4-(2-phenylnitrone) analog under similar conditions using **52a** resulted in the formation of decomposition products. This may be due to the instability of the nitrone product. Also attempts to prepare compounds **53e** and **53f** (R=*i*-Pr) by the reaction of **52f** and **52g** with benzylmagnesium chloride resulted in the recovery of mostly unre



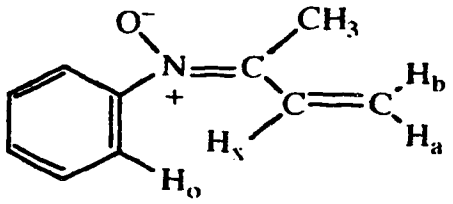
acted starting materials. The reaction of **52e** with cyclohexylmagnesium chloride or 4-methylphenylmagnesium chloride or ethylmagnesium bromide, also resulted in the recovery of mostly unreacted starting materials.

Bartoli *et al*[173-180] have reported extensive studies pertaining to the synthesis of nitrones by the reaction of nitro compounds with Grignard reagents, and the (*E*)- and (*Z*)-stereochemistry of the nitrone products. For example the reaction of 2-butenylmagnesium chloride with nitrobenzene[175,181] followed by an aqueous ammonium chloride quench afforded the (*E*)-nitrone (**57**) as illustrated on the next page, in 82% yield.



55 56 57

This latter reaction proceeded with high stereospecificity, since the (*E*)-isomer (**57**) was formed exclusively. The (*E*)-stereochemistry for (**57**) was assigned based on nuclear Overhauser effects (nOe), determined using the ROESY (Rotating-Frame Overhauser Effect Spectroscopy) technique.[182]



57

^{1}H nmr spectral data also confirmed the (*E*)-nitrone stereochemistry for **57**. For (*E*)- and (*Z*)-nitrones, of this type, Bartoli observed that the methyl resonance appeared in the δ 1.76–1.91 range for the (*Z*)-nitrones. It was stated that the nitrone oxygen deshielded the methyl group in the (*E*)-stereoisomers which appeared in the δ 2.25–2.31 range.

It was further stated that, remarkable cross-peaks were detected between the two geminal vinylic protons, H_a-H_b, the methyl group and H_b, and also between H_b and H_o in the aromatic ring. This demonstrated that the molecule, in its preferred conformation, had the H_b proton almost equidistant from the methyl group and the H_o proton in the aromatic ring, which is possible only for the (*E*)-isomer. Bartoli *et al*[175] concluded that the lack of any nOe effect between the methyl group and the protons of the aromatic ring ruled out the possibility of a (*Z*)-isomer. However, despite these observations by Bartoli *et al*[175], we suggest that, there is still the possibility that the methyl group in the (*Z*)-nitrone could be shielded by a *cis*-aryl group, resulting in a lower ^1H nmr resonance of the *Z*-isomer. Other reports which described the synthesis of nitrones by reaction of arylnitro compounds with Grignard reagents such as benzylmagnesium chloride showed that the (*Z*)-nitrone was the exclusive product.[173] Steric repulsion between the negative oxygen and the aromatic ring in the (*Z*)-nitrone may constrain the phenyl group to assume an orthogonal orientation with a consequent decrease in conjugative efficiency.[183] However, the preferred (*Z*)-stereochemistry for nitrones prepared by reaction of aryl nitro compounds with benzylmagnesium chloride, or other arylmagnesium halides, could be due to reduced steric interactions relative to that which would be present in the (*E*)-nitrone where the two aromatic rings are *cis* to each

other. In the (*Z*)-nitrone the two phenyl, or aromatic groups, are *trans* to each other.

On the basis of these considerations, it is expected that the 1,4-DHP C-4-aryl nitrones (**53a–h**) possess the (*Z*)-nitrone stereochemistry in which the two aromatic moieties attached to the nitrone group are *trans* to each other (Figure 5). **53i**, which has a vinyl substituent in place of the phenyl or aryl moiety, is expected to have the (*E*)-stereochemistry (Figure 5). Attempts to confirm the (*Z*)-nitrone stereochemistry using a ^{1}H nOe difference experiment for compound **53d** did not show any nOe effect. Although this study did not provide any confirmatory stereochemical data, it does not rule out the possibility that **53a-h** possess a (*Z*)-nitrone group observed and confirmed by other workers.[174] Our failure to observe any nOe effects could be due to the fact that the nitrone CH or C-phenylnitrone ring protons do not fall within the range that nOe effects to the C-4 phenyl protons *ortho*- to the nitrone nitrogen could be observed. However, the Hyperchem 3 (PC version for Microsoft Windows) molecular mechanics (MM^{+}) program was used to calculate the energies for the (*E*)- and (*Z*)-nitrones of **53d** (detailed discussion given in section 3.2.0.0.). These calculations gave E_{min} values of 36.55 and 38.27 kcal/mol for the (*E*)- and (*Z*)-nitrones of **53d**, respectively. These values indicate that the (*E*)-nitrone is more stable and should be expected to be preferentially formed. This contrasts with the observations of other workers that reported the preferential formation of the (*Z*)-nitrone, as

discussed previously.[173] Further studies are therefore required to unambiguously assign the stereochemistry about the nitrone group.

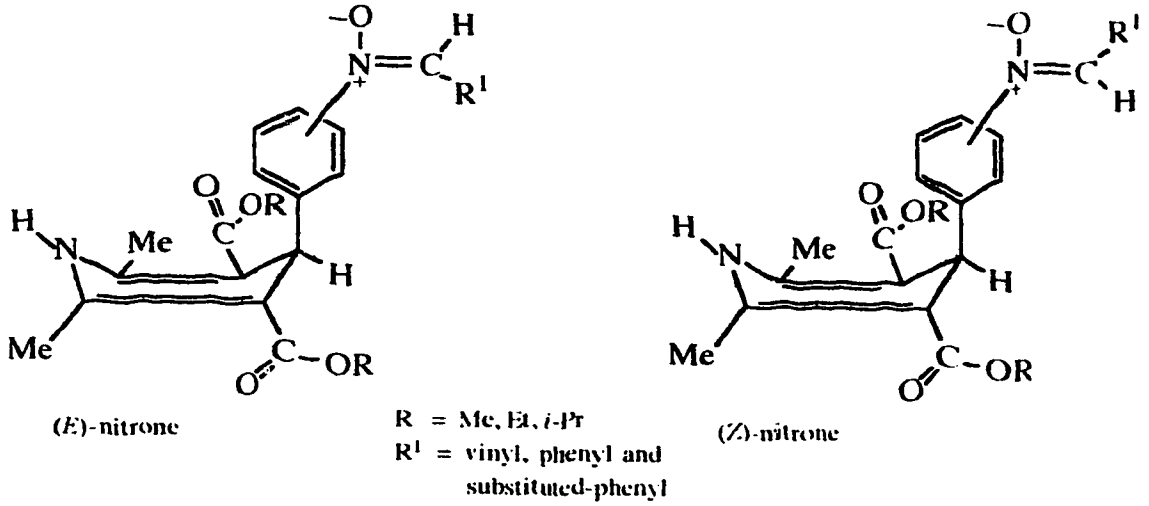
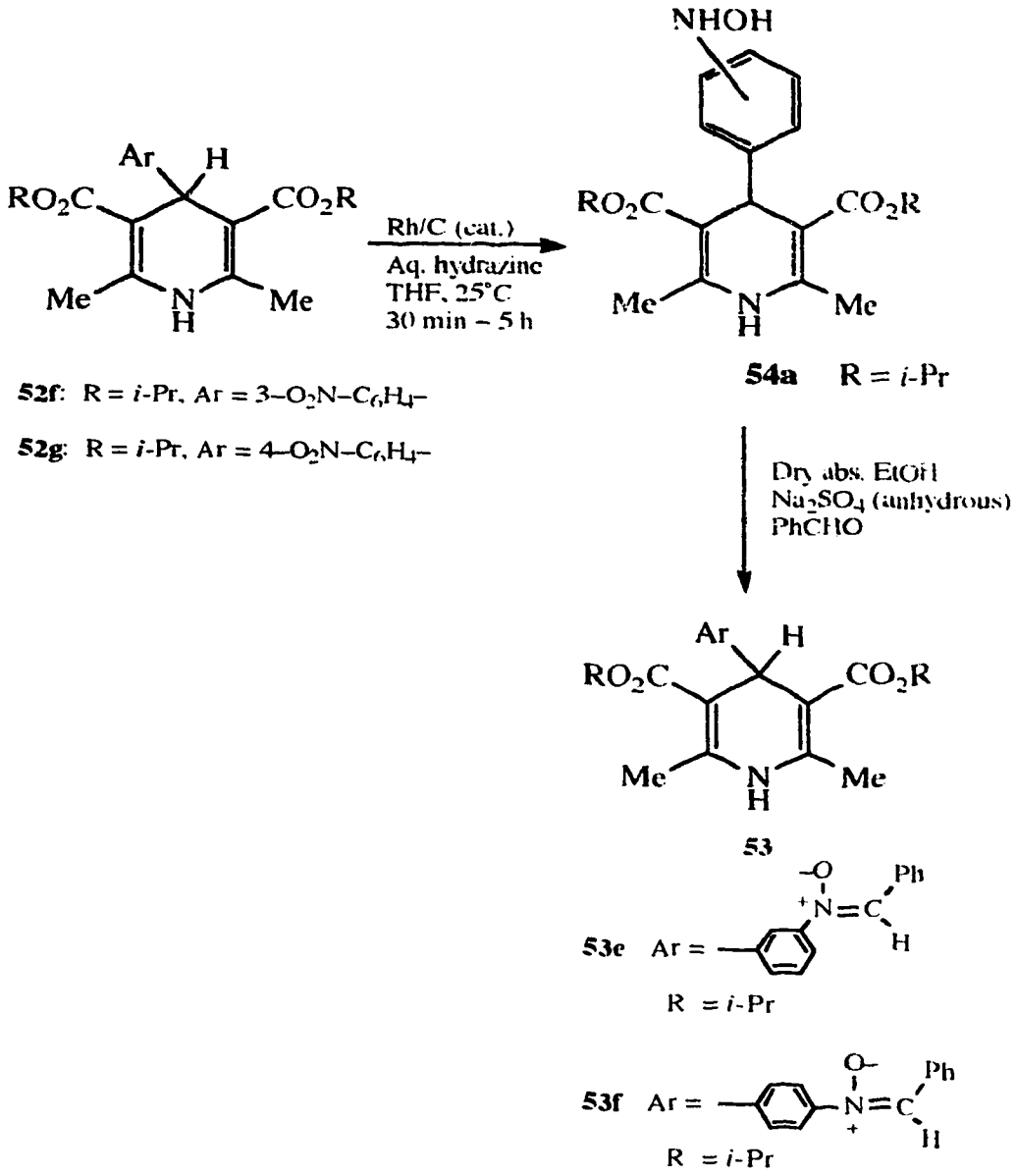


Figure 5. Possible Geometrical Isomers for Compounds **53a–53i.**

It is also expected that the 1,4-DHP C(4)-arylnitrones prepared via the reaction of arylhydroxylamines with the aryl aldehydes (**53e–i**, discussed below) possess the (*Z*)-stereochemistry about the carbon-nitrogen unsaturated bond. If this is the case, it would be consistent with other studies where the two N- [or C(4)] and C-aromatic substituents attached to the nitrone moiety are *trans* to each other.[169,174]

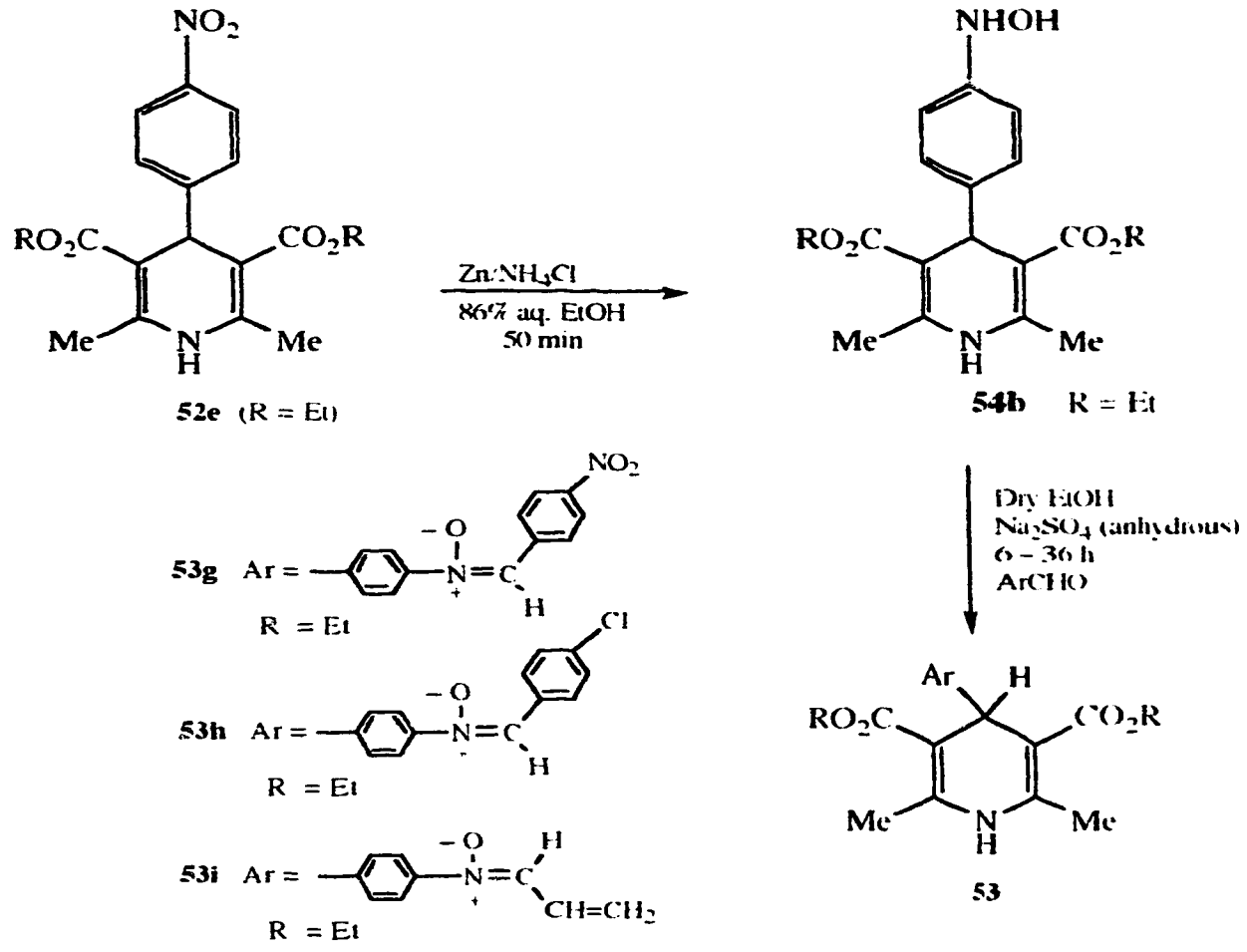
Other 1,4-DHP arylnitrones (**53e-i**) were synthesized using a reaction sequence which involved the reduction of the nitrophenyl compounds (**52d-g**) to the corresponding hydroxyl-

amine derivative (**54a** and **54b**) which was allowed to react with a selected aldehyde to yield the nitrone product (**53e-i**). Two methods were used to reduce the nitro compounds to the corresponding hydroxylamines. Thus, reduction of the diisopropylesters having a C(4)-3-nitrophenyl (**52f**) or 4-nitrophenyl (**52g**) substituent using a 5% rhodium on charcoal catalyst and aqueous (65%) hydrazine hydrate[184] in THF yielded the corresponding hydroxylamine derivative (**54a**) after quenching with water. The progress of the reaction was followed by tlc and the reaction was quenched when most of the starting nitro compound had been consumed (usually about 30 minutes to 5 hours). Extraction with ether afforded the corresponding hydroxylamine product, which without further purification, was allowed to react with benzaldehyde to afford the respective arylnitrones (**53e,53f**) in 54 and 62% yields, respectively. The latter reaction was carried out using absolute ethanol containing anhydrous sodium sulfate (Na_2SO_4)[172] to remove water that is produced during the reaction. The arylhydroxylamines were used without further purification because attempts to purify the compounds by silica gel chromatography led to extensive decomposition and dimerization products. About 80-90% of the arylnitro compounds (**52f,52g**) were reduced to the corresponding hydroxylamines before reaction with benzaldehyde. The reaction sequence is shown on the next page.



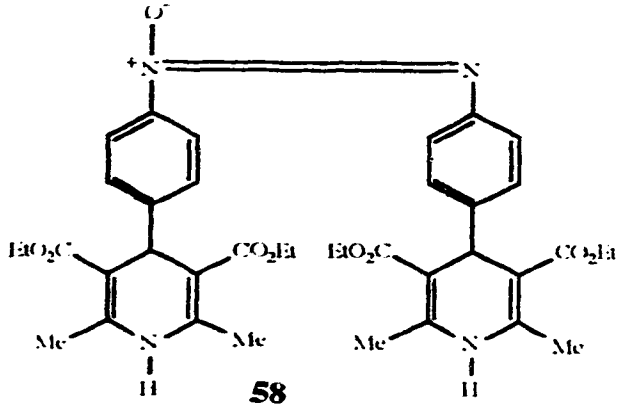
The nitro group present in diethyl 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)-3,5-pyridinedicarboxylate(**52e**) was reduced to the corresponding hydroxylamine substituent, **54b**, in 80 to 90% yield using a zinc/ammonium chloride mixture in 86% aqueous ethanol with vigorous stirring at 25°C for 50 minutes.[172] Reaction of the crude hydroxylamine product (**54b**) with either 4-nitrobenzaldehyde, 4-chlorobenzaldehyde or

acrolein in absolute ethanol in the presence of anhydrous sodium sulfate yielded the corresponding arylnitrone products (**53g–53i**) in 35 to 42% yields, after purification by silica gel column and preparative thin layer chromatography using ethyl acetate/hexane as eluent. About 10–15% of the starting nitrophenyl compound (**52e**) was recovered, while the dimerization products were formed in about 40 to 50%. Compound **53g** was recrystallized from ethyl acetate/hexane. Crystals could not be obtained for **53h** (though a solid) and **53i** (a sticky semi-solid), which were therefore used in the pharmacological assays without re-crystallisation and/or crystallisation, respectively. An earlier attempt to convert **52e** to the corresponding hydroxylamine using 5% rhodium-on-activated charcoal catalyst and 65% hydrazine hydrate, followed by the reaction of the resulting hydroxylamine with an aldehyde as previously described, resulted in the formation of dimeric products in about 80 to 85% yields. In this later reaction, the desired arylnitrone products (**53g-i**) were formed in less than 1% yield. Attempts to prepare nitrone products by the reaction of **54a** and/or **54b** with cyclohexanal, 4-methylbenzaldehyde, or 4-methoxybenzaldehyde, respectively, also resulted in the formation of dimeric products in about 80 to 85%. The yield of the target nitrone product was less than 1%.



A possible structure for the dimeric product **58** is shown on the next page. The ^1H nmr spectrum of **58** exhibited dual resonances for the phenyl, C-3 and C-5 ester ethyl, 1,4-DHP C-2 and C-6 methyl and and N-1 protons, that could be explained by the proposed azoxy structure. There was no evidence of the nitrone hydrogen (hydrogen attached to the carbon-nitrogen double bond) which usually appears at about δ 7.9. Compound **58** is most likely formed by the reaction of hydroxylamine (**54b**), with the corresponding intermediate nitroso compound. However, further analysis was not carried

out to unambiguously assign the structure for the dimeric product.



58

There was no evidence of the formation of the (*E*)-nitrone using any of the methods described above. The ^{1}H nmr chemical shift of the nitrone hydrogen was identical in all the arylnitrone compounds (**53a-h**). This proton resonance appeared at about δ 7.9, whereas in compound **53i**, which is expected to have the opposite (*E*)-stereochemistry, the chemical shift of this proton occurs at about δ 5.7 (**53i** has a C-vinyl substituent in place of a C-aryl substituent on the nitrone moiety for **53a-h**). However, the ^{1}H nmr chemical shift for the nitrone proton reported in the literature[169] for the (*Z*)-C-4-(C-arylnitrones) appears at about δ 6.5-6.8. The spectral data (^{1}H nmr, IR and ^{13}C-nmr in some cases) were consistent with the assigned structures for compounds **53a-53i**. Representative ^{1}H nmr spectral data for compound **53b** in $CDCl_3$ is as follows: δ 8.34 (d, $J_{2,3} = J_{5,6} = 7.6$ Hz, 2H, 1,4-DHP C-4 phenyl H-3 and H-5), 7.88 (s, 1H, =CH), 7.61 (dd, $J_{2,3} = J_{5,6} = 8.5$ Hz, $J_{2,4} = J_{4,6} = 1.7$Hz, 2H, C-phenyl H-2 and H-

6), 7.48 - 7.50 (m, 3H, C-phenyl H-3, H-4 and H-5), 7.41 (dd, $J_{2,3} = J_{5,6} = 7.6$ Hz; $J_{3,5} = 1.8$ Hz, 1,4-DHP C-4 phenyl H-2 and H-6), 6.54 (s, 1H, NH), 5.04 (s, 1H, 1,4-DHP H-4), 3.65 (s, 6H, CO_2Me), 2.05 (s, 6H, 1,4-DHP C-2 and C-6 Me). The spectral and physical data for compounds (**53a-i**) are given in the Experimental section (Section 4.0.0.0). The *in vitro* CC smooth muscle antagonist and cardiac agonist activities of these nitrones is presented in Section 3.3.0.0. of this thesis.

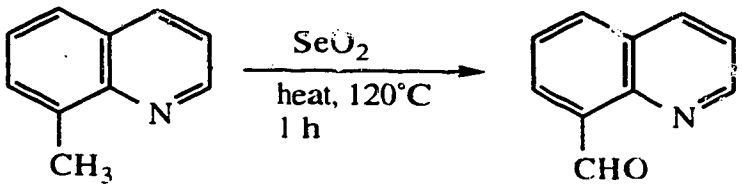
3.1.3.0. Synthesis of Dialkyl 1,4-dihydro-2,6-dimethyl-4-(quinolinyl)-3,5-pyridinecarboxylates (61a–61e)

It has been determined by Knaus *et al.*[129] that a C(4) pyridinyl substituent is bioisosteric with a 4-(nitrophenyl) substituent on a 1,4-DHP ring, where *ortho*-, *meta*-, and *para*-nitrophenyl are bioisosteric with 2-, 3-, and 4-pyridyl, respectively. A study by Knaus *et al*[161] describing 1-(5-quinolyloxy)-3-alkylamino-2-propanols and 1-(5-isoquinolyloxy)-3-alkylamino-2-propanols provided further evidence showing how charge distribution affects β-adrenergic antagonist activities. This study showed that the quinolyl compounds were ten-fold more potent than the corresponding isoquinolyl analogs when their β_1-adrenergic antagonists activities were compared. These results indicated that the position of the heteroaryl nitrogen atom might be an important determinant of β_1-adrenergic antagonist activity. In a re-

cent report by Knaus *et al*,[163] further evidence was obtained to support the postulate that the position of nitrogen in the heteroaryl ring of a C-4 pyridinyl moiety is a major determinant of calcium channel agonist-antagonist modulation activities of C-4 pyridinyl 1,4-DHPs. Determination of the *in vitro* CC smooth muscle antagonist and cardiac agonist activities for isopropyl 5-nitro-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates showed that the (±)-2-pyridyl racemate acted as a dual cardioselective calcium channel agonist (GPLA)/smooth muscle selective calcium channel antagonist (GPILSM). In contrast, the (±)-3-pyridyl and (±)-4-pyridyl racemates acted as calcium channel agonists on both GPLA and GPILSM. *In vitro* studies also showed that the (+)-2-pyridinyl enantiomer exhibited agonist activity on both GPILSM and GPLA, whereas the (-)-2-pyridinyl enantiomer exhibited agonist activity on GPLA and antagonist activity on GPILSM. It was therefore of interest to synthesize 4-(4-quinolyl)- and 4-(8-quinolyl)-1,4-dihydropyridines for pharmacological evaluation as CC agonists and/or CC antagonists, respectively. The quinolinyl nitrogen atom in the 4-(4-quinolyl) compound (**61a**) is located in the same position as the corresponding 4-(4-pyridyl) and/or 4-(4-nitrophenyl) 1,4-DHP diesters, and the quinolinyl nitrogen atom in the 4-(8-quinolyl) compounds (**61b-e**) is similar to that in the 4-(2-aryl/heteroaryl substituted) compounds. These studies would provide useful structure-activity relationships pertaining to

the position of the quinolinyl nitrogen atom on CC smooth muscle antagonist and/or cardiac agonist activity.

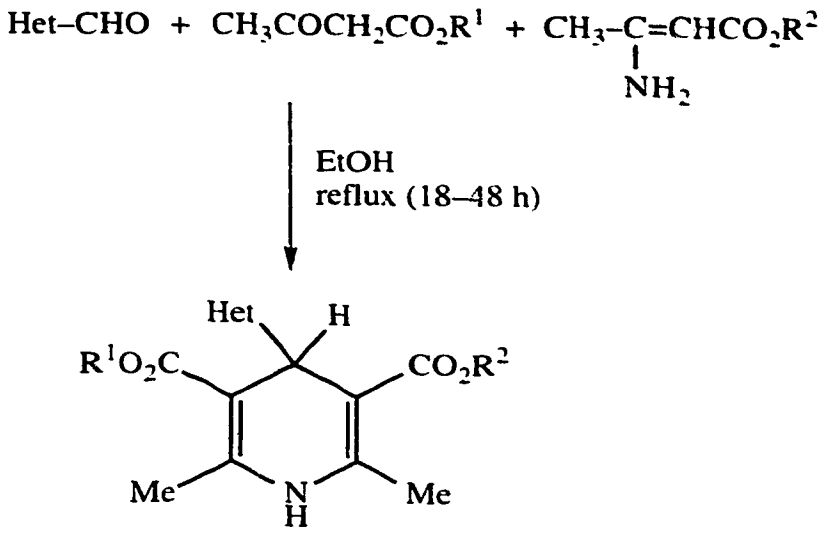
These target 4-(quinolinyl)-1,4-DHP compounds were synthesized using a three component modified Hantzsch reaction,[143,144] which involved condensation of an alkyl acetoacetate with an alkyl 3-aminocrotonate and either 4- or 8-quinolinecarboxaldehyde. The alkyl acetoacetates and alkyl 3-aminocrotonates were prepared using the methods previously described (Section 3.1.2.0). 4-Quinolinecarboxaldehyde was purchased from the Aldrich Chemical Co. and 8-quinolinecarboxaldehyde was prepared by oxidation of 8-methylquinoline (**59**) using freshly prepared selenium dioxide (SeO_2). A mixture of 8-methylquinoline (**59**) and selenium dioxide was heated at an oil bath temperature maintained at 120°C for about one hour. The resulting dark solution was extracted with ethyl acetate and the solvent was removed *in vacuo* to afford the crude product which was purified by silica gel column chromatography and then recrystallized from ethyl acetate/hexane to give the target compound (**60**) in 35% yield. The reaction scheme is shown below.



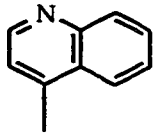
59 **60**

The 4-(quinolinyl)-1,4-DHPs (**61a-61e**), prepared as described below were purified by silica gel column chromatography and then recrystallized from ethyl acetate/hexane to afford the title compounds (**61a-61e**) in 28 to 78% yields (see scheme that follows). The major side-products formed in the reaction used to prepare **61c** and **61d** (unsymmetrical esters) were the corresponding symmetrical esters which were formed in about 50% yield. The use of a modified Hantzch reaction enhanced the yield of the unsymmetrical diesters (**61c** and **61d**). Unreacted starting materials were also recovered (10 to 40%).

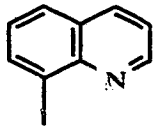
The spectral data for **61a-61e** were consistent with their assigned structures. Representative ^{1}H nmr spectral data for **61a** in $CDCl_3$ is as follows: δ 8.80 (d, $J_{2,3}$ = 4.8 Hz, 1H, quinolyl H-3), 8.[illegible] (d, $J_{5,6}$ = 8.0 Hz, 1H, quinolyl H-5), 8.09 (dd, $J_{7,8}$ = [illegible], $J_{6,8}$ = 1.0 Hz, 1H, quinolyl H-8), 7.[illegible] (ddd, $J_{5,6}$ = $J_{6,7}$ = 8.0 Hz, $J_{6,8}$ = 1.0 Hz, 1H, quinolyl [illegible]), 7.59 (ddd, $J_{6,7}$ = $J_{7,8}$ = 8.0 Hz; $J_{5,7}$ = 1.0 Hz, 1H, quinolyl H-7), 7.47 (d, $J_{2,3}$ = 4.8 Hz, 1H, quinolyl H-2), 5.47 (s, 1H, NH), 5.84 (s, 1H, 1,4-DHP H-4), 3.90 (q, $J_{CH2,Me}$ = 7.1 Hz, 4H, CH_2Me), 2.38 (s, 6H, 1,4-DHP C-2 and C-6 Me), 0.95 (t, $J_{Me,CH2}$ = 7.1 Hz, 6H, CH_2Me). The IR and ^{1}H nmr



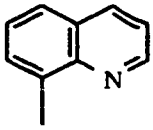
61

61a Het = 

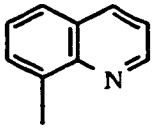
$R^1, R^2 = Et$

61b Het = 

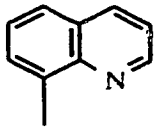
$R^1, R^2 = Et$

61c Het = 

$R^1 = Me,\ R^2 = i\text{-Pr}$

61d Het = 

$R^1 = Et,\ R^2 = i\text{-Pr}$

61e Het = 

$R^1, R^2 = i\text{-Pr}$

spectral and physical data for **61a-61e** are given in the Experimental Section. The CC modulation activities for **61a-61e** will be described later in the thesis (Section 3.3.0.0).

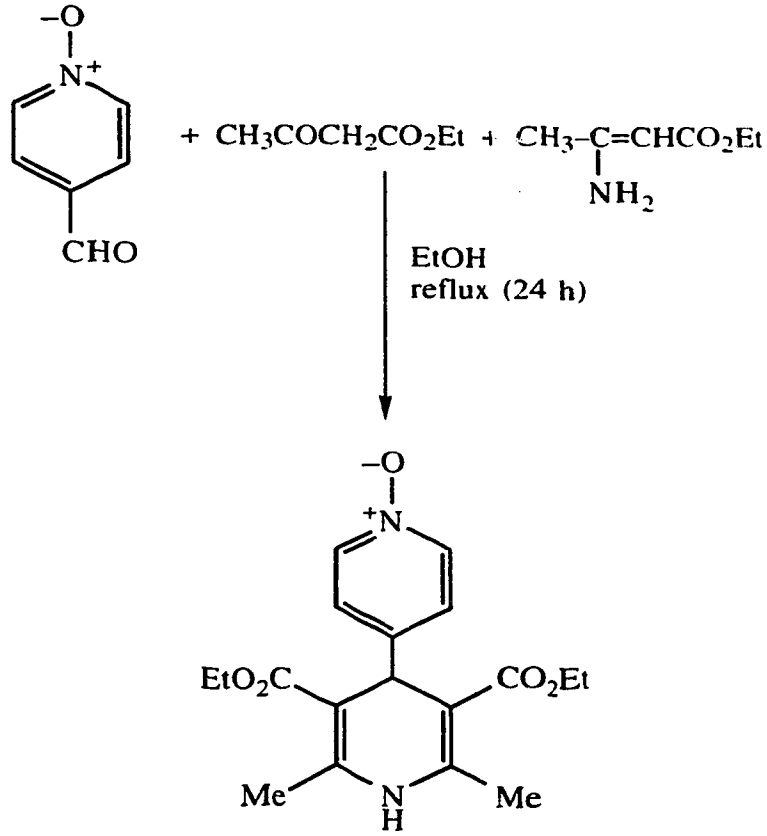
3.1.4.0. Synthesis of Diethyl 1,4-dihydro-2,6-dimethyl-4-(1-oxido-4-pyridyl)-3,5-pyridinedicarboxylate (62)

Knaus *et al*[185] reported that replacement of the 4-(2-nitrophenyl) substituent of nifedipine (**1**) by a 4-(1-oxido-2-pyridyl) substituent decreased calcium channel antagonist activity by greater than 100-fold. Subsequent to this study, other structure-activity correlations obtained for 1,4-DHP CC modulators suggested that the negative minimum at a position *para* to the point of attachment of a heteroaryl ring (for example 4-pyridinyl) reduced CC antagonist and increased CC agonist activity [127] It was therefore anticipated that a C-4 1-oxido-4-pyridinyl moiety would provide a negative minimum due to the 1-oxido group to possibly enhance CC cardiac agonist activity and decrease CC antagonist activity. The CC modulating activity of the 4-(1-oxido-4-pyridyl) compound (**62**) could be compared to those of the corresponding 4-(arylnitrone) compounds described earlier (**53b,53d,53f, 53g-i**) and the 4-(1-oxido-2-pyridyl) analog reported earlier,[185] to develop additional structure-activity correlations. In compound **62**, the quaternary nitrogen is part of the pyridyl ring system, whereas in the 4-(arylnitrone) class of compounds the quaternary nitrogen atom is attached directly to the phenyl ring system.

The title compound **62** was synthesized using a modified Hantzsch reaction involving the condensation of ethyl acetoacetate, ethyl 3-aminocrotonate and 1-oxido-4-pyridine car-

boxaldehyde in 85% yield, after purification by silica gel column chromatography and recrystallization from ethyl acetate/hexane. The IR and ^1H nmr spectra for **62** were consistent with the assigned structure. The ^1H nmr spectra for **62** in $CDCl_3$ exhibited the following resonances: δ 8.10 (dd, $J_{2,3} = J_{5,6} = 7.0$ Hz, $J_{2,6} = 1.5$ Hz, 2H, 1-oxido-4-pyridyl H-2 and H-6), 7.31 (d, $J_{2,3} = J_{5,6} = 7.0$ Hz, 2H, 1-oxido-4-pyridyl H-3 and H-5), 7.21 (s, 1H, NH), 5.02 (s, 1H, 1,4-DHP H-4), 4.12 (q, J = 7.1 Hz, 4H, CH_2CH_3), 2.38 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.23 (t, J = 7.1 Hz, 6H, CH_2Me).

The IR spectral and physical data for **62** are listed in the Experimental Section (Section 4.0.0.0).



62

3.2.0.0. CONFORMATION OF 1,4-DIHYDROPYRIDINES

Extensive research has been performed to acquire structure-activity relationships for the 1,4-dihydropyridine calcium channel modulators (Section 1.4.3.0). The conformation of the dihydropyridine ring, the configuration at C-4, and the particular substituents and their orientation at the C-3 and C-5 positions, are important factors that influence the pharmacological properties of 1,4-dihydropyridines. In 1,4-DHP CC antagonists such as nifedipine (**1**), the carbonyl moieties of the ester groups at the C-3 and C-5 positions are synperiplanar (sp) or *cis* to the double bonds of the 1,4-DHP ring, whereas in the 1,4-DHP CC agonist Bay K8644 (**3a**), the ester and nitro groups at the C-3 and C-5 positions are antiperiplanar (ap) or *trans* to the double bonds of the 1,4-DHP ring.[121,138,186-188] Generally, 1,4-dihydropyridine CC modulators exist in the boat conformation, where the C(4)-substituted-aryl ring is perpendicular to the 1,4-dihydropyridine ring (Figures 2 and 3; Section 1.4.3.0). Changes in the substitution pattern at the C(3), C(4) and C(5) positions alter the conformation (degree of ring pucker) of the 1,4-dihydropyridine ring, and the nature of these substituents are determinants of the resulting agonist or antagonist effect. The 1,4-DHP ring is strained due to non-bonded interactions between substituents at the C(3), C(4) and C(5) positions. This ring strain is relieved by puckering of the 1,4-DHP ring and distortion of the bond angles particularly about

C(4).[138,186,187] Knaus *et al*[125-130] have reported several studies describing many 1,4-DHPs containing C(4) pyridinyl substituents to determine structure-activity correlations and bioisosteric relationships.

In the present study, the conformation of alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates (compounds **38a-38f**) were determined using the Hyperchem Version 3 for Windows minimizer (MM^+) program from Autodesk (IBM PC Version). This program was used to measure selected interatomic distances, torsion angles, and local minimum energy (E_{min}) after minimization. A general structure for this class of compounds is shown in Figure 6 and the theoretical data obtained in these calculations are shown in Table 1.

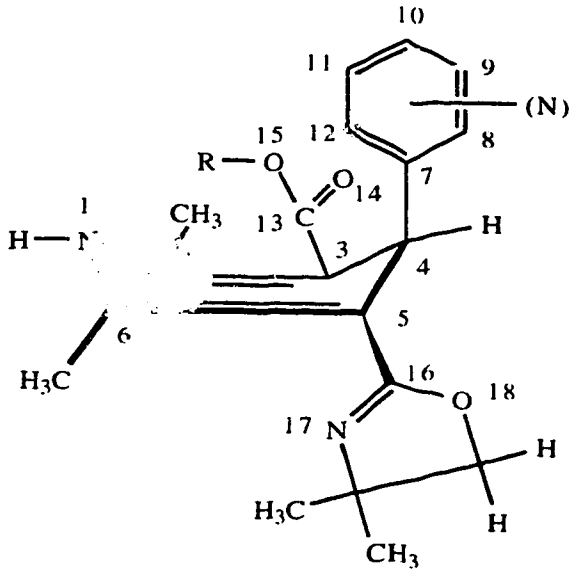
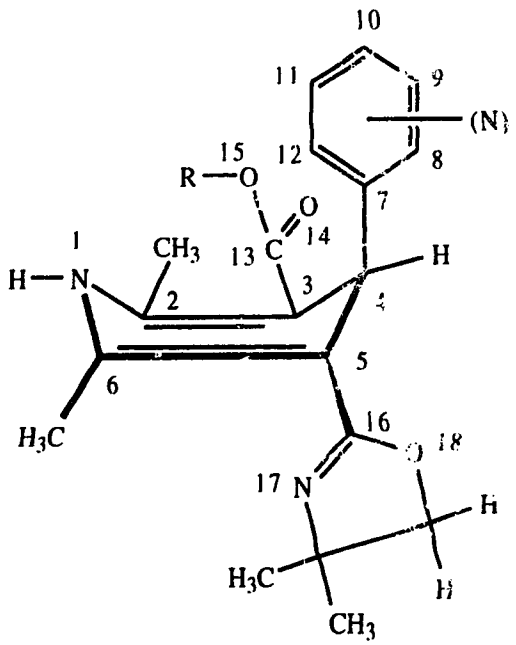
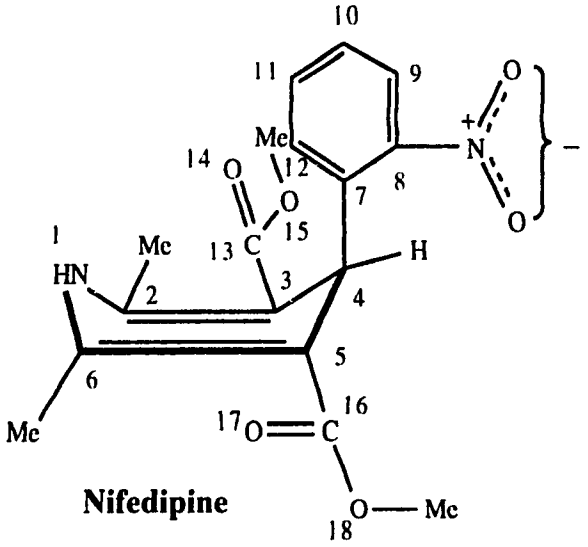


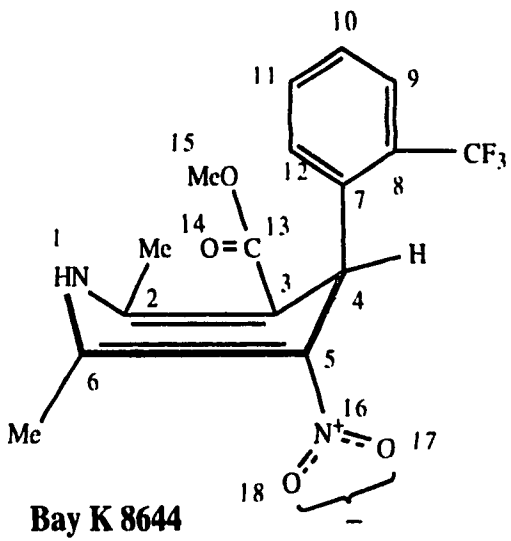
Figure 6. Proposed Boat Conformation for Alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates (**38a-38f**).

Table 1. Interatomic distances (Å), torsion angles (°), and local minimum energy (kcal/mol) for **38a-38f**, nifedipine and Bay K 8644.[a]





Nifedipine



Bay K 8644

R	Me	Me	i-Pr	i-Pr	i-Pr	i-Bu		
Het	3-pyr	4-pyr	2-pyr	3-pyr	4-pyr	3-pyr	Nifedipine	Bay K 8644
Compound	**38a**	**38b**	**38c**	**38d**	**38e**	**38f**		
Interatomic Distances (Å)								
N1-C4	2.68	2.86	2.66	2.68	2.68	2.68	2.71	2.76
N1-C8	4.77	4.77	4.63	4.80	4.80	4.78	4.71	4.93

R	Me	Me	*i*-Pr	*i*-Pr	*i*-Pr	*i*-Bu		
Het	3-pyr	4-pyr	2-pyr	3-pyr	4-pyr	3-pyr	Nifedipine	Bay K 8644
Compound	**38a**	**38b**	**38c**	**38d**	**38e**	**38f**		
N1-C12	3.29 (N1-N12)	3.29	3.04 (N1-N12)	3.31	3.31	3.31	3.27	3.32
N1-C13	3.68	3.68	3.68	3.69	3.69	3.69	3.70	3.69
N1-O14	4.60	4.60	4.60	4.62	4.63	4.63	4.27	4.29
N1-O15	4.30	4.30	4.31	4.28	4.28	4.28	4.69	4.64
N1-C16	3.69	3.69	3.68	3.69	3.69	3.69	3.70	3.62
N1-N17	4.36	4.37	4.36	4.36	4.36	4.36	4.24	4.37
N1-O18	4.77	4.77	4.77	4.77	4.77	4.77	4.70	4.52
Torsion Angles (˚)								
N1-C2-C3-C4	-1.35	5.22	3.77	8.81	8.46	8.65	5.54	8.39
N1-C6-C5-C4	4.77	-1.30	-0.71	-1.88	-1.49	-1.69	-3.23	-3.52
C2-C3-C4-C7	95.69	-96.83	-90.38	-100.75	100.65	-100.21	-93.62	-100.79
C6-C5-C4-C7	-96.41	96.04	89.39	97.99	97.26	97.33	94.22	99.00
C2-C3-C13-O14	154.28	155.96	156.50	176.40	177.39	178.49	-34.20	-48.66
C6-C5-C16-N17	12.45	10.49	15.84	2.78	8.15	4.07	-45.71 (C6-C5-C16-O17)	111.44 (C6-C5-N16-O18)
								-83.60 (C6-C5-N16-O17)
Energy	133.38	133.22	132.30	134.62	134.43	136.12	28.56	28.09

[a]Determined using the Hyperchem Version 3 for Windows minimizer program from Autodesk (IBM PC Version) using the MM^+ force field. The energy (in kcal/mol) relative to the same atoms that are not interacting (in a stress-free state) was obtained according to the Polak-Rebiere procedure for the Allinger MM^+ molecular mechanics force field.

There are four possible conformations for compounds **38a-38f**, depending on the orientation of the carbonyl (C=O) moiety of the ester group at C-3 and the C=N moiety of the oxazolinyl group at C-5 of the 1,4-DHP ring. These include :

1. Both the C=O of ester group and the C=N of the oxazolinyl group are antiperiplanar (ap) to the olefinic bond to which they are attached.
2. The C=O of the ester is antiperiplanar (ap) while the N=C of the oxazolinyl group is synperiplanar (sp) to the olefinic bond to which they are attached.
3. Both the O=C of the ester and the N=C of the oxazolinyl group are synperiplanar (sp) to the olefinic bond to which they are attached.
4. The O=C of the ester group is sp and C=N of the oxazolinyl group ap to the olefinic bond to which they are attached.

The minimum energy for all four possible orientations were calculated using the Hyperchem Version 3 program mentioned above. The calculations were performed for structures with the (S)-configuration at C-4 and N-1 (as in Figure 6). A difference of 1.4 kcal/mol is equivalent to a ten-fold difference in stability. For the C-4 (2-pyridyl) compound **38c**, and the C-4 (3-pyridyl) compounds, **38a**, **38d** and **38f** respectively, the calculations were performed with the pyridyl nitrogen (N) oriented closer to N-1 and also with pyridyl nitrogen (N) oriented away from N-1. It was observed that for C-4 (2-pyridyl) compound, (**38c**), the most stable (lower energy) conformation was obtained when the pyridyl ni-

trogen was closer to N-1. In contrast, the most stable (lower energy) conformation was obtained in each case, for C-4 (3-pyridyl) compounds (**38a**, **38d**, and **38f**) when the pyridyl nitrogen was further from N-1. For example the following minimum energy (E_{min}) data were obtained for **38c**.

(a) For ap C=O and ap C=N (pyridyl N closer to N-1), E_{min} = 145.06 kcal/mol (146.56 kcal/mol with pyridyl N further away from N-1).

(b) For sp O=C and ap C=N (pyridyl N closer to N-1), E_{min} = 148.58 kcal/mol (149.10 kcal/mol for pyridyl N further away from N-1).

(c) For sp O=C and sp N=C (pyridyl N closer to N-1), E_{min} = 134.42 kcal/mol (135.55 kcal/mol for pyridyl N further away from N-1).

(d) For ap C=O and sp N=C (pyridyl N closer to N-1), E_{min} = 132.30 kcal/mol (133.24 kcal/mol for pyridyl N further away from N-1).

For all the four possible orientations for compounds **38a-38f**, it was observed that the most stable conformation (lower energy) in each case, was the one with an ap C=O ester and a sp N=C oxazolinyl group. The data presented in Table 1 for interatomic distances, torsion angles and minimum energy for compounds **38a-38f** were calculated in each case, from this most stable conformation.

The data obtained are consistent with that for a flat boat conformation, with the C(4) pyridinyl substituent pseudoaxial to the plane of the 1,4-DHP ring. The N(1)-C(4) bond dis-

tance gives a measure of the flatness of the boat. In nifedipine (**1**), the N(1)-C(4) distance is reported to be 2.66 Å (2.71 Å using Hyperchem 3) This is comparable to the N(1)-C(4) distance of about 2.66 Å to 2.68 Å obtained for compounds **38a-38f**. Other bond distances that provide relevant conformation information are N(1)-C(8) and N(1)-C(12) which show the orientation of the nearly planar pyridyl ring; N(1)-C(13), N(1)-O(14) and N(1)-O(15) which show the direction of C=O (antiperiplanar or synperiplanar); N(1)-C(16), N(1)-N(17) and N(1)-O(18) which show the direction of C=N (antiperiplanar or synperiplanar). The extent of 1,4-DHP ring puckering (degree of flatness) can be deduced from the N(1)-C(2)-C(3)-C(4) and N(1)-C(6)-C(5)-C(4) torsion angles. The values obtained indicate a very flat ring (-0.7 to 8.81 Å). The C(2)-C(3)-C(4)-C(7) and C(6)-C(5)-C(4)-C(7) torsion angles indicate whether the pyridyl ring is pseudoaxial or pseudoequatorial. The values obtained are close to 90° (89.39-100.78), reflecting the pseudoaxial orientation of the 1,4-DHP C-4 (pyridinyl) substituent relative to the plane of the C(6)-C(5) or C(2)-C(3) olefinic bond. The C(2)-C(3)-C(13)-O(14) torsion angle tells whether the C=C double bond (of 1,4-DHP ring) and C=O of the ester moiety are in the same plane, while the C(6)-C(5)-C(16)-N(17) torsion shows whether the C=C and C=N bond (of oxazolinyl group) are in the same plane (if close to 0° or 180°). The values obtained for these angles indicate that these groups are not in the same plane (see Table 1).

The conformation of the 1,4-DHP aryl nitrones (**53a-53j**) is also expected to be a flat boat shape, with the C-4 (aryl nitrone) moiety being pseudoaxial to the 1,4-DHP ring (Figure 7).

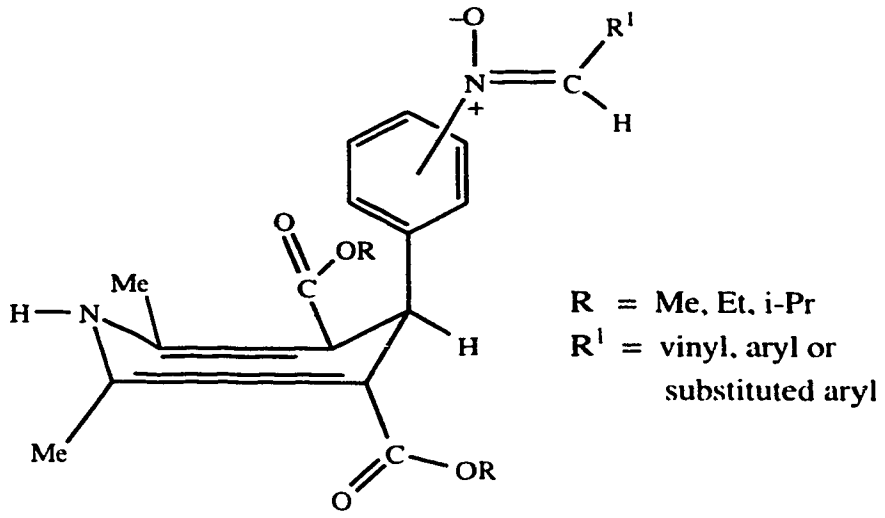


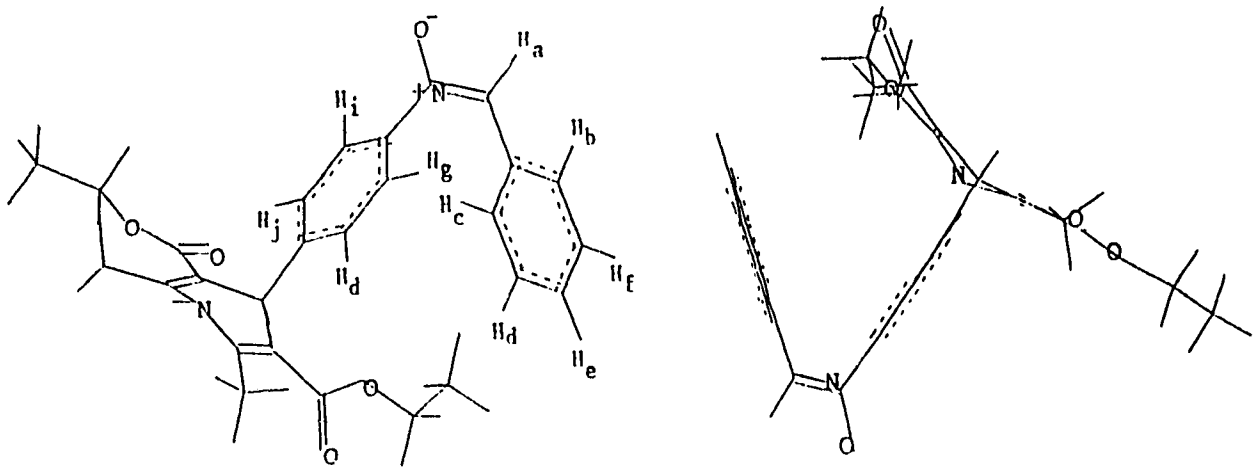
Figure 7 .Proposed Boat Conformation for the Dialkyl 1,4-dihydro-2,6-dimethyl-4-{[(*Z*)-N-Oxo-N-(aryl/vinylmethylene)-λ^5-azanyl]-phenyl}-3,5-pyridinedicarboxylates (**53a-i**).

The Hyperchem Version 3 for Windows Minimizer Program from Autodesk (IBM PC Version) using the Allinger MM$^+$ force field was also used to acquire conformational information for the 4-arylnitrone compounds (**53a-53i**) prepared in this study. The (*E*)- and (*Z*)-stereoisomers of compound **53d** gave calculated E_{min} of 36.55 and 38.27 kcal/mol, respectively. It was unexpected that (*E*)-stereoisomer of **53d**, in which the two phenyl rings attached to the nitrone moiety are *cis* to each

other, would have a lower energy than the (*Z*)-stereoisomer of **53d** where the two phenyl rings are *trans* to each other (see Figures 8 and 9). Although the two phenyl rings in the (*E*)-stereoisomer of **53d** are *cis* to each other, there is a sufficient separation between the two phenyl rings, which are in the same plane, to preclude any steric interactions (Figure 8). For example, the distance between the closest protons in the two phenyl rings (H_C and H_i) of 3.17 Å is greater than the distance between two *ortho* phenyl protons on the same ring (approx. 2.44 Å). In contrast, the two phenyl rings in (*Z*)-stereoisomer of **53d** are in different planes. Examination of selected interatomic spacial distances for (*E*)- and (*Z*)-stereoisomers of **53d**, as summarized in Figures 8 and 9, respectively show that steric effects should not be relevant with respect to stability. If a true global minimum energy was determined in these calculations, a factor(s) other than steric effects must be operative to explain the lower energy of the (*E*)-nitrone stereoisomer.

The conformation for the (*E*)- and (*Z*)-stereoisomers for **53i** (vinylnitrone) was similarly calculated to provide local energy minima of 31.95 and 31.29 kcal/mol, respectively. In this case, the (*Z*)-stereoisomer of the vinylnitrone (**53i**), in which the vinyl and phenyl substituents are *trans* to each other, was more stable than the (*E*)-vinylnitrone where the vinyl and phenyl substituents are *cis* to each other (see Figures 10 and 11). These results differ from those reported in the literature[172,173] in which the arylnitrones are as

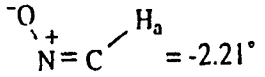
Figure 8. Different stereo-views of diethyl 1,4 dihydro-2,6-dimethyl-4-[(*E*)-N-oxo-N-(phenylmethylene)-λ^5-azanyl)phenyl}-3,5-pyridinedicarboxylate (**53d**).

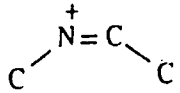


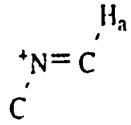
Interatomic Spacial Distances (Å)

ab = 2.75	cg = 3.99
ac = 3.53	ch = 4.93
ad = 5.38	bg = 3.54
ae = 5.90	bi = 6.08
af = 4.90	ci = 3.17
ag = 3.87	bh = 5.64
ah = 6.10	
ai = 4.33	
aj = 6.40	

Torsion Angles (Å)


= -2.21°


= -1.76


= 177.83

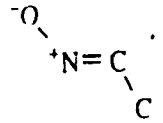
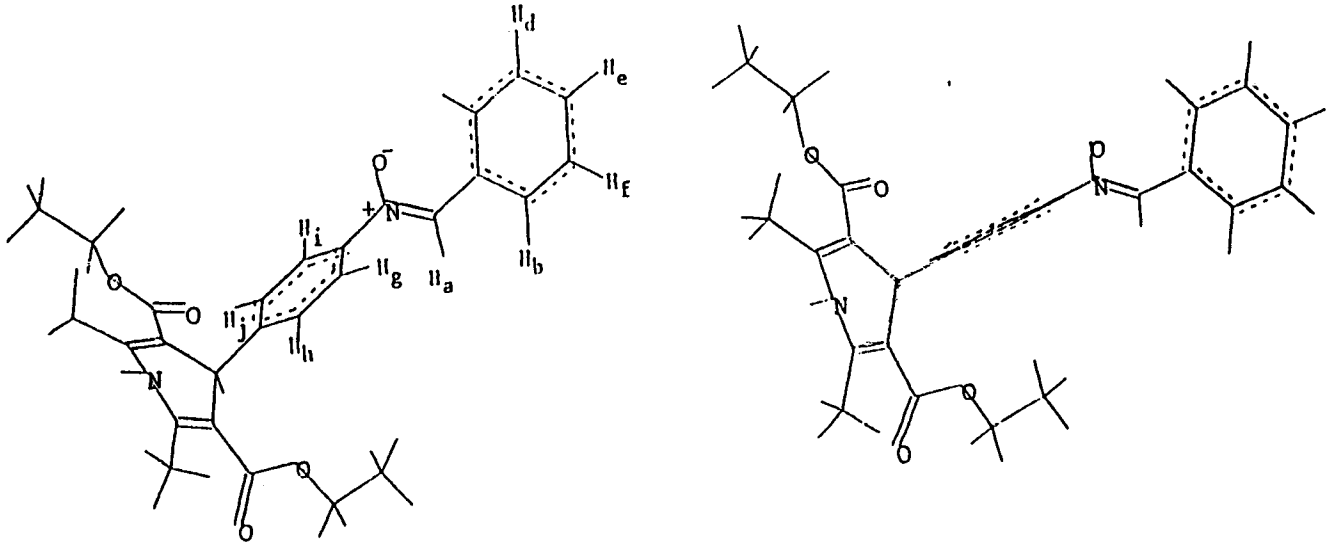

= 178.84

Figure 9. Different stereo-views of diethyl 1,4 dihydro-2,6-dimethyl-4-[(*Z*)-N-oxo-N-(phenylmethylene)-λ^5-azanyl)phenyl]-3,5-pyridinedicarboxylate (**53d**).



Interatomic Spacial Distances (Å)

ab = 2.62	bg = 5.05 Å
ac = 3.63	cg = 4.52
ad = 5.44	ci = 5.16
ae = 5.89	bi = 5.72
af = 4.82	
ag = 2.44	
ah = 4.55	
ai = 4.22	
aj = 5.71	

Torsion Angles (Å)

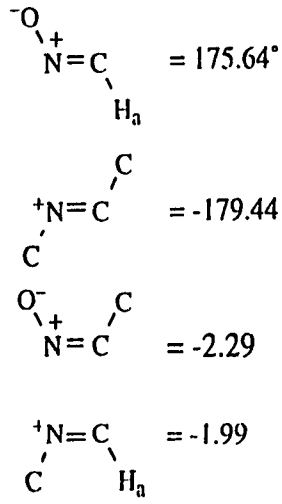
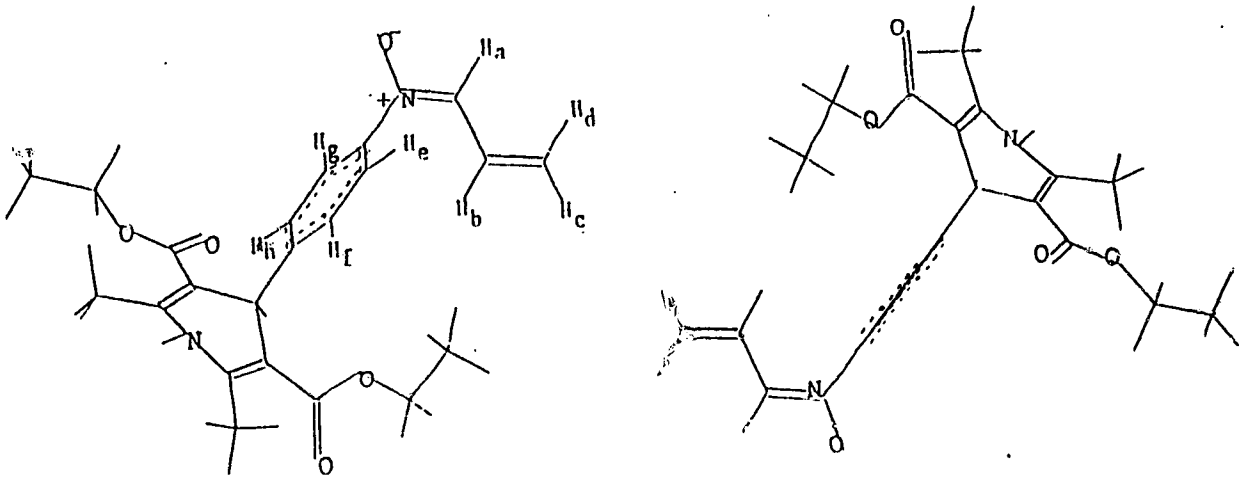


Figure 10. Different stereo-views for diethyl 1,4 dihydro-2,6-dimethyl-4-[(*E*)-N-oxo-N-(vinylmethylene)-λ^5-azanyl)phenyl}-3,5-pyridinedicarboxylate (**53i**).



Interatomic Spacial Distances (Å)

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ac = 3.72

ad = 2.45

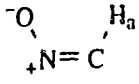
ae = 3.96

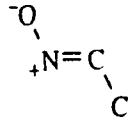
ag = 4.24

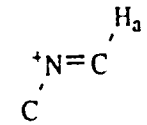
af = 6.17

ah = 6.35

Torsion Angles (Å)


= -0.69°


= 178.36


= 179.89

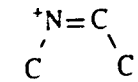
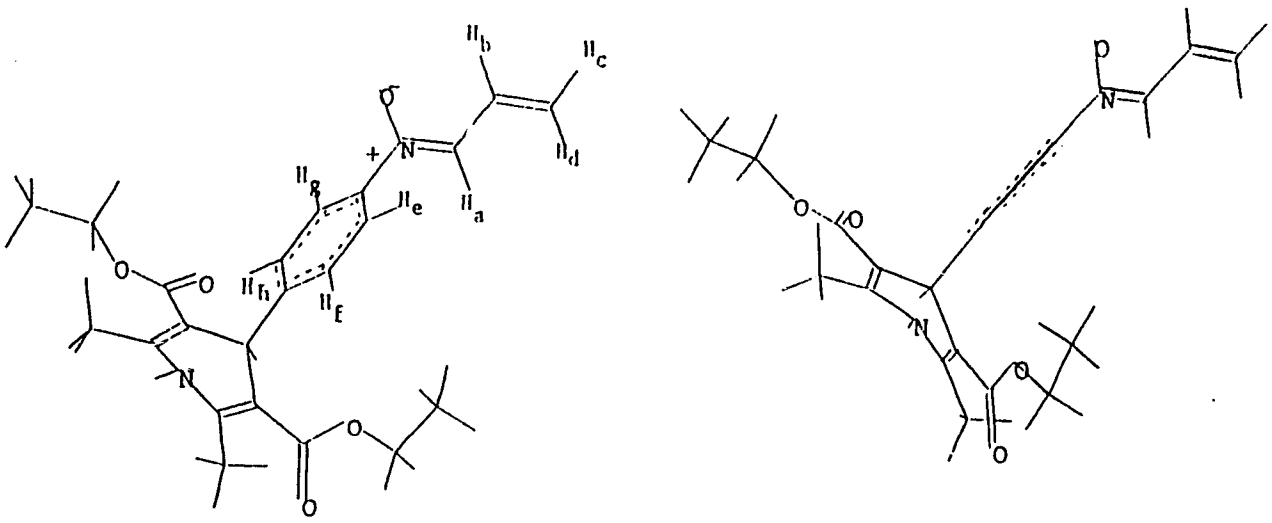

= -1.06

Figure 11. Different stereo-views for diethyl 1,4 dihydro-2,6-dimethyl-4-{[(Z)-N-oxo-N-(vinylmethylene)-λ^5-azanyl)phenyl}-3,5-pyridinedicarboxylate (**53i**).



Interatomic Spacial Distances (Å)

ab = 3.10

ac = 3.72

ad = 2.45

ae = 2.48

af = 4.56

ag = 4.18

ah = 5.66

Torsion Angles (Å)

$^{-}O_{\backslash}^{+}N{=}C_{\backslash}^{\backslash}H_a$ = 178.25°

$^{-}O_{\backslash}^{+}N{=}C_{\backslash}^{H_b}$ = -0.84

$^{+}N{=}C_{\backslash}^{/}C$ = -179.17

$^{+}N{=}C_{/}^{\backslash}C$ $\quad$ H_a = -0.09

signed the (*Z*)-stereochemistry and (*E*)-stereochemistry for the allylnitrones, respectively. Further studies and analysis are required to resolve these differences.

3.3.0.0. PHARMACOLOGICAL EVALUATION OF THE 1,4-DIHYDROPYRIDINES

3.3.1.0. *In Vitro* Calcium Channel Antagonist and Agonist Assays

Calcium channel antagonist smooth muscle activity (CCA) was determined using a guinea pig ileal longitudinal smooth muscle (GPILSM) assay according to the procedure previously reported.[129] CCA activity is expressed as an IC_{50} value, where IC_{50} is defined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction (tonic response) of GPILSM. For each concentration of the test compound, three values (tonic response) were obtained from tissues obtained from three different animals (n = 3). The IC_{50} value (± SEM, Standard Error of the Mean) was determined graphically from the dose-response curve.

Calcium channel cardiac agonist (CCag) activity of the test compounds was determined using a guinea pig left atria assay (GPLA) where CCag activity (positive inotropic effect) was calculated as the percentage increase in the contractile force of the isolated guinea pig left atrium relative to its basal contractile force in the absence of the test compound. The chronotropic effect, which was determined using the

guinea pig right atrium (GPRA), was calculated as the percentage change in the rate of isolated guinea pig right atrium by the test compound, relative to its normal rate that was predetermined in the absence of the test compound.

The tissues (GPILSM, GPLA and GPRA) used in these *in vitro* assays were isolated from male Hartley strain guinea pigs (Charles River Canada Inc., St. Constant, Quebec) weighing 350-700 g. The isolated tissues were suspended in an organ bath filled with HPSS (HEPES buffered physiological saline solution) of the following composition in mM/L: NaCl 137.0, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.6, D-glucose 11.9, HEPES 9.0, that was adjusted to pH 7.4 with normal NaOH. The bath was maintained at 37°C by a water circulator (Haake Model E52 or Braun Thermomix 11) and aerated with pure oxygen. Tissues were allowed to equilibrate for about 45 minutes with a change of the HPSS HEPES solution in the organ bath every fifteen minutes, before any measurements were carried out. Solutions of the test compounds were prepared in DMSO-H_2O where the ratio used was dependent upon the test compound solubility. In the GPLA atrium CCag assay, the response for the solvent (aqueous-DMSO) was subtracted as indicated in some latter studies since the solvent increased the contractile response of GPLA.

3.3.2.0. Pharmacological Evaluation of Alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridine carboxylates (38a-38f)

Since the discovery that Bay K8644 acts as a non-selective CC agonist on both cardiac and vascular smooth muscle, extensive research has been performed to find new analogs with better pharmacological properties. The goal was to design compounds that exhibit cardioselective CC agonist properties and which may exhibit, or be devoid of, an antagonist effect on vascular smooth muscle. Compounds exhibiting these dual properties would be very effective in the treatment of congestive heart failure. As mentioned earlier, "No CC activator has been reported that is cardioselective, that is able to increase myocardial contractility without also increasing vascular resistance and blood pressure".[74] The future clinical use of calcium agonists to treat congestive heart failure will be dependent upon separating their vasoconstricting from their cardiostimulant properties.[76]

In a recent report by Knaus et al[163], the *in vitro* CC modulating activity of a novel group of racemic (±) and individual enantiomers [(+) and (-)] of isopropyl 5-nitro 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates was determined using GPLA (agonist) and GPILSM (antagonist) assays. These authors observed that the (±)-2-(pyridinyl) isomer acted as a dual cardioselective CC agonist/smooth muscle selective CC antagonist. In contrast, the (±)-3-

(pyridinyl) and the (±)-4-(pyridinyl) racemates exhibited CC agonist activity on both GPLA and GPILSM. The (+)-2-(pyridinyl) enantiomer acted as an agonist on both GPLA and GPILSM, whereas the (-)-2-(pyridinyl) enantiomer exhibited agonist activity on GPLA and antagonist activity on GPILSM.

The 1,4-DHP C-3 and C-5 regions have been found to be significant determinants of binding to receptor site. There are differences in the molecular electrostatic potentials in these regions between activator and antagonist compounds, and this may constitute a mechanism whereby the receptor distinguishes between activator and antagonist ligands. It has been reported that CC activators have a strong negative potential in the region adjacent to their C-5 nitro substituent, whereas antagonists exhibit a positive potential in this region when a C-5 ester group was present.[189] In compounds (**38a-38f**), the 1,4-DHP 5-nitro group of Bay K8644 is replaced by a 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)] moiety. It was expected that the 5-oxazolinyl sustituent may be bioisosteric with the 5-nitro group of Bay K8644 and/or the ester group of nifedipine. In addition, the 4-[2-(trifluoromethyl)phenyl substituent present in Bay K8644 was simultaneously replaced by a C-4-2-pyridinyl, 3-pyridinyl or 4-pyridinyl substituent in compounds **38a-38f**. It was anticipated that the position of the pyridinyl nitrogen and its ability to act as an additional electron donor for hydrogen bonding to the CC receptor may influence or modulate CC binding and/or tissue specificity.

The orientations of the substituents at the C(3), C(4) and C-5 positions of the 1,4-DHP ring are important determinants of CC modulation. Studies have shown that in both CC agonists (*eg* Bay K8644) and CC antagonists (*eg* nifedipine), the C(4) aryl or heteroaryl ring is perpendicular or pseudoaxial to the flat boat-shaped 1,4-DHP ring. However, differences have been found in the orientations of the groups attached to C(3) and C(5) positions. In CC antagonists such as nifedipine, both ester groups are thought to be preferentially oriented in a plane that intersects the plane of the DHP ring at angle of between 30° and 60°. The carbonyl moiety (C=O) of the ester groups in CC antagonists are synperiplanar (sp) or *cis* to the C(5)-C(6) and C(2)-C(3) double bonds of the 1,4-DHP ring. In the CC agonist, Bay K8644, the 5-nitro group (NO_2) is in the same plane as the as the C(5)-C(6) double bond, and it has an antiperiplanar (ap) or *trans* orientation with respect to the double bond to which it is attached. It has been suggested that the NH moiety present in Bay K8644 is much more acidic (due to delocalization of the lone pair of electrons on the DHP ring nitrogen) and that it is capable of forming stronger H-bonds than the undelocalized NH moiety of DHP diester antagonists.[121,188,190] Thus, the NH moiety of Bay K8644 has the potential to form three types of geometrically favorable H-bonds, depending on whether the NH is sp^2 hybridized (due to electron delocalization into the ring) or sp^3 hybridized with the NH hydrogen either above or below the DHP plane. These conformational

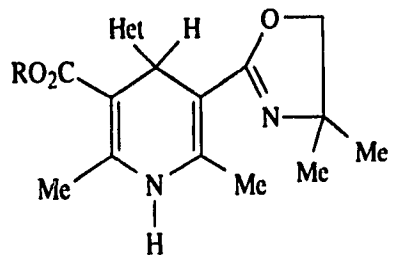
differences affect binding to the receptor site and hence the difference between CC agonists and antagonists. Using the Hyperchem Version 3 for Windows Minimizer (MM^+) Program from Autodesk (IBM PC Version), it has been determined that, in the most stable conformation for compounds **38a-38f**, the carbonyl (O=C) of the ester at C-3 is antiperiplanar (ap) and the C=N of the oxazolinyl group is synperiplanar (sp) to the planes of the double bonds of the 1,4-DHP ring(see section 3.2.0.0.). However, the Hyperchem Program shows that the most stable conformation calculated for Bay K8644 has a synperiplanar (sp) C=O, while one of the ester groups' C=O of nifedipine is antiperiplanar. These calculations contrast with the X-ray crystallography determinations which show that the C=O of the Bay K8644 ester group is antiperiplanar, and both O=C in nifedipine are synperiplanar to the double bonds to which they are attached. These differences may be due to the fact that X-ray crytallography involves the use of crystalline solids whereas calculations using the Hyperchem Program are for compounds in the vapor state. Thus, there may be some conformational differences in the solid and vapor state for these compounds.

Compounds (**38a-38f**) were evaluated for both CC antagonist and CC agonist properties using GPILSM and GPLA, repectively. The chronotropic effect was determined using GPRA.

All compounds (**38a-38f**) exhibited a calcium channel antagonist effect on vascular smooth muscle. Compared to nifedipine [(**1**), $IC_{50} = 1.43 \pm 0.19 \times 10^{-8}$ M], compounds **38a-**

38c showed a 1000-fold decrease and compounds **38d-38f** a 100-fold decrease, respectively in their CCA properties (see Table 2). For compounds with the same ester moiety at the C-3 position of the 1,4-DHP ring (*eg* **38c**, **38d** and **38e**), the C(4)-(2-pyridinyl) compound **38c**, was the least active (IC_{50} = 2.17 ± 0.05 x 10^{-5} M) as a CCA, while the C(4)-(3-pyridinyl), **38d** (IC_{50} = 3.27 ± 0.09 x 10^{-6} M), and C(4)-(4-pyridinyl), **38e** (IC_{50} = 2.59 ± 0.06 x 10^{-6} M) isomers, were approximately equipotent. Compared to the corresponding 3,5-diisopropyl 1,4-DHP diesters,[130] compound **38c**, is 100-fold less active than the corresponding diester, while **38d** and **38e** are both 10-fold less active than their corresponding diesters. However, in the 1,4-DHP diester series, the C-4-(2-pyridinyl) analog (IC_{50} = 1.25 ± 0.20 x 10^{-7} M), is the most active CCA, while [C-4-(3-pyridinyl), IC_{50} = 2.57 ± 0.60 x 10^{-7} M] and [C(4)-(4-pyridinyl), IC_{50} = 2.31 ± 0.67 x 10^{-7} M] were nearly equipotent. The reversal of the normal observed potency order for the C-4-(2-pyridinyl) compound, which was normally the most active in the diester series, compared to the C-5 oxazolinyl series (**38c**, **38d** and **38e**) investigated in this study, indicates that the oxazolinyl moiety may bind less effectively to the receptor site than the ester group.[130] However, the fact that compounds **38a-38f** still exhibit CC antagonist properties, indicates that the oxazolinyl group, to a certain extent, is bioisosteric with an ester moiety. For compounds with the same C(4)-pyridinyl substituent and different C-3 ester substituents, (*eg* **38a**,

Table 2. Pharmacological data for racemic alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates (**38a-38f**).



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Entry	R	Het	Calcium channel antagonist act: IC_{50}[a]	Calcium channel agonist act[b]	Chronotropic effect[c]
38a	Me	3-pyr	$1.60 \pm 0.06 \times 10^{-5}$ M	(+) 11% @ 3.0×10^{-10} M to (+) 23% @ 1.6×10^{-7} M	(-) 2% @ 3.0×10^{-10} M to (-) 29% @ 1.6×10^{-7} M
38b	Me	4-Pyr	$2.78 \pm 0.04 \times 10^{-5}$ M	(+) 1.2% @ 3.0×10^{-10} M to (-) 14% @ 1.6×10^{-7} M	(-) 3% @ 3.0×10^{-10} M to (+) 51% @ 1.6×10^{-7} M
38c	i-Pr	2-Pyr	$2.17 \pm 0.05 \times 10^{-5}$	(+) 0.2% @ 3.0×10^{-10} M to (+) 10% @ 1.64×10^{-7} M	(-) 11% @ 3.0×10^{-10} M to (+) 36% @ 1.64×10^{-7} M

Entry	R	Het	Calcium channel antagonist act: IC_{50}[a]	Calcium channel agonist act[b]	Chronotropic effect[c]
38d	i-Pr	3-Pyr	$3.27 \pm 0.09 \times 10^{-6}$ M	(+) 5% (42%)[d] @ 3.0×10^{-10} M to (+) 16% (153%)[d] @ 1.64×10^{-7} M	(-) 0.3% (5%)[e] @ 3.0×10^{-10} M to (-) 2% (+31%)[e] @ 1.64×10^{-7} M
38e	i-Pr	4-Pyr	$2.59 \pm 0.06 \times 10^{-6}$ M	(+) 3% (9%)[d] @ 3.0×10^{-10} M to (+) 3% (40%)[d] @ 1.64×10^{-7} M	(-) 1% (+1%)[e] @ 3.0×10^{-10} M to (-) 2% (+1%)[e] @ 1.64×10^{-7} M
38f	i-Bu	3-Pyr	$3.22 \pm 0.03 \times 10^{-6}$ M	(+) 6% @ 3.0×10^{-10} M to (+) 50% @ 1.64×10^{-7} M	(+) 0.5% @ 3.0×10^{-10} M to (-) 10% @ 1.64×10^{-7} M
Nifedipine (**1**)			$1.43 \pm 0.19 \times 10^{-8}$ M		
(±)-Bay K8644				(+) 50% @ 7.7×10^{-7} M	(+) 26% @ 7.7×10^{-7} M

[a]The molar concentration of the test compound required to produce a 50% decrease in the slow component or tonic contractile response ($IC_{50} \pm$ SEM) in GPILSM by the muscarinic agonist carbachol (1.64×10^{-7} M) was determined graphically from the dose-response curves (n = 3).

[b]The % change in the contractile response of GPLA induced by the test compound relative to its basal contractile force produced in the absence of the test compound. A (+) value

denotes an increase, whereas a (-) value denotes a decrease in the contractile response of GPLA (n = 3).

[c]The % change in the chronotropic rate produced by the test compound on GPRA, relative to its rate in the absence of test compound. A (+) value indicates an increase, whereas a (-) value indicates a decrease in chronotropic rate.

[d]The value for CC agonist activity obtained when the compound was tested initially using a calculation which involved subtracting the DMSO effect (see Section 3.3.3.0 for a detailed explanation).

[e]The chronotropic rate obtained when the compound was first tested using the initial method when DMSO effect was subtracted (see Section 3.3.3.0 for a detailed explanation).

38d and **38f**), the ester with the smallest alkyl substituent (**38a**, R = Me) was the least active (IC_{50} = 1.60 ± 0.06 x 10^{-5} M) and the compound with the largest ester moiety (**38f** ,R = *i*-Bu) was the most active (IC_{50} = 3.22 ± 0.03 x 10^{-6} M). This agrees with the reported trend in the literature where larger ester substituents provide a greater potency.

Compounds **38a-38f** also exhibited varying degrees of CC agonist properties (see Table 2). The CC agonist effect exhibited by the majority of these compounds was lower than that of Bay K8644 (CCag effect = (+) 50% @ 7.7 x 10^{-7} M), except for compound **38f** (CCag effect = (+) 50% @ 1.64 x 10^{-7} M), which was slightly more potent that Bay K8644. It is pertinent to note that, unlike Bay K8644 (which is an agonist on both heart and smooth muscle), compounds **38a-38f** exhibited a CC antagonist effect on smooth muscle and a CC agonist cardioselective properties. It is also significant to note that these compounds exhibited their CC agonist effect at concentrations that are about 10 to 100 fold lower than their corresponding CCA IC_{50} concentration. Thus, these compounds could exert CCA properties and positive inotropic effects independent of each other, depending on the molar concentrations. Most of the compounds also exhibited a significant CC agonist effect on the heart, at their IC_{50} CCA concentrations. These are desirable antagonist-agonist properties which are valuable for the development of "lead-compounds" for the treatment of congestive heart failure.

A comparison of compounds with the same C-3 ester moiety (**38a** and **38b** or **38c**,**38d** and **38e**) showed that the C(4)-3-(pyridinyl) analog was always more active than the corresponding C(4)-2-(pyridinyl) isomer, while C(4)-4-(pyridinyl) compounds were the least active as CCag agonists on cardiac muscle. It is not obvious from available literature data and/or Hyperchem calculations why this should be the case. However, it could be deduced that the presence of a negative minimum in the *meta*-position of the C(4)-3-(pyridyl) moiety may be an important determinant of CC agonist activity for compounds **38a-38f**. A comparison of the compounds with the same C(4) pyridinyl substituent and different C(3) ester substituents (**38a**, **38d**, and **38f**), gave the following CCag potency order, when considering the size of the alkyl group on the ester moiety : *i*-Bu > Me > *i*-Pr. It is not possible to explain this on the basis of the size of the ester alkyl groups.

From the foregoing results, it can be concluded that the oxazolinyl group is bioisosteric with both the nitro (NO_2) and ester (CO_2R) groups, respectively. In other words, it induces pharmacological characteristics of both NO_2 and CO_2R functionalities. Thus, this class of oxazolinyl compounds **38a-38f**, except **38b** exhibit dual CC smooth muscle antagonist /CC cardioselective agonist properties which is a combination of the pharmacological profiles exhibited by nifedipine and Bay K8644. Compounds **38a-38f**, except for **38b**, are weak CC agonists which could serve as good leads for

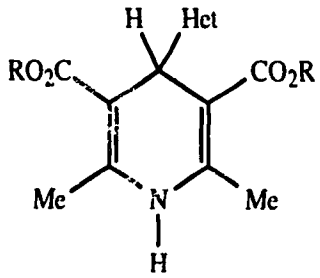
the development of more potent drugs for the treatment of congestive heart failure and as probes to study the drug-receptor interaction with respect to antagonist-agonist activity.

3.3.3.0. Pharmacological Evaluation of Dialkyl 1,4-dihydro-2,6-dimethyl-4-[(*Z*)-N-oxo-N-(aryl/vinylmethylene)-λ^5-azanyl]phenyl}-3,5-pyridinedicarboxylates (53a-53i)

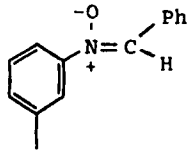
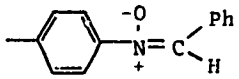
The CCA and CCag activities for compounds **53a-53i** were determined using the assays described previously and the results are presented in Table 3.

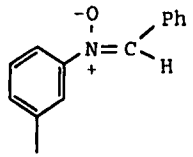
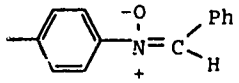
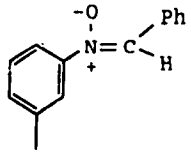
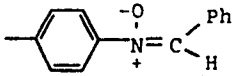
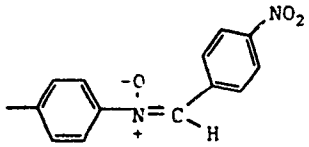
All **53a-53i** compounds exhibited a significant calcium channel antagonist effect on GPILSM. The CCA IC_{50} values for these compounds were generally about 10 to 1000 (10^{-5} to 10^{-8} M range) fold lower than that for nifedipine, except for compound **53c** (IC_{50} = 3.69 ± 0.17 x 10^{-8} M), which was of the same order of potency as nifedipine (IC_{50} = 1.43 ± 0.19 10^{-8} M). Generally, the C(4)-3-(aryl nitrones) were more potent than the corresponding C(4)-4-(arylnitrones). For example, **53a** (IC_{50} = 2.21 ± 0.01 x 10^{-7} M) was 125-fold more active than the corresponding **53b** (IC_{50} = 2.77 ± 0.21 x 10^{-5} M) as CCA. Also **53c** was 751-fold more active than **53d**; and **53e** was 7-fold more active than **53f**. This is consistent with the trend reported in the literature by Knaus *et al*[125-130] where the order of CCA potency for C-4 (substituted aryl/or

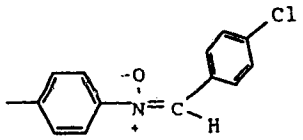
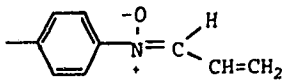
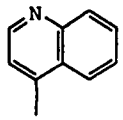
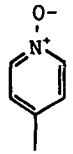
Table 3. Pharmacological data for dialkyl 1,4-dihydro-2,6-dimethyl-4-{[(*Z*/*E*)-N-oxo-N-(aryl/vinylmethylene)-λ^5-azanyl]phenyl}-3,5-pyridinedicarboxylates (**53a-53i**); **61a** and **62**.



53a-i, 61a, 62

Entry	R	Het	Calcium channel antagonist act: IC_{50}[a]	Calcium channel agonist act[b]	Chronotropic effect[c]
53a	Me		2.21 ± 0.01 × 10^{-7} M	(+) 24% @ 2.99 × 10^{-10} M to (+) 79% @ 1.64 × 10^{-7} M	(+) 8% @ 2.99 10^{-10} M to (+) 43% @ 1.64 × 10^{-7} M
53b	Me		2.77 ± 0.21 × 10^{-5} M	(+) 8% @ 2.99 × 10^{-10} M to (+) 34% @ 1.64 × 10^{-7} M	(+) 4% @ 2.99 10^{-10} M to (+) 8% @ 1.64 × 10^{-7} M

Entry	R	Het	Calcium channel antagonist act: IC_{50}[a]	Calcium channel agonist act[b]	Chronotropic effect[c]
53c	Et		3.69 ± 0.17 × 10^{-8} M	(+) 0% (41%)[d] @ 2.99 × 10^{-10} M to (+) 56% (138%)[d] @ 1.64 × 10^{-7} M	(+) 0% (10%)[e] @ 2.99 10^{-10} M to (-) 14% (+42%)[e] @ 1.64 × 10^{-7} M
53d	Et		3.53 ± 0.07 × 10^{-5} M	(+) 8%(32%)[d] @ 2.99 × 10^{-10} M to (+) 30%(205%)[d] @ 1.64 × 10^{-7} M	(-) 5% (0%)[e] @ 2.99 10^{-10} M to (+) 6% (-1%)[e] @ 1.64 × 10^{-7} M
53e	i-Pr		1.55 ± 0.11 × 10^{-7} M	(+) 11% @ 3.0 × 10^{-10} M to (+) 17% @ 1.64 × 10^{-8} M	(-) 2% @ 3.0 × 10^{-10} M to (-) 29% @ 1.64 × 10^{-7} M
53f	i-Pr		1.04 ± 0.02 × 10^{-6} M	(+) 21% @ 2.99 × 10^{-10} M to (-) 1% @ 1.64 × 10^{-7} M	(-) 4% @ 2.99 × 10^{-10} M to (-) 4% @ 1.64 × 10^{-7} M
53g	Et		4.30 ± 0.11 × 10^{-7} M	(+) 2% @ 2.99 × 10^{-10} M to (+) 48% @ 1.64 × 10^{-7} M	(-) 1% @ 2.99 × 10^{-10} M to (+) 23% @ 1.64 × 10^{-7} M

Entry	R	Het	Calcium channel antagonist act: IC_{50}[a]	Calcium channel agonist act[b]	Chronotropic effect[c]
53h	Et		1.10 ± 0.55 x 10^{-5} M	(+) 7% @ 2.99 x 10^{-10} M to (-) 17 @ 1.64 x 10^{-7} M	(-) 1% @ 2.99 x 10^{-10} M to (+) 3% @ 1.64 x 10^{-7} M
53i	Et		4.41 ± 0.19 x 10^{-5} M	(-) 18% @ 2.99 x 10^{-10} M to (-) 125% @ 1.64 x 10^{-7} M	(-) 0.1% @ 2.99 x 10^{-3} M to (+) 5% @ 1.64 x 10^{-7} M
61a	Et		1.21 ± 0.11 x 10^{-6} M	(+) 6% @ 2.99 x 10^{-10} M to (+) 46% @ 1.64 x 10^{-7} M	(+) 5% @ 2.99 x 10^{-10} M to (+) 11% @ 1.64 x 10^{-7} M
62	Et		Inactive, IC_{50} > 1.0 x 10^{-4} M	(+) 11% @ 2.99 x 10^{-10} M to (+) 27% @ 1.64 x 10^{-7} M	(+) 7% @ 2.99 x 10^{-10} M to (+) 16% @ 1.64 x 10^{-7} M
Nifedipine (**1**)			1.43 ± 0.19 x 10^{-8} M		
Bay K8644				(+) 87% @ 10^{-7} M	(+) 26% @ 10^{-7} M

[a]The molar concentration of the test compound required to produce a 50% decrease in the slow component or tonic contractile response (IC_{50} ± SEM) in GPILSM by the muscarinic agonist carbachol (1.64×10^{-7} M) was determined graphically from the dose-response curves (n = 3).

[b]The % change in the contractile response of GPLA induced by the test compound relative to its basal contractile force produced in the absence of the test compound. A (+) value denotes an increase, whereas a (-) value denotes a decrease in the contractile response of GPLA (n = 3).

[c]The % change in chronotropic rate produced by the test compound on GPRA, relative to its rate in the absence of test compound. A (+) value indicates an increase, whereas a (-) value indicates a decrease in chronotropic rate.

[d]The initial value obtained for CC agonist activity when the test compound was first tested using the first method of subtracting the DMSO effect for the stock solution (see Section 3.3.3.0. for a detailed explanation).

[e]The initial value obtained for chronotropic rate when the test compound was first tested using the first method of subtracting DMSO effect for the stock solution (see Section 3.3.3.0 for a detailed explanation.

pyridinyl) compounds is generally: 2-substituted > 3-substituted > 4-substituted for 1,4-DHP-3,5-diesters. The replacement of the C-phenyl substituent on the nitrone moiety with a *para*-substituted-phenyl or vinyl group (compounds **53g-53i**), altered CCA activity, which was dependent upon the nature of the *para*-substituent. In compound **53g** (IC_{50} = 4.30 ± 0.11 x 10^{-7} M), with a para-substituted nitro substituent on the C-phenyl moiety of the nitrone, the CCA activity was increased by 82-fold, when compared to that of the corresponding unsubstituted phenyl compound, **53d** (IC_{50} = 3.53 ± 0.07 x 10^{-5} M). In contrast, the *para*-chlorophenyl [(**53h**, IC_{50} = 1.07 ± 0.16 x 10^{-5} M)] and vinyl [(**53i**, IC_{50} = 4.41 ± .19 x 10^{-5} M)] analogs, exhibited CCA activity comparable to the unsubstituted phenyl compound **53d**. The increased potency of **53g** may be due to the electronic properties of NO_2 group, which has additional positive (+) and negative (-) charges that could be involved in binding to the CC receptor. These electronic effects are not present in the *para*-chlorophenyl and vinyl substituents, which may acount for their reduced CCA activity. It would be necessary to synthesize additional *meta*- and *para*-phenyl substituted analogs in order to obtain more meaningful structure-activity correlations.

Compounds **53a-53i** also exhibited a CC agonist effect on GPLA. These compounds were generally less potent than Bay K8644 as a CC agonist. A comparison of compounds with identical ester groups at the C-3 and C-5 positions (**53a** and **53b**, **53c** and **53d**, **53e** and **53f**) showed that in each case,

the C(4)-3-(arylnitrone) analog was more potent than the corresponding C(4)-4-(arylnitrone) analog as a CC agonist. The relative potency was **53a** > **53b**; **53c** ≥ **53d**; **53e** > **53f**, respectively. For example, the CCag effect exhibited by **53c** at 1.64 x 10-7 M was (+) 56%, whereas that for **53d** at the same concentration was (+) 30%. These observations indicate that the presence of a negative minimum at or about the *meta*-position of the aryl ring tend to increase CC agonist activity more than when the negative minimum is located at the *para*-position of the aryl ring. This is in partial agreement with earlier observations by Knaus[161] and Testa[162] that, the presence of negative minimum at or beyond the *meta*-position of heteroaryl and/or an aryl ring increased β_1-selective antagonist activity. The negative minimum at the *para*-position of the aryl ring in these C(4)-arylnitrones may be a less relevant determinant of CCag activity, since the C(4)-4-(arylnitrones) are less active than the corresponding C(4)-3-(arylnitrones).

A comparison of the CC agonist activities for compound **53d** and compounds **61a** and **62**, could provide important structure-activity correlations. In compound **53d**, the quaternary nitrogen atom and negative oxygen atom of the nitrone moiety are attached to the aryl ring system, whereas in compound **62**, the quaternary nitrogen atom of the N-oxide moiety is part of the pyridyl ring system and the negative oxygen atom is attached to the ring nitrogen atom. Compound **61a**, has a tertiary nitrogen atom, which lacks a positive charge (as in **53d**

and **62**) and the nitrogen atom is part of the quinolinyl ring system. The common feature shared by these compounds (**53d**, **61a** and **62**), is the presence of a negative minimum at or near the *para*-position of the C(4) aryl or heteroaryl ring, respectively. The test results showed that, at a concentration of 1.64×10^{-7} M, compounds **53d**, **61a** and and **62** exhibited CC agonist activity of (+) 30%, (+) 46% and (+) 27%, respectively. These results suggest that the presence of an additional positive minima at the *para*-position of the aryl and/or heteroaryl ring system (as in **53d** and **62**) appears to decrease CCag activity for the nitrone (**53d**) and N-oxide (**62**) compounds. This observation is a contrast to earlier observations by Testa et al[162] that, the presence of an additional positive minimum at the *para*-position of aryloxypropanolamines enhanced β_1-adrenergic antagonist activity. It is possible that a negative nitrogen atom located in the heteroaryl ring system (**61a**), enhances binding to the receptor site for CCags. Further studies are necessary to determine whether the positive charge and/or the interspacial distance to the negative charge is/are determinants(s) of CCag activity for the nitrone and N-oxide classes of compounds.

Para-substitution on the C-phenyl ring of the nitrone or replacement of the C-phenyl group with a vinyl group altered the CCag activity exhibited by compounds **53a-53i**. For example, replacement of the C-phenyl group present in compound **53d** with a *para*-nitrophenyl group (**53g**) appears to provide

an increase in CCag activity. At a concentration of 1.64 x 10^{-7} M, the CCag activity exhibited by **53d** was 30%, whereas that induced by **53g** was 48%. This increase in CCag activity may be due to the introduction of an additional negative minima (from the NO_2 group) at the *para*-position of the phenyl ring. However, when the C-phenyl group of compound **53d** was replaced by either a *para*-chlorophenyl (**53h**) or vinyl (**53i**) substituent, these compounds acted as negative inotropes. At a concentration of 1.64 x 10^{-7} M, **53h** decreased the contractile force by -17% and **53i** decreased contractile force by -125% relative to a (+) 30% increase for compound **53d** at the same concentration. Thus, there is a complete reversal of the pharmacological profile, from a positive inotropic effect (**53d**) to a negative inotropic effect (**53h** and **53i**). The *para*-chlorophenyl and vinyl groups do not introduce an additional negative minima. Further studies, involving more phenyl substituted analogs and/or replacement of the C-phenyl group with other moieties are required to obtain a more definitive structure-activity correlations.

Compounds **53a-53g**, exhibited respectable CC agonist activity at their CCA IC_{50}, and at lower concentration. For example, compound **53c** with a CCA IC_{50} of 3.69 ± 0.17 x 10^{-8} M, exhibited a CCag effect of 24% at a concentration of 4.58 x 10^{-8} M and 18% at 1.63 x 10^{-8} M. Also, compound **53d** with a CCA Ic_{50} of 3.53 ± 0.07 x 10^{-5} M exhibited a CCag effect of 58% at 4.52 x 10^{-5} M and 44% at 1.62 x 10^{-5} M. These dual CC

smooth muscle antagonist/CC cardioselective agonist properties would be desirable for drugs that are used for the treatment of congestive heart failure. Thus, the nitrone moiety present in this class of C(4)-arylnitrones is a suitable CCag isotere which could provide a good pharmacophore for the development of superior drugs for the treatment of congestive heart failure.

It must be pointed out that during the pharmacological evaluation of the nitrone compounds (and also **38a-38f**) for CCag activity that dimethylsulfoxide (DMSO) was used to dissolve the test compounds. Initially, the DMSO was purified by double distillation, and stored for use as solvent, until the batch was all used. At this time, a new batch was prepared in a similar way. However, it was observed that the DMSO-HEPES solution used to dissolve the test compounds exhibited a positive inotropic effect on heart. Consequently, the positive inotropic effect exhibited by the DMSO-HEPES solution (at the ratio used for the test compound) was determined for each new batch of DMSO as soon as it was distilled. This value was subtracted from CCag effect of the test compound to determine the effect induced solely by the drug. It was assumed that, for each batch of DMSO, the value of the positive inotropic effect due to the DMSO only would remain constant for the period of time the DMSO stock-solution was used. However, it was later found that, the DMSO effect fluctuated considerably over a period of time, even for the same batch. This prompted a change in the procedure. The

DMSO effect was then determined at the same time and on the same day that the compound was tested, and a dose-response curve prepared for the DMSO effect. For each concentration of the test compound, the positive inotropic effect due to DMSO was calculated (from the dose-response curve) and subtracted from the overall agonist effect, in order to obtain the effect due to the drug alone. This eliminated the error due to fluctuation of the DMSO positive inotropic effect over a period of time. Some of the compounds had to be re-tested to determine the extent of this potential error due to fluctuation in the DMSO-HEPES vehicle effect. For example, when compound **53d** was first tested using the initial procedure, it exhibited a CCag effect of (+) 32% at a concentration of 2.99×10^{-10} M and (+) 205% at 1.64×10^{-7} M. On re-testing, using the new procedure, **53d** exhibited a CCag effect of (+) 8% at 2.99×10^{-10} M and (+) 30% at 1.64×10^{-7} M. Also, when **53c** was initially tested using the first method, it exhibited a CCag effect of (+) 41% at 2.99×10^{-10} M and (+) 138% at 1.64×10^{-7} M. On re-testing using the new method, **53c** exhibited a CCag effect of 0% at 2.99×10^{-10} M and (+) 56% at 1.64×10^{-7} M. In Table 3, the re-test values are shown in brackets. These results indicate that a considerable error was operative when the initial method was used. It has not been possible to re-test all of the nitrone compounds (and compounds **38a-38f**), but the results obtained for the two nitrone compounds that have been re-tested (**53c**, **53d**) show that compounds **53a-53g** exhibit CCag activity,

though much less than the values obtained earlier and reported for compounds **53a-53f** in Table 3. Compounds **53g-53i** were each tested using the latter procedure, thus the values reported in Table 3 for these compounds are considered to be accurate. The positive inotropic effect exhibited by the DMSO-HEPES will be investigated as a "lead" to design a potentially new class of CCag drug.

3.3.4.0. Pharmacological Evaluation of Dialkyl 1,4-dihydro-2,6-dimethyl-4-(quinolinyl)-3,5-pyridinedicarboxylates (61a-61e)

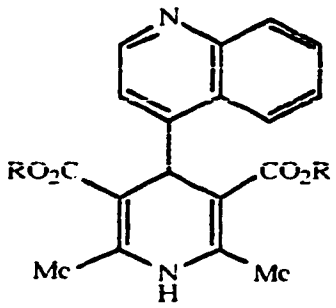
Compounds **61b-61e** were evaluated as calcium channel antagonists only, whereas **61a** was tested as both a CC agonist and a CC antagonist to obtain further structure-activity correlations. The CCag effect of **61a** is shown in Table 3 The CCA pharmacological results for **61a-61e** are presented in Table 4. For compound **61a**, the position of the nitrogen atom in the C(4)-4-(quinolinyl) substituent is equivalent to the position of the nitrogen atom in the C(4)-4-(pyridinyl) substituent of compounds reported earlier.[125-128,130] Also, the position of the nitrogen atom in the C(4)-8-(quinolinyl) substituent for compounds **61b-61e** is equivalent to that of 1,4-DHP C(4)-2-(pyridinyl) compounds reported previously[125-128,130].

A comparison of **61a** (IC_{50} = 1.21 ± 0.11 x 10^{-7} M) and **61b** (IC_{50} = 1.20 ± 0.12 x 10^{-7} M), which have identical ester moieties at 1,4-DHP C-3 and C-5 positions, showed that these

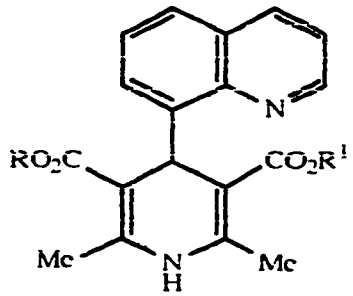
isomers were equipotent CC antagonists. Thus, the position of nitrogen atom in the quinolinyl ring does not affect CCA activity. Compound **61b** was also equipotent with the corresponding 1,4-DHP C(4)-2-(pyridinyl) analog (IC_{50} = 1.77 ± 0.03 x 10^{-7} M).[130] No data was available for comparison of the corresponding 1,4-DHP C(4)-4-(pyridinyl) analog of compound **61a**. An increase in the size of alkyl group of the ester moiety on the 1,4-DHP ring at the C(3) and C(5) positions, resulted in a reduction of CC antagonist activity. For example, **61b** (R = R^1 = Et) has a CCA IC_{50} = 1.20 ± 0.12 10^{-7} M and **61e** (R = R^1 = *i*-Pr) exhibited an IC_{50} of 3.23 ± 0.48 x 10^{-5} M, a 269-fold reduction. Also **61c** (R = Me, R^1 = *i*-Pr) is 13-fold more potent than **61d** (R = Et, R^1 = *i*-Pr). When compared to nifedipine, compounds **61a-61e** are 10 to 1000-fold less potent with respect to CCA activity.

The results obtained also suggest that, when a large 1,4-DHP C(4) substituent such as the quinolinyl group is present, a smaller alkyl ester substituent at the C(3) and C(5) positions increases CCA activity. Consequently, the C(4)-quinolinyl compound in which R = R^1 = Me would be expected to be the most active as a CCA. Chiral compounds for the C-4 quinolinyl series (**61a-61e**) in which R ≠ R^1 (**61c**, **61d**), do not appear to exhibit better CCA potencies when compared to the corresponding symmetrical diesters in which R = R^1 (**61a**, **61b**, **61e**); whereas in the 1,4-DHP C(4)-pyridinyl compounds, the chiral compounds normally tend to exhibit superior CCA effects than the corresponding symmetrical diesters.[130]

Table 4. Pharmacological data for Dialkyl 1,4-dihydro-2,6-dimethyl-4-(quinolinyl)-3,5-pyridinedicarboxylates **(61a–61e)**



61a, R = Et



61b–e

Entry	R	R^1	IC_{50}
61a	Et	Et	$1.21 \pm 0.11 \times 10^{-7}$ M
61b	Et	Et	$1.20 \pm 0.12 \times 10^{-7}$ M
61c	Me	i-Pr	$5.23 \pm 0.17 \times 10^{-6}$ M
61d	Et	i-Pr	$6.75 \pm 0.28 \times 10^{-5}$ M
61e	i-Pr	i-Pr	$3.23 \pm 0.48 \times 10^{-5}$ M
Nifedipine			$1.43 \pm 0.19 \times 10^{-8}$ M

In conclusion, the pharmacological results obtained for compounds **38a-38f** and **53a-53g** are very encouraging. Both classes of compounds (except **38b, 53h, 53i**) exhibit dual CC smooth muscle antagonist/CC cardioselective agonist activity at their IC_{50} concentration. This dual activity would be desirable for drugs that are used for the treatment of CHF. Further elaboration of these two classes of compounds may

produce more potent and efficacious drugs. For example, in compounds **38a-38f**, it would be of interest to investigate additional analogs where R = Et, $PhCH_2$-, substituted-aryl/phenethyl- etc; and where the C(4)-pyridinyl substituent was replaced by a substituted phenyl and other heteroaryl groups. In the **53a-53i** series of compounds, the nitrone C-phenyl group could be replaced by 2- and 3-substituted phenyl moieties which would alter physicochemical properties (π, σ, E_S) particularly of the nitrone moiety.

4.0.0.0. EXPERIMENTAL

4.1.0.0. Physical Constants and Spectroscopy

Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ^{1}H nmr and ^{13}C nmr spectra were recorded using a Bruker AM-300 spectrometer for solutions in deutero-chloroform ($CDCl_3$) or dimethylsulfoxide-d_6 (DMSO-d6) with tetramethylsilane (TMS) as internal standard. High resolution mass (exact mass) spectra (HRMS) were recorded using an AEI M550 spectrometer and these exact mass determinations are in some cases used in the place of elemental analysis. Infrared (IR) spectra were recorded on a Nicolet 5DX FT spectrophometer, using KBr discs. Microanalytical Laboratory personnel at the University of Alberta performed the microanalyses (C, H, N).

4.2.0.0. Reagents and Solvents

Most reagents were purchased from the Aldrich Chemical Company. These include all of the Grignard reagents, pyridinecarboxaldehydes, 2,4,4-trimethyl-2-oxazoline, nitro arylcarboxaldehydes, diketene, and 8-methylquinoline. Reagents were distilled prior to use. Solvents were purified and dried (if required) before use. For example tetrahydrofuran (THF) and diethyl ether were dried over sodium-benzophenone and distilled prior to use. Distilled THF was stored in sealed bottles over molecular sieves for use within one week. Absolute ethanol was prepared from 98% ethanol by refluxing over magnesium turnings and distillation

immmediately prior to use. Benzene was dried by distillation from calcium hydride and stored over molecular sieves.

4.3.0.0 Chromatography

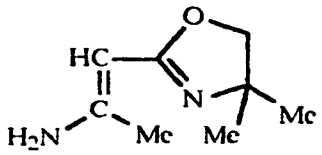
Silica gel column chromatography was carried out using Merck 7734 (60-200 mesh) silica gel. Preparative thin layer chromatography (TLC) was performed using Camag kieselgel DF-5 plates, 1.00 mm in thickness that were activated at 120°C for 6 hours or overnight prior to use. The progress of the reactions and the purity of products prepared were monitored using Whatman and/or Machery-Nagel precoated silica gel microslides (250 μm in thickness). The spots were detected by shortwave ultraviolet light visualization.

4.4.0.0 Synthetic Chemistry

4.4.1.0 Synthesis of 1-[2-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)]-1-propen-2-amine(32)

To a solution of 5.56 g (55 mmol) of diisopropylamine in 20 ml of dry THF at 0°C under a nitrogen atmosphere, 55 mmol (22 ml of a 2.5 M solution in hexanes) of *n*-butyllithium was added dropwise via a syringe. After 10 minutes at 0°C, the solution was cooled to -78°C (acetone/dry ice mixture) and a solution of 2,4,4-trimethyl-2-oxazoline (**31**, 5.66 g, 50 mmol) in 50 ml THF was added slowly. After the addition was complete (about 15 minutes), the yellow solution was stirred at -78°C for a further 2 hours. Acetonitrile (100 mmol, 4.1 g) was added quickly and the reaction mixture was allowed to

warm to room temperature. The reaction mixture was quenched with 10 ml of saturated ammonium chloride solution and sufficient water (15 ml) and ether (50 ml) were added to dissolve the solids. The organic and aqueous phases were separated and the aqueous layer was further extracted with ether (3 x 30 ml). The combined ether extracts were washed with saturated brine solution (30 ml) and dried over anhydrous sodium carbonate. After filtration, the solvent was removed *in vacuo* to afford the crude product which was distilled under reduced pressure (70-75°C at 0.5 mm Hg) to afford **32** (800 mg, 12%); lit[164] bp = 105-112°C, at 4 mm Hg.



32

^{1}H nmr ($CDCl_3$): δ 4.90 (br s, 2H, NH_2), 4.37 (s, 1H, =CH), 3.80 (s, 2H, oxazolinyl H-5), 1.90 (s, 3H, C=CMe), 1.26 (s, 6H, C-4 oxazolinyl methyls).

4.4.2.0 Synthesis of Alkylacetoacetates (49). General Procedure.

To an ice cold solution of the alkyl alcohol (100 mmol) and a catalytic amount of DMAP (5-8 mmol) in anhydrous THF (25 ml) was added slowly, freshly distilled diketene (**47**, 200 mmol). The reaction mixture was stirred for 5 minutes to 2 hours at 0°-65°C. The reaction progress was monitored on

tlc, and the reaction was allowed to proceed until most of the starting materials had disappeared. The solvent was removed *in vacuo* and the crude product was distilled under reduced pressure to afford the target alkylacetoacetates (**49**) in 80-90% yields. This method was used to prepare isopropyl acetoacetate and isobutyl acetoacetate. Ethyl acetoacetate and methyl acetoacetate were purchased from the Aldrich Chemical Company. ^{1}H nmr spectra for the compounds are given below.

Isopropyl acetoacetate

$CH_3COCH_2CO_2CH(CH_3)_2$

Distilled at 58°C, at 0.5 mm Hg. Yield = 90%.
(Lit[191] bp = 184°C).
^{1}H nmr ($CDCl_3$): δ 4.95 (septet, J = 6.1 Hz, 1H, $\underline{CH}Me_2$), 3.32 (s, 2H, $CO\underline{CH_2}CO_2$-), 2.16 (s, 3H, $\underline{Me}CO$), 1.16 (d, J = 6.1 Hz, $CH\underline{Me_2}$.

Isobutyl acetoacetate

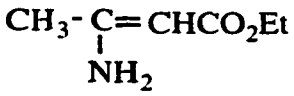
$CH_3COCH_2CO_2CH_2CH(CH_3)_2$

Distilled at 62°C, at 0.5 mm Hg. Yield = 80% (lit[191] b.p. = 202-206°C).
^{1}H nmr ($CDCl_3$): δ 3.77 (d, J = 6.8 Hz, 2H, $\underline{CH_2}CH$), 3.33 (s, 2H, $CO\underline{CH_2}CO_2$), 2.1 (s, 3H, $\underline{CH_3}CO$), 1.80 (m, J = 6.8 Hz, 1H, $CH_2\underline{CH}Me_2$), 0.78 (d, J = 6.8Hz, 6H, $CH\underline{Me_2}$).

4.4.3.0 Synthesis of Alkyl 3-aminocrotonates (50). General Procedure.

Ammonia gas was passed through a stirred solution of 0.1 mole of the alkyl acetoacetate (**49**), in 50 ml of methanol at 25°C. The progress of the reaction was monitored by thin layer chromatography (tlc), and the reaction was stopped when almost all the starting material had been consumed. Removal of the solvent *in vacuo* gave the target alkyl 3-aminocrotonates (**50**) in yields of 95% or greater. These compounds were used without further purification. The ^{1}H nmr spectra and physical data for these compounds are given below.

Ethyl 3-aminocrotonate



^{1}H nmr ($CDCl_3$): δ 7.8 (br s, 2H, -$\underline{NH_2}$), 4.34 (s, 1H, =$\underline{CH}CO_2$), 3.94 (q, J = 7.0 Hz, 2H, $\underline{CH_2}$Me), 1.74 (s, 3H, $\underline{Me}$C=CH), 1.10 (t, J = 7.0 Hz, 3H, $CH_2\underline{Me}$).

Yield = 98%.

Isopropyl 3-aminocrotonate

$$CH_3\text{-}\underset{\underset{NH_2}{|}}{C}{=}CHCO_2CH(CH_3)_2$$

^{1}H nmr ($CDCl_3$): δ 7.8 (br s, 2H, $\underline{NH_2}$), 4.99 (septet, J = 6.2 Hz, 1H, $\underline{CH}Me_2$], 4.44 (s, 1H, =$\underline{CH}$), 5.02 (s, 3H, $\underline{Me}CO$), 1.24 (d, J = 6.2 Hz, $CH\underline{Me_2}$). Yield = 95%.

Isobutyl 3-aminocrotonate

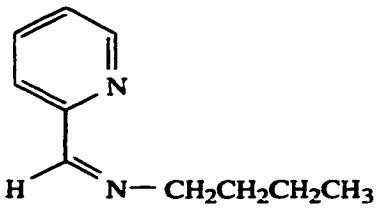
$$CH_3\text{-}\underset{\underset{NH_2}{|}}{C}{=}CHCO_2CH_2CH(CH_3)_2$$

^{1}H nmr ($CDCl_3$): δ 7.8 (br s, 2H, $\underline{NH_2}$), 4.54 (s, 1H, =$\underline{CH}$), 3.82 (d, J = 6.70, 2H, -$CO\underline{CH_2}CH$-), 1.85-1.86 (m J = 1H, (-$\underline{CH}Me_2$), 1.90 (s, 3H, $\underline{CH_3}CO$-), 0.93 (d, J = 6.70 Hz, -$CHMe_2$). Yield = 95%.

4.4.4.0 Synthesis of Pyridinyl-N-(*n*-butyl)-imines (35)

A mixture of 0.1 mole (10.71 g) of 2- 3- or 4-pyridinecarboxaldehyde and 0.1 mole (7.31 g) of *n*-butylamine in benzene (100 ml) was refluxed for 3-5 hours using a Dean-Stark apparatus. Removal of the solvent *in vacuo* and distillation of the crude product under reduced pressure afforded the target imines (**35**) in 80-92% yields.

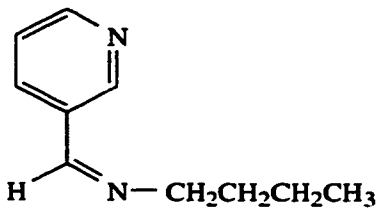
2-Pyridyl-N-(n-butyl)-imine



Distilled under reduced pressure at 65°C, and 0.9 mm Hg. Yield = 85%.

^{1}H nmr ($CDCl_3$): δ 8.64 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.38 (s, 1H, CH=N), 7.98 (d, $J_{3,4}$ = 8.0 Hz, 1H, pyridyl H-3), 7.72 (ddd, $J_{3,4} = J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 1.40 Hz, 1H, pyridyl H-4), 7.23 (ddd, $J_{4,5} = J_{5,6}$ = 5.0 Hz; $J_{3,5}$ = 1.60 Hz, 1H, pyridyl H-5), 3.68 (t, J = 7.1 Hz, 2H, =N-$CH_2CH_2CH_2Me$), 1.72 (quintet, J = 7.1 Hz, 2H, $CH_2CH_2CH_2Me$), 1.40 (sextet, J = 7.1 Hz, 2H, $CH_2CH_2CH_2Me$), 0.95 (t, J = 7.1 Hz, 3H, $CH_2CH_2CH_2Me$).

3-Pyridyl-N-(n-butyl)-imine

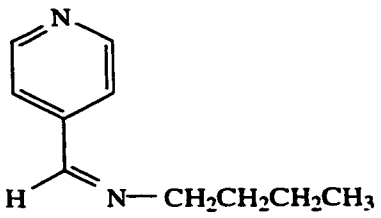


Distilled under reduced pressure at 79-81°C, and 5 mm Hg. Yield = 87%.

^{1}H nmr ($CDCl_3$): δ 8.87 (d, $J_{2,4}$ = 1.8 Hz, 1H, pyridyl H-2), 8.62 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 1.7 Hz, 1H, pyridyl H-6),

8.29 (s, 1H, $\underline{CH}$=N), 8.08 (ddd, $J_{4,5}$ = 8.0 Hz, $J_{2,4}$ = 1.8 Hz, $J_{4,6}$ = 1.7 Hz, 1H, pyridyl H-4), 7.31 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 3.63 (t, J = 7.1 Hz, 2H, =N$\underline{CH_2}CH_2CH_2Me$), 1.69 (quintet, J = 7.1 Hz, 2H, $CH_2\underline{CH_2}CH_2Me$), 1.39 (sextet, J = 7.1 Hz, 2H, $CH_2CH_2\underline{CH_2}CH_3$), 0.95 (t, J = 7.1 Hz, 3H, $CH_2CH_2CH_2\underline{CH_3}$).

4-pyridyl-N-(n-butyl)-imine



The product was distilled under reduced pressure at 75-80°C and 0.4 mm Hg. Yield = 90%.

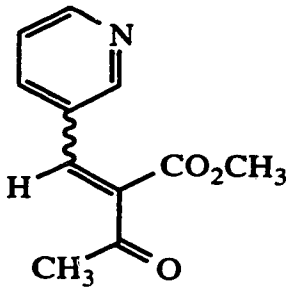
^1H nmr ($CDCl_3$): δ 8.27 (s, 1H, $\underline{CH}$=N), 7.73 (dd, $J_{2,3}$ = $J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-2 and H-6), 7.40 (ddd, $J_{2,3}$ = $J_{5,6}$ = 5.0 Hz, $J_{3,5}$ = 1.5 Hz, 2H, pyridyl H-3 and H-5), 3.62 (t, J = 7.1 Hz, 2H, =N$\underline{CH_2}CH_2CH_2Me$), 1.68 (quintet, J = 7.1 Hz, 2H, $CH_2\underline{CH_2}CH_2Me$), 1.36 (sextet, J = 7.1 Hz, 2H, $CH_2CH_2\underline{CH_2}Me$), 0.94 (t, J = 7.1 Hz, 3H, $CH_2CH_2CH_2\underline{Me}$).

4.4.5.0 Synthesis of 2-Alkoxycarbonyl-1-(pyridinyl)-but-1-ene-3-ones (37). General Procedure

To 100 mmol of the pyridinyl imine **35** and alkyl acetoacetate **37** (100 mmol), was added 2 ml of acetic

anhydride. The reaction mixture was stirred at room temperature for about 18-24 hours, at which time the reaction was complete. Water (20 ml) was added. Extraction with ethyl acetate (3 x 50 ml), washing the combined ethyl acetate extracts with brine solution (30 ml), drying (anhydrous magnesium sulfate) and removal of the solvent *in vacuo* afforded the crude product (a liquid or oil), which was used to prepare 1,4-DHPS without separating the (*E*)- and (*Z*)-isomers. The crude product was purified either by distillation or silica gel column chromatography. The ^{1}H nmr spectra, which were consistent with the assigned structures, were similar except for resonances due to the different alkyl and pyridinyl groups. The ^{1}H nmr spectra of the compounds are given below.

2-Methoxycarbonyl-1-(3-pyridyl)-but-1-ene-3-one

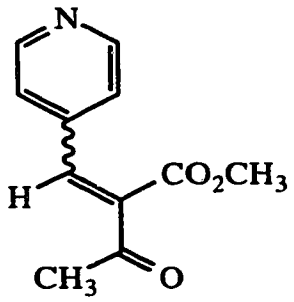


The product was purified by silica gel column chromatography using ethyl acetate/hexane (1:3, v/v) as eluent, R_f = 0.35. Yield = 65% , [(*E*):(*Z*) = 2:3 as determined

by integration of the methyl protons at δ 2.40 and 2.46, respectively].

^{1}H nmr ($CDCl_3$): δ 8.68 and 8.69 (two s, 1H total, (*E*)- and (*Z*)- isomers, pyridyl H-2), 8.61-8.65 (m, 1H, pyridyl H-6), 7.74-7.80 (m, 1H, pyridyl H-4), 7.63 and 7.67 (two s, 1H total, (*E*)- and (*Z*)- isomers, CH=CCO_2Me), 7.34 - 7.41 (m, 1H, pyridyl H-5), 3.80 and 3.86 (two s, 3H total, (*E*)- and (*Z*)- isomers, MeCO_2), 2.40 and 2.46 (two s, 3H total, (*E*)- and (*Z*)- isomers, COMe).

2-Methoxycarbonyl-1-(4-pyridyl)-but-1-ene-3-one

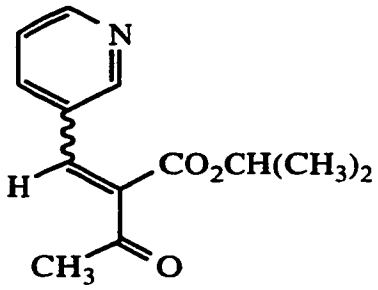


The product was purified by silica gel column chromatography using ethyl acetate/hexane (1:3, v/v) as eluent, R_f 0.37, yellow viscous oil. Yield = 85%, [(*E*):(*Z*) = 2:3 as determined by integration of the methyl protons at δ 2.37 and 2.45, respectively].

1H nmr ($CDCl_3$): δ8.66 and 8.68 (two d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, (*E*)- and (*Z*)- pyridyl H-2 and and H-6), 7.60 and 7.55 (two s, 1H total, (*E*)- and (*Z*)- CH=CCO_2Me), 7.27 and 7.29 (two d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, (*E*)- and (*Z*) H-3 and H-5), 3.83 and

3.86 (two s, 3H total, (*E*)- and (*Z*)- $\underline{Me}CO_2$), 2.37 and 2.45 (two s, 3H total, (*E*)- and (*Z*)- CO$\underline{Me}$)

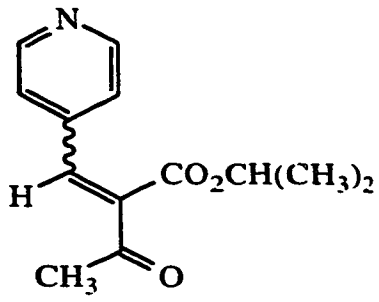
2-Isopropoxycarbonyl-1-(3-pyridyl)-but-1-ene-3-one



The product was distilled at 80-82°C, and 0.7 mm Hg pressure. Yield = 78%, [(*E*):(*Z*) = 2:3 as determined by the integration of methyl protons at δ 2.18 and 2.23, respectively].

^{1}H nmr ($CDCl_3$): δ 8.48 and 8.48 (two s, 1H total, (*E*) and (*Z*)- pyridyl H-2), 8.36-8.44 (m, 1H, pyridyl H-6), 7.48-7.66 (m, 1H, pyridyl H-4), 7.36 and 7.40 (two s, 1H total, (*E*)- and (*Z*)- $\underline{CH}$=CCO_2), 7.06-7.26 (m, 1H, pyridyl H-5), 4.90-5.08 (m, 1H, $\underline{CH}Me_2$), 2.18 and 2.23 (two s, 3H total, (*E*)- and (*Z*)- isomers, CO$\underline{Me}$), 1.01 and 1.09 (two d, $J_{CH,Me}$ = 7.0 Hz, 6H total, $CH\underline{Me_2}$).

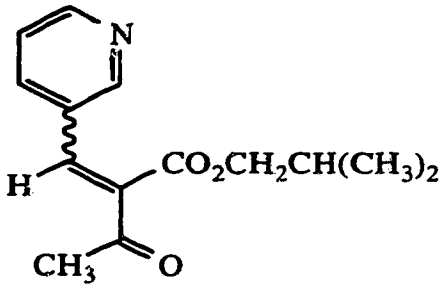
2-Isopropoxycarbonyl-1-(4-pyridyl)-but-1-ene-3-one



The product was distilled at 85-87°C at a pressure of 1.5 mm Hg. Yield = 80%, [(*E*):(*Z*) = 1:1 as determined by integration of the methyl protons at δ 2.20 and 2.27 respectively].

^{1}H nmr ($CDCl_3$): δ 8.48 and 8.50 (two d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H total, (*E*)- and (*Z*)- pyridyl H-2 and H-6), 7.34 and 7.38 (two s, 1H total, (*E*)- and (*Z*)- $\underline{CH}=CCO_2$), 7.09 and 7.21 (two d, 2H total, $J_{2,3} = J_{5,6}$ = 5.0 Hz, pyridyl H-3 and H-5), 5.03 (septet, J = 6.2 Hz, $\underline{CH}Me_2$), 2.20 and 2.27 (two s, 3H total, (*E*)- and (*Z*)- $\underline{Me}CO$), 1.08 and 1.16 [(two d, 3H each, J = 6.2 Hz, $CH\underline{Me_2}$).

2-Isobutoxycarbonyl-1-(3-pyridyl)-but-1-ene-3-one



The product was distilled under reduced pressure at 80°C and 0.5 mm Hg. Yield = 75%, [(*E*):(*Z*) = 1:1 as determined by integration of methyl protons at δ 2.40 and 2.46, respectively].

^{1}H nmr ($CDCl_3$): δ 8.60-8.69 (m, 2H, pyridyl H-2 and H-6) 7.72-7.79 (m, 1H, pyridyl H-4), 7.58 and 7.65 (two s, 1H total, (*E*)- and (*Z*)- isomers, $\underline{CH}=CCO_2$), 7.30-7.36 (m, 1H, pyridyl H-5), 4.08 and 4.08 (two d, 2H total, (*E*)- and (*Z*)- isomers, $CO_2\underline{CH_2}CH$), 2.40 and 2.46 (two s, 3H total, (*E*)- and (*Z*)- isomers, $\underline{Me}CO$), 1.85-2.10 (m, 1H total, $CH_2\underline{CH}Me_2$).

4.4.6.0 Synthesis of N-(1,1-Dimethyl-2-hydroxyethyl) acetoacetamide (46) and N-(1,1-Dimethyl-2-hydroxyethyl)aminocrotonamide (39).

Method A: To a solution of 8.90 g (100 mmol) of 2-amino-2-methyl-1-propanol in 10 ml of xylene in an open round-bottomed flask was added 14.2 g (100 mmol) of 2,2,6-trimethyl-*4H*-1,3-dioxin-4-one. The reaction mixture was heated with an oil bath maintained at 150°C for about 2 hours (or until there was no more evolution of acetone vapour).

The solvent was removed *in vacuo* and the crude product was purified by silica gel column chromatography using ethyl acetate/hexane (1:3, v/v) as eluent to yield **46** as a viscous light-yellow oil, R_f 0.49 (ethyl acetate/hexane, 1:3, v/v). Yield = 60%.

Method B: To a solution of 8.90 g (100 mmol) of 2-amino-2-methyl-1-propanol in 30 ml of THF was added dropwise, 8.40 g (100 mmol) of diketene. When the addition was completed, the reaction mixture was stirred at room temperature for a further 18 hours. The solvent was removed *in vacuo* and the crude product (**46**) was purified by silica gel column chromatography using ethyl acetate/hexane as described for Method A. Yield = 90%.

The product (**46**), was converted to N-(1,1-dimethyl-2-hydroxyethyl)-3-aminocrotonamide (**39**) by passing ammonia gas through a solution of **46** (7.9 g, 50 mmol), in methanol,as described in Section 4.4.3.0. Removal of the solvent *in vacuo* afforded **39** as a white solid (mp 99-101°C) which was used immediately without further purification.

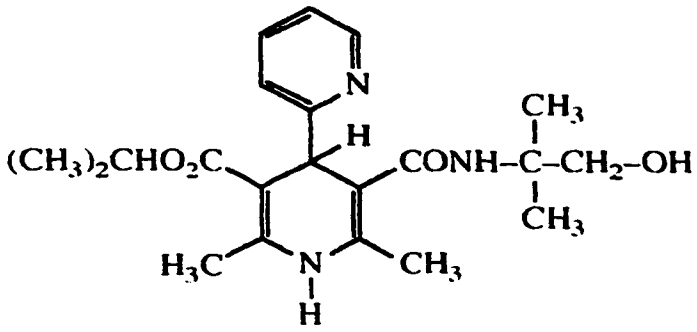
N-(1,1-Dimethyl-2-hydroxyethyl)-3-aminocrotonamide (39)

^{1}H nmr ($CDCl_3$): δ 6.0-6.4 (br s, 3H, CO<u>NH</u> and =C-<u>NH_2</u>), 5.02 (s, 1H, <u>OH</u>), 4.31 (s, 1H, =<u>CH</u>), 3.56 (s, 2H, <u>CH_2</u>OH), 1.84 (s, 3H, <u>Me</u>C=CH), 1.28 [s, 6H, NHC<u>Me_2</u>)

Yield = 95%; mp 99-101°C.

4.4.7.0 Synthesis of Isopropyl 5-{N-[1-(1,1-Dimethyl-2-hydroxyethyl)aminocarbonyl]} 1,4-dihydro-2,6-dimethyl-3-pyridinecarboxylate (41)

A mixture of **39** (180 mg, 1.1 mmol), isopropyl acetoacetate (150 mg, 1.1 mmol) and 2-pyridinecarboxaldehyde (112 mg, 1.1 mmol) was heated at reflux in ethanol for about 48 hours. The solvent was removed *in vacuo* and the crude product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent to give **41** (R_f 0.44, 5.81 g) in 30% yield, mp 170-172°C. The ^{1}H nmr and IR spectral and microanalytical data are given on the next page.



IR (KBr): 3160-3311 (NH and OH), 1671 (CONH), 1592 (NH) cm^{-1}; ^1H nmr ($CDCl_3$): δ 9.36 (s, 1H, CO<u>NH</u>), 8.43 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 7.65 (dd, $J_{3,4} = J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4), 7.07-7.13 (m, 2H, pyridyl H-3 and H-5), 6.32 (t, $J_{CH,OH}$ = 4.2 Hz, 1H, CH_2<u>OH</u>), 6.13 (s, 1H, 1,4-DHP <u>NH</u>), 4.85-5.0 [m, 2H, 1,4-DHP H-4 and <u>CH</u>Me_2), 3.6 (d, $J_{CH2,OH}$ = 4.2 Hz, 2H, <u>CH_2</u>OH), 2.14 and 2.43 (two s, 3H each, 1,4-DHP C-2 and C-6 <u>CH_3</u>), 1.35 and 1.43 (two s, 3H each, CONHC<u>Me_2</u>) 1.01 and 1.20 (two d, 3H each, CH<u>Me_2</u>).

Anal. Calcd. for $C_{21}H_{29}N_3O_4.1/2\ H_2O$: C, 63.62; H, 7.62; N, 10.60. Found: C, 63.66; H, 7.60; N, 10.27

4.4.8.0 Synthesis of alkyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3-pyridinecarboxylates (35a-35f).

General Method A

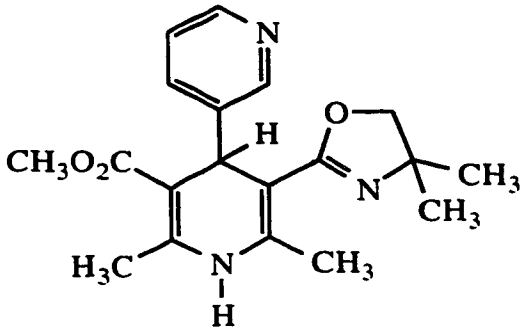
A solution of **32** (460 mg, 2 mmol) and the appropriately functionalized Knoevenagel derived adduct (**34**, 2 mmol) in ethanol (30ml) was refluxed for 18-48 hours. Removal of the solvent *in vacuo* afforded the crude product which was

purified using silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane as eluent. The product was then recrystallized from ethyl acetate/hexane. The ^{1}H nmr and IR spectral data, and the physical data, are given below.

General Method B

To a solution of **41** (388 mg, 1 mmol) in chloroform (25 ml) containing pyridine (10 ml), thionyl chloride (179mg, 1 mmol, 0.13 ml) was added dropwise. The reaction temperature was maintained at -10 to -5°C using a salt/ice bath. After the addition was completed, the reaction mixture was maintained at the same temperature for a further 1.5 hours. The reaction mixture was allowed to warm up to 0°C, and the solvent was removed *in vacuo*. The crude product was purified by silica gel column and then preparative thin layer chromatography, and recrystallization from ethyl acetate/hexane. This latter method used to prepare compound **38c** only.

Methyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3-pyridine-carboxylate (38a).



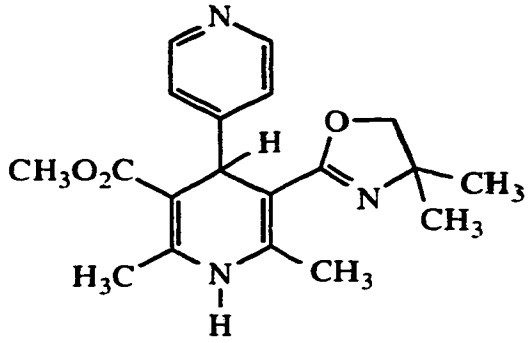
The product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent and then recrystallized from ethyl acetate/hexane (2:1, v/v) to afford a 30% yield of **38a**, [R_f 0.52 (ethyl acetate/hexane, 3:1, v/v), mp 140-142°C].

IR (KBr):3197 (NH), 1679 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.55 (s, 1H, pyridyl H-2), 8.36 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 7.63 (d, $J_{4,5}$ = 7.9 Hz, pyridyl H-4), 7.12-7.16 (m, 1H, pyridyl H-5), 5.93 (s, 1H, NH), 5.05 (s, 1H,1,4-DHP H-4), 3.83 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 3.60 (s, 3H, $CO_2\underline{CH_3}$), 2.22 and 2.35 (two s, 3H each, 1,4-DHP C-2 and C-6 Me), 1.17 and 1.22 (two s, 3H each, oxazolinyl C-4 Me).

Anal. Calcd. for $C_{19}H_{23}N_3O_3$: C, 66.84; H, 6.79; N, 12.31. Found C, 66.65; H, 6.96; N, 11.99.

Methyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3-pyridine-carboxylate (38b).



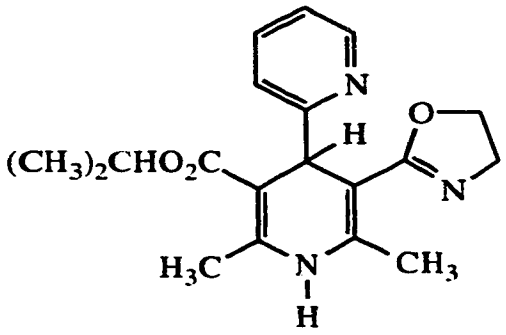
The product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent and recrystallization from ethyl acetate/hexane (2:1, v/v) to afford **38b**, [R_f 0.49 (ethyl acetate/hexane, 3:1, v/v), mp 193-195°C, 57% yield).

IR (KBr): 3201 (NH), 1683 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.42 (d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-2 and H-6), 7.23 (d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-3 and H-5), 5.84 (s, 1H, NH) 5.07 (s, 1H, 1,4-DHP H-4), 3.85 (q, J = 8.0 Hz, 2H, oxazolinyl H-5) 3.61 (s, 3H, CO_2CH_3), 2.23 and 2.37 (two s, 3H each, C-2 and C-6 Me), 1.20 and 1.23 (two s, 3H each, oxazolinyl C-4 Me).

Anal. Calcd. for $C_{19}H_{23}N_3O_3$: C,66.84; H,6.79; N, 12.31. Found: C, 66.47; H, 6.86; N, 12.18.

Isopropyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3-pyridinecarboxylate (**38c**).



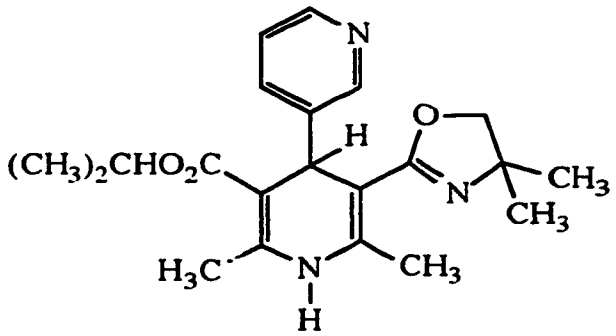
The product was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent, and recrystallization from ethyl acetate/hexane (1:1, v/v) afforded **38c**, [R_f 0.47 (ethyl acetate/hexane, 2:1, v/v), mp 187-189°C, 54% yield].

IR (KBr): 3206 (NH), 1682 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.46 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.09 (s, 1H, NH), 7.46 (dd, $J_{3,4} = J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4), 7.25 (d, $J_{3,4}$ = 7.5 Hz, 1H, pyridyl H-3), 7.05 (dd, $J_{4,5}$ = 7.5 Hz, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 5.06 (s, 1H, 1,4-DHP H-4), 4.83 (septet, J = 6.1 Hz, 1H, CHMe$_2$), 3.73 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 2.06 and 2.22 (two s, 3H each, 1,4-DHP C-2 and C-6 Me), 1.06 and 1.18 (two s, 3H each, oxazolinyl C-4 Me), 0.93 and 1.14 (two d, J = 6.1 Hz, 3H each, CHMe$_2$).

^{13}C nmr ($CDCl_3$): (δ) 167.40 (CO_2), 165.50 (oxazolinyl C-2), 162.73 (pyridyl C-2), 148.21 (pyridyl C-6), 147.04 and 138.97 (1,4-DHP C-2 and C-6), 135.08 (pyridyl C-4), 124.41 and 121.25 (pyridyl C-3 and C-5), 100.45 and 99.83 (O<u>C</u>H_2 and oxazolinyl C-4), 66.43 (1,4-DHP C-3 and C-5), 66.27 (1,4-DHP C-4), 44.29[(<u>C</u>HMe_2), 28.25 (1,4-DHP C-2 and C-6 <u>C</u>H_3), 22.13 and 21.70 (oxazolinyl C-4 <u>C</u>H_3), 19.16 and 17.90 (<u>C</u>HMe_2).
Exact Mass calcd. for $C_{21}H_{27}N_3O_3$: 369.2052
Found: (HRMS): 369.2046

Isopropyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3-pyridinecarboxylate (38d).



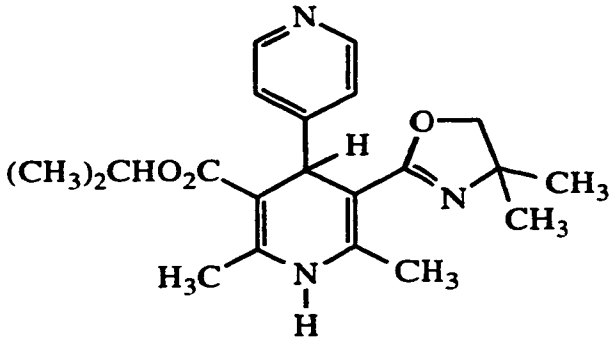
The product was purified by silica gel column chromatography using ethyl acetate/hexane (4:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **38d**, [R_f 0.44 (ethyl acetate/hexane, 4:1, v/v), mp 200-202°C, 51% yield].
IR (KBr): 3206 (NH), 1685 (CO_2) cm^{-1}.

^{1}H nmr (CDCl3): δ 8.53 (s, 1H, pyridyl H-2), 8.33 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 1.6 Hz, 1H, pyridyl H-6), 7.62-7.68 (m, 1H, pyridyl H-4), 7.13 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-5), 6.17 (s, 1H, NH), 4.99 (s, 1H, 1,4-DHP H-4), 4.90 (septet, J = 6.2 Hz, 1H, CHMe), 3.79 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 2.19 and 2.32 (two s, 3H each, 1,4-DHP C-2 and C-6 Me), 1.10 and 1.21 (two d, J = 6.2 Hz, 3H each, $CHMe_2$).

^{13}C nmr (CDCl3): δ 166.89 (CO_2), 162.13 (oxazolinyl C-2), 149.66 and 146.91 (pyridyl C-2 and C-6), 145.65 and 143.20 (1,4-DHP C-2 and C-6), 138.01 (pyridyl C-3), 135.85 (pyridyl C-4), 122.95 (pyridyl C-5), 101.53 and 101.23 (oxazolinyl OCH_2 and C-4), 66.84 (1,4-DHP C-4), 66.70 (1,4-DHP C-3 and C-5), 39.05 $CHMe_2$), 28.32 and 28.21 (1,4-DHP C-2 and C-6 CH_3), 22.09 and 21.71 (oxazolinyl C-4 C), 19.54 and 18.41 [$CH(CH_3)_2$].

Anal. Calcd. for $C_{21}H_{27}N_3O_3$: C, 68.27; H, 7.37; N,11.37. Found: C, 68.22; H, 7.45; N, 11.12.

Isopropyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)-3-pyridinecarboxylate (38e).



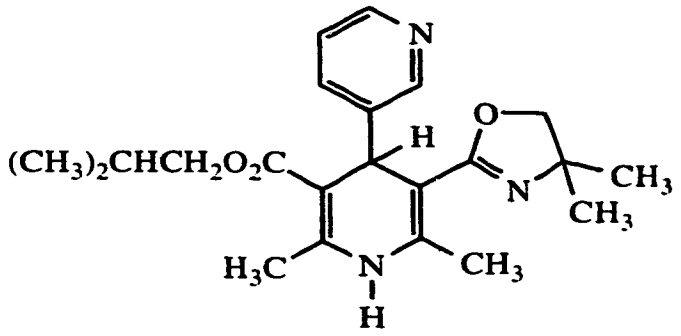
Compound **38e** was purified by silica gel column chromatography using ethyl acetate/hexane (4:1, v/v) as eluent and recrystallized from ethyl acetate/hexane (3:1, v/v). [R_f 0.43 (ethyl acetate/hexane, 4:1, v/v), mp 207-208°C, 45% yield].

IR (KBr): 3197 (NH), 1708 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.4 (d, $J_{2,3} = J_{5,6} = 5.0$ Hz, 2H, pyridyl H-2 and H-6), 7.24 (d, $J_{2,3} = J_{5,6} = 5.0$ Hz, 2H, pyridyl H-3 and H-5), 6.30 (s, 1H, N<u>H</u>), 5.01 (s, 1H, 1,4-DHP H-4), 4.91 (septet, J = 6.2 Hz, 1H, <u>CH</u>Me_2), 3.81 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 2.19 and 2.33 (two s, 3H each, 1,4-DHP C-2 and C-6 <u>Me</u>), 1.02 and 1.22 [two d, J = 6.2 Hz, 3H each, CH<u>Me</u>$_2$) 1.17 and 1.20 (two s, 3H each, oxazolinyl C-4 <u>Me</u>).

Anal. Calcd. for $C_{21}H_{27}N_3O_3$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.26; H, 7.30; N, 11.48.

Isobutyl 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)-3-pyridinecarboxylate (38f).



The product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent and recrystallization from ethyl acetate/hexane (2:1, v/v) yielded **38f**. [R_f 0.51 (ethyl acetate/hexane, 3:1, v/v), mp 181-183, 49% yield].

IR (KBr): 3219 (NH), 1702 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.56 (d, $J_{2,4}$ = 2.0 Hz, 1H, pyridyl H-2), 8.35 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 1.5 Hz, 1H, pyridyl H-6), 7.63 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 1.5 Hz, 1H, pyridyl H-4), 7.13 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,5}$ = 8.0 Hz, 1H, pyridyl H-5), 5.56 (s, 1H, N<u>H</u>), 5.05 (s, 1H, 1,4-DHP H-4), 3.83 (d, J = 6.1 Hz, 2H, CH<u>CH$_2$</u>CO_2), 3.73 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 2.22 and 2.37 (two s, 3H each, C-2 and C-6 <u>Me</u>) 1.85 (m, 1H, CH_2<u>CH</u>Me_2), 1.19 and 1.21 (two s, 3H each, oxazolinyl C-4 <u>Me</u>), 0.78 and 0.82 (two d, 3H each, CH<u>Me$_2$</u>).

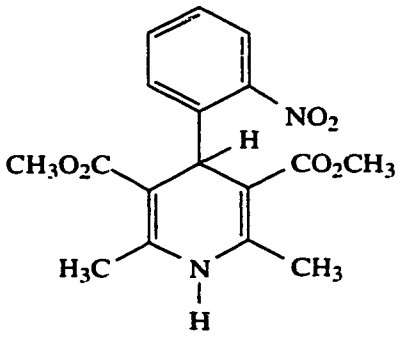
Anal. Calcd. for $C_{22}H_{29}N_3O_3$: C, 68.90; H, 7.62; N, 10.96. Found :C, 68.89; H, 7.70; N, 11.19.

4.4.9.0 Synthesis of Dialkyl 1,4-dihydro-2,6-dimethyl-4-(nitrophenyl)-3,5-pyridinedicarboxylates (52a-52g).

General Procedure.

A mixture of the alkyl acetoacetate **49** (2 mmol), enamine **50** (2 mmol) and appropriate nitrobenzaldehyde **51** (2 mmol), in ethanol (30 ml) was refluxed for 18-48 hours. Removal of the solvent *in vacuo* afforded the crude product which was purified by silica gel column chromatography prior to recrystallization from ethyl acetate/hexane. The ^{1}H nmr and IR spectral data for compounds **52a-52g** are given below.

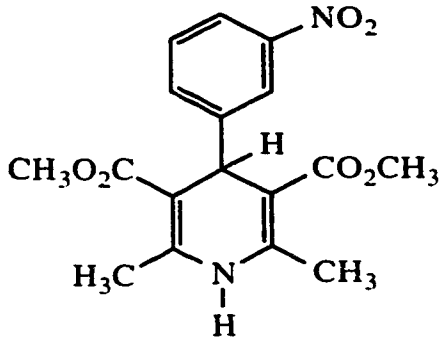
Dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (52a).



The product was purified by silica gel column chromatography using ethyl acetate/hexane (1:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:2, v/v) to afford **52a**, [mp 172-173°C (lit[192] mp 172-174°C), R_f 0.53 (ethyl acetate/hexane, 1:1, v/v), 85% yield].

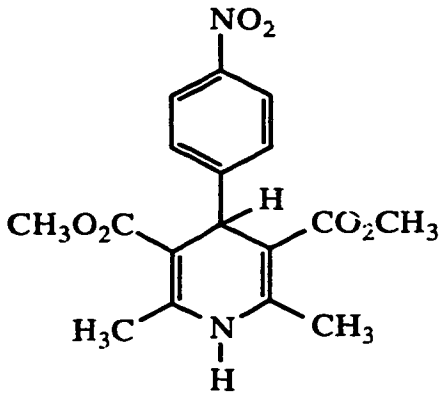
IR (KBr): 3342 (NH), 1697 (CO_2), 1523 and 1342 (NO_2) cm^{-1}.
^1H nmr ($CDCl_3$): δ 7.69 (d, $J_{3,4}$ = 8.2 Hz, 1H, phenyl H-3), 7.43-7.53 (m, 2H, phenyl H-4 and H-5), 7.23-7.28 (m, 1H, phenyl H-6), 5.75 ((s, 1H, NH), 5.73 (s, 1H, 1,4-DHP H-4), 3.59 (s, 6H, 1,4-DHP C-3 and C-5 CO_2Me), 2.35 (s, 6H, 1,4-DHP C-2 and C-6 Me).

Dimethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (52b).



Compound **52b** was purified by silica gel column chromatography using ethyl acetate/hexane (1:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:2, v/v), [R_f 0.51 (ethyl acetate/hexane, 1:1, v/v), mp 204-205°C, (lit[192] mp 206-208°C), 96% yield].
IR (KBr): 3350 (NH), 1704 (CO_2), 1523 and 1433 (NO_2) cm^{-1}.
^1H nmr ($CDCl_3$): δ 8.10-7.99 (m, 2H, phenyl H-2 and H-4), 7.63 (d, $J_{5,6}$ = 7.6 Hz, 1H, phenyl H-6), 7.40-7.34 (m, 1H, phenyl H-5), 5.82 (s, 1H, NH), 5.10 (s, 1H, 1,4-DHP H-4), 3.65 (s, 6H, CO_2Me), 2.37 (s, 6H, 1,4-DHP C-2 and C-6 Me).

Dimethyl 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)-3,5-pyridinedicarboxylate (52c).

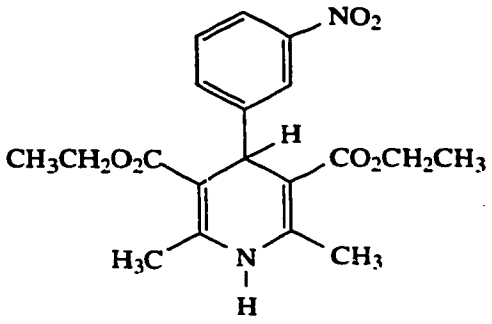


This product was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **52c**, [mp 197-198°C (lit[192] mp 196-197°C), R_f 0.56 (ethyl acetate/hexane, 2:1, v/v), 86% yield].

IR (KBr): 3170 (NH), 1704 (CO_2), 1525 and 1345 (NO_2), cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.10 (dd, $J_{2,3} = J_{5,6} = 8.8$ Hz, $J_{3,5} = 1.9$ Hz, 2H, phenyl H-3 and H-5), 7.44 (dd, $J_{2,3} = J_{5,6} = 8.8$ Hz; $J_{2,6} = 1.9$ Hz, 2H, phenyl H-2 and H-6), 5.73 (s, 1H, NH), 5.11 (s, 1H, 1,4-DHP H-4), 3.65 (s,6H, CO_2Me), 2.37 (s, 6H, 1,4-DHP C-2 and C-6 Me).

Diethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (52d).

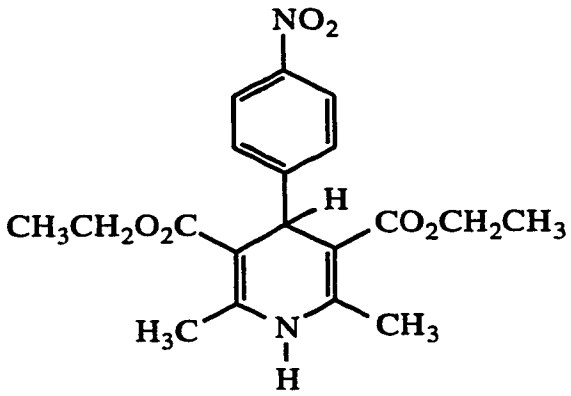


This product was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v), [mp 160-161°C (lit[192] mp 161°C), R_f 0.54 (ethyl acetate/hexane, 2:1, v/v), 90% yield].

IR (KBr): 3349 (NH), 1698 (CO_2), 1523 and 1343 (NO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.12 (s, 1H, phenyl H-2), 7.99 (d, $J_{4,5}$ = 8.0 Hz, 1H, phenyl H-4), 7.64 (d, $J_{5,6}$ = 8.0 Hz, 1H, phenyl H-6), 7.35 (dd, $J_{4,5} = J_{5,6}$ = 8.0 Hz, 1H, phenyl H-5), 5.96 (s, 1H, NH), 5.10 (s, 1H, 1,4-DHP H-4), 4.10 (q, J = 6.2 Hz, 4H, $CO_2\underline{CH_2}Me$), 2.25 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.23 (t, J = 6.2 Hz, 6H, $CO_2CH_2\underline{Me}$).

Diethyl 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)-3,5-pyridinedicarboxylate (52e).

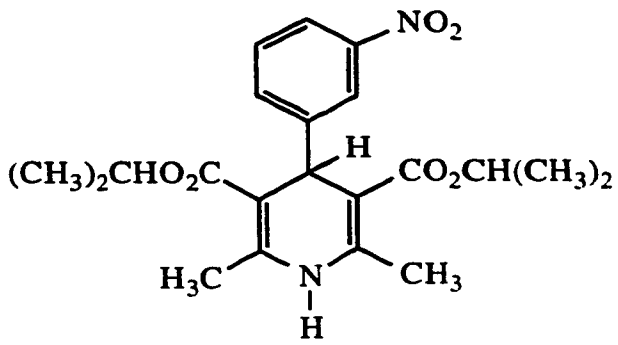


This compound was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **52e**, [mp 133-134°C (lit[192] mp 132-134°), R_f 0.52 (ethyl acetate/hexane, 2:1, v/v), 88% yield].

IR (KBr): 3345 (NH), 1696 (CO_2), 1524 and 1343 (NO_2).

^1H nmr ($CDCl_3$): δ 8.09 (dd, $J_{2,3}$ = 8.7 Hz, $J_{3,5}$ = 1.8 Hz, 1H, phenyl H-3 and H-5) 7.45 (dd, $J_{2,3} = J_{5,6}$ = 8.7 Hz, $J_{2,6}$ = 1.8 Hz, 2H, phenyl H-2 and H-6), 5.72 (s, 1H, NH), 5.10 (s, 1H, 1,4-DHP H-4), 4.09 (q, J = 7.1 Hz, 4H, $CO_2\underline{CH_2}Me$), 2.36 (s, 6H 1,4-DHP C-2 and C-6, Me), 1.22 (t, J = 7.1 Hz, 6H, $CO_2CH_2\underline{Me}$).

Diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-(pyridinedicarboxylate (52f).

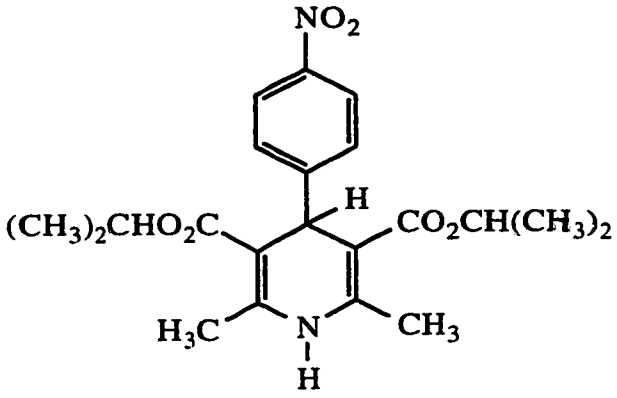


This compound was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **52f**, [mp 121-122°C (lit[192] mp 123°C), R_f 0.56 (ethyl acetate/hexane, 3:1, v/v), 85% yield].

IR (KBr): 3346 (NH), 1698 (CO_2), 1524 and 1343 (NO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.13 (s, 1H, phenyl H-2), 8.01 (d, $J_{4,5}$ = 8.0 Hz, 1H, phenyl H-4), 7.65 (d, $J_{5,6}$ = 7.8 Hz, 1H, phenyl H-6), 7.37 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 7.8 Hz, phenyl H-5), 5.70 (s, 1H, NH), 5.06 (s, 1H, 1,4-DHP H-4), 4.95 [septet, J = 6.2 Hz, $CHMe_2$, 2.36 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.10 and 1.26 (two d, J = 6.2 Hz, 3H each, $CHMe_2$).

Diisopropyl 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)-3,5-pyridinedicarboxylate (52g).



This product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **52g**, [mp 153-154°C (lit[192] mp 152°C), R_f 0.55 (ethyl acetate/hexane, 3:1, v/v), 70% yield].

IR (KBr): 3349 (NH), 1698 (CO_2), 1525 and 1342 (NO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.08 (dd, $J_{2,3} = J_{5,6} = 8.8$ Hz, $J_{3,5} = 2.0$ Hz, 2H, phenyl H-3 and H-5), 7.46 (dd, $J_{2,3} = J_{5,6} = 8.8$ Hz, $J_{2,6} = 1.9$ Hz, 2H, phenyl H-2 and H-6), 5.68 (s, 1H, NH), 5.07 (s, 1H, 1,4-DHP H-4), 4.95 (septet, J = 6.2 Hz, 1H, $CHMe_2$), 2.35 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.1 and 1.24 (two d, J = 6.2 Hz, 3H each, $CHMe_2$).

4.4.10.0 Synthesis of Dialkyl 1,4-dihydro-2,6-dimethyl-4-[(Z)-N-oxo-N-aryl/vinylmethylene)-λ^5-azanyl]phenyl-3,5-pyridinedicarboxylates (53a-53i).

General Method A.

To a solution of 2 mmol of the 1,4-DHP 4-(nitrophenyl) diester (**52b-52d**) in 50 ml THF, under a nitrogen atmosphere at -78°C, was added slowly 5 mmol (2.5 equivalents) of benzylmagnesium chloride in THF. The reaction was maintained at -78°C for a further 30 minutes to 5 hours, depending upon the rate of the reaction which was monitored by tlc. The reaction mixture was quenched with a saturated ammonium chloride solution and extracted with ethyl acetate (3 x 50 ml). The organic layer was dried with anhydrous magnesium sulfate, the solvent was removed *in vacuo* to give the crude product. Purification by silica gel column chromatography and then preparative thin layer chromatography afforded the target compounds **53a-53d** in 30 - 45% yields. The IR and ^1H nmr spectral and physical data of these compounds are given below.

General Method B

To a solution of 2 mmol (802 mg) of the diisopropyl 1,4-DHP 4-(3- and 4-nitrophenyl) diester (**52f** or **52g**) in 50 ml of dry THF, was added slowly 2 mmol of aqueous (65%) hydrazine hydrate in the presence of 20 mg of 5% rhodium-on-

charcoal catalyst. The progress of the reaction was monitored by tlc and the reduction was allowed to proceed until most of the starting materials had reacted. The mixture was filtered into water (30 ml) prior to extraction with ether (3 x 50 ml). The organic layer was dried (anhydrous sodium sulfate) and the solvent was removed *in vacuo* to afford the crude product **54a**, which was used without further purification.

To a solution of the unpurified hydroxylamine **54a** in 30 ml of dry absolute ethanol containing 400 mg of anhydrous sodium sulfate, was added 2 mmol (180 mg) of benzaldehyde. The reaction mixture was stirred overnight at 25°C, diluted with 100 ml of chloroform and dried (anhydrous magnesium sulfate). Removal of the solvent *in vacuo* gave the crude product, which was purified by silica gel column chromatography using ethyl acetate/hexane as eluent to afford the target compound (**53e** or **53f**). The IR and ^{1}H nmr spectral and physical data of these two compounds are given below.

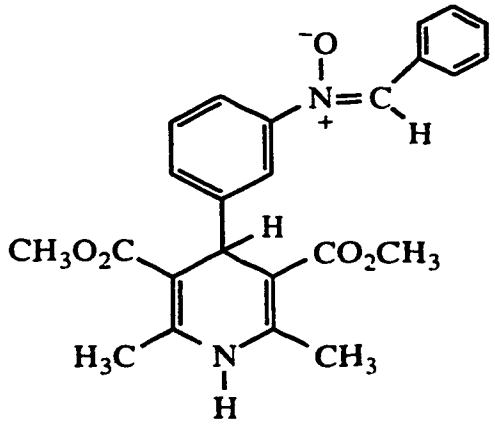
General Method C.

To a solution of 2 mmol (748 mg) of the diethyl ester (**52e**) in 22 ml of 86% aqueous ethanol was added zinc powder (520 mg, 7.95 mmol) and ammonium chloride (260 mg, 4.84 mmol). The resulting mixture was stirred vigorously at room temperature for 50 minutes. The reaction mixture was filtered and the filtrate was poured into 100 ml of dichloromethane. This solution was washed with water (2 x 30 ml) and dried (anhydrous magnesium sulfate). Removal of the solvent *in*

vacuo yielded the crude product **54b** which was used without further purification in the following reaction.

To a solution of the crude hydroxylamine **54b** in 30 ml of dry absolute ethanol containing 400 mg of anhydrous sodium sulfate, was added 2 mmol of 4-nitrobenzaldehyde, 4-chlorobenzaldehyde or acrolein, respectively. The reaction mixture was stirred overnight at 25°C, diluted with 100 ml of chloroform and dried (anhydrous magnesium sulfate). Removal of the solvent *in vacuo* gave the crude product, which was purified by silica gel column chromatography using ethyl acetate/hexane as eluent to afford the target compounds (**53g - 53i**). The spectral and physical data for these three compounds are given below.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-{3-[(Z)-N-oxo-N-(phenylmethylene)-λ^5-azanyl]phenyl}-3,5-pyridinedicarboxylate (53a).



The product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as eluent prior to

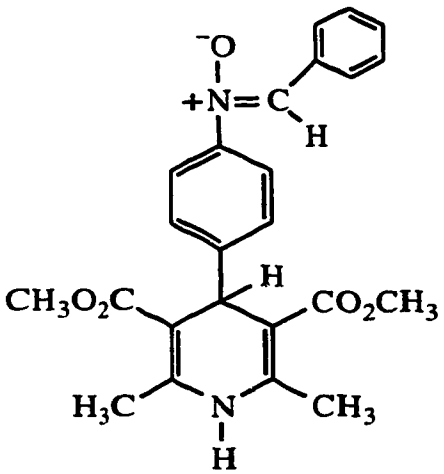
recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **53a**, [mp 140-142°C, R_f 0.51 (ethyl acetate/hexane, 3:1, v/v), 30% yield].

IR (KBr): 3336 (NH), 1696 (CO_2), 122[illegible] (NO) cm^{-1}

^1H nmr ($CDCl_3$): δ 8.36-8.40 (m, 2H, C-4 phenyl H-2 and H-4), 7.87 (s, 1H, nitrone =CH), 7.69 (d, $J_{5,6}$ = 8.0 Hz, 1H, C-4 phenyl H-6), 7.39-7.50 (m, 6H, C-4 phenyl H-5 and C-phenyl hydrogens), 6.07 (s, 1H, NH), 5.09 (s, 1H, 1,4-DHP H-4), 3.66 (s, 6H, CO_2Me), 2.33 (s, 6H, 1,4-DHP C-2 and C-6 Me).

Anal. Calcd. for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, [illegible]3; H, 5.74; N, 6.64.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-{4-[(Z)-N-oxo-N-(phenylmethylene)-λ⁵-azanyl]phenyl}-3,5-pyridinedicarboxylate (53b).



This product was purified by silica gel column chromatography using ethyl acetate/hexane (3:1, v/v) as

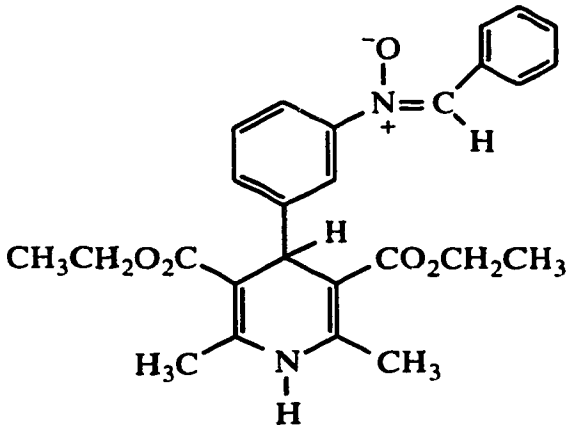
eluent prior to recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **53b**, [mp 240-242°C, Rf 0.51 (ethyl acetate/hexane, 3:1, v/v), 40% yield].

IR (KBr): 3345 (NH), 1692 (CO_2), 1213 (NO).

^1H nmr ($CDCl_3$): δ 8.37-8.40 (m, 2H, C-4 phenyl H-3 and H-5), 7.88 (s, 1H, nitrone =CH), 7.61 (d, $J_{2,3} = J_{5,6} = 8.0$ Hz, 2H, C-phenyl H-2 and H-6) 7.45-7.50 (m, 3H, C-phenyl H-3, H-4 and H-5) 7.41 (d, $J_{2,3} = J_{5,6} = 8.0$ Hz, 2H, C-4 phenyl H-4 and H-6), 6.54 (s, 1H, NH), 5.04 (s, 1H, 1,4-DHP H-4), 3.65 (s, 6H, CO_2Me), 2.05 (s, 6H, 1,4-DHP C-2 and C-6 Me).

Anal. Calcd. for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.34; H, 5.89; N, 6.57.

Diethyl 1,4-dihydro-2,6-dimethyl-4-{3-[(Z)-N-oxo-N-(phenylmethylene)-λ⁵-azanyl]phenyl}-3,5-pyridinedicarboxylate (53c).



This product was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (3:1, v/v) as eluent prior to

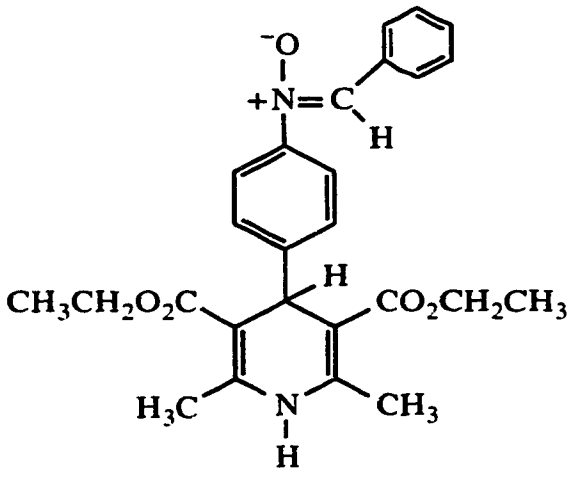
recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **53c**, [mp 183-185°C, R_f 0.50 (ethyl acetate/hexane, 3:1, v/v), 42% yield].

IR (KBr): 3345 (NH), 1698 (CO_2), 1228 (NO).

^1H nmr ($CDCl_3$): δ 8.32-8.40 (m, 2H, C-4 phenyl H-2 and H-4), 7.86 (s, 1H, nitrone =CH), 7.70-7.74 (m, 1H, C-4 phenyl H-6), 7.27-7.50 (m, 6H, C-4 phenyl H-5 and C-phenyl hydrogens), 5.81 (s, 1H, NH), 5.08 (s, 1H, 1,4-DHP H-4), 4.12 (q, J = 7.1 Hz, 2H, CO_2CH_2Me), 2.33 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.23 (t, J = 7.1 Hz, 6H, CO_2CH_2Me).

Anal. Calcd. for $C_{26}H_{28}N_2O_5$: C, 69.63; H, 6.29; N, 6.25. Found : C, 69.42; H, 6.32; N, 6.12.

Diethyl 1,4-dihydro-2,6-dimethyl-4-{4-[(Z)-N-oxo-N-(phenylmethylene)-λ⁵-azanyl]phenyl}-3,5-pyridinedicarboxylate (53d).



This product was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (3:1, v/v) as eluent prior to

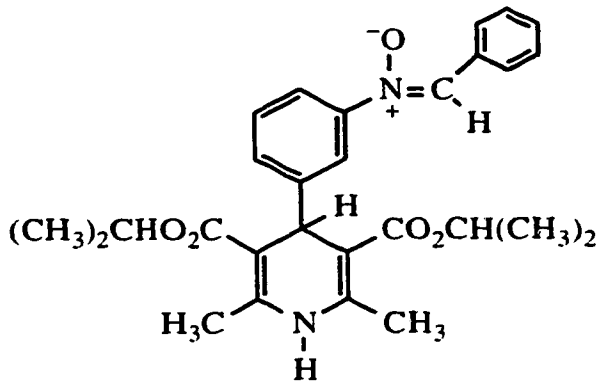
recrystallization from ethyl acetate/hexane (2:1, v/v) to yield **53d**, [mp 214-215°C, R_f 0.48 (ethyl acetate/hexane, 3:1, v/v), 45% yield].

IR (KBr): 3337 (NH), 1696 (CO_2), 1221 (NO).

^1H nmr ($CDCl_3$): δ 8.35-8.40 (m, 2H, C-4 phenyl H-3 and H-5), 7.89 (s, 1H, nitrone =CH), 7.59 (d, $J_{2,3} = J_{5,6} = 8.60$ Hz, 2H, C-phenyl H-2 and H-6), 7.46-7.51 (m, 3H, C-phenyl H-3, H-4 and H-5), 7.41 (dd, $J_{2,3} = J_{5,6} = 8.6$ Hz, 2H, C-4 phenyl H-2 and H-6), 6.53 (br s, 1H, NH), 5.03 (s, 1H, 1,4-DHP H-4), 4.1 (q, J = 7.1 Hz, 4H, CO_2CH_2Me), 2.31 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.25 (t, J = 7.1 Hz, 6H, CO_2CH_2Me).

Anal. Calcd. for $C_{26}H_{28}N_2O_5$: C, 69.63; H, 6.29; N, 6.25. Found : C,69.93; H, 6.19; N, 6.23.

Diisopropyl 1,4-dihydro-2,6-dimethyl-4-{3-[(Z)-N-oxo-N-(phenylmethylene)-λ⁵-azanyl]phenyl}-3,5-pyridinedicarboxylate (53e).



This compound was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (4:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (2:1, v/v) to

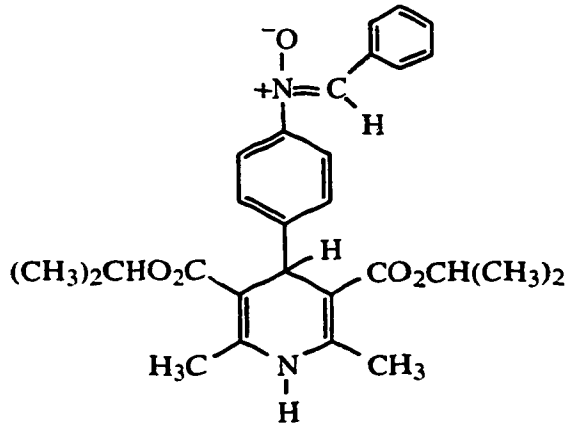
yield **53e**, [mp 95-97°C, Rf 0.56 (ethyl acetate/hexane, 4:1, v/v), 54% yield].

IR (KBr): 3338 (NH), 1697 (CO_2), 1225 (NO).

^1H nmr ($CDCl_3$): (δ): 8.30-8.40 (m, 2H, C-4 phenyl H-2 and H-4), 7.87 (s, 1H,, nitrone =CH), 7.67-7.70 (m, 1H, C-4 phenyl H-6), 7.26-7.47 (m, 6H, C-4 phenyl H-5 and C-phenyl hydrogens), 6.29 (s, 1H, NH), 5.05 (s, 1H, 1,4-DHP H-4), 4.96 (septet, J = 6.2 Hz, 1H, $CHMe_2$), 2.29 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.13 and 1.24 (two d, J = 6.2 Hz, 3H each, $CHMe_2$).

Anal. Calcd. for $C_{27}H_{30}N_3O_5.1/2\ H_2O$: C, 69.19; H, 6.38; N, 5.77. Found: C, 69.31; H, 6.95; N, 6.01

Diisopropyl 1,4-dihydro-2,6-dimethyl-4-{4-[(Z)-N-oxo-N-(phenylmethylene)-λ^5-azanyl]phenyl-3,5-pyridinedicarboxylate (53f).



This product was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (4:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (2:1, v/v) to

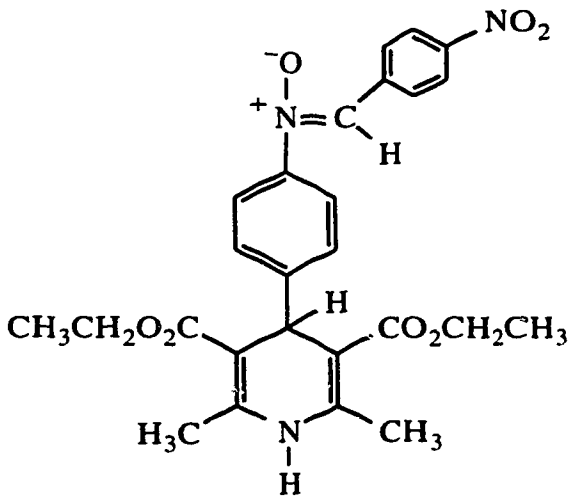
yield **53f**, [mp 169-171°C, R_f 0.55 (ethyl acetate/hexane, 4:1, v/v), 62% yield].

IR (KBr): 3337 (NH), 1697 (CO_2), 1221 (NO).

^1H nmr ($CDCl_3$): δ 8.32-8.40 (m, 2H, C-4 phenyl H-3 an H-5), 7.89 (s, 1H, nitrone =CH), 7.62 (d, $J_{2,3} = J_{5,6} = 8.60$ Hz, 2H, C-phenyl H-2 and H-6), 7.42-7.50 (m, 3H, C-phenyl H-3, H-4 and H-5), 7.39 (d, $J_{2,3} = J_{5,6} = 8.60$ Hz, C-4 phenyl H-6 and H-6), 6.20 (s, 1H, NH), 5.02 (s, 1H, 1,4-DHP H-4), 4.96 (septet, J = 6.2 Hz, 1H, $CHMe_2$), 2.31 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.14 and 1.25 (two d, J = 6.2 Hz, 3H each, $CHMe_2$).

Anal. Calcd. for $C_{27}H_{30}N_2O_5.3/4\ H_2O$: C, 68.56; H, 6.40; N, 5.71. Found: C,68.78; H, 6.70; N, 5.37

Diethyl 1,4-dihydro-2,6-dimethyl-4-{4-[(*Z*)-N-oxo-N-(4-nitrophenylmethylene)-λ⁵-azanyl]phenyl}-3,5-pyridine-dicarboxylate (53g).



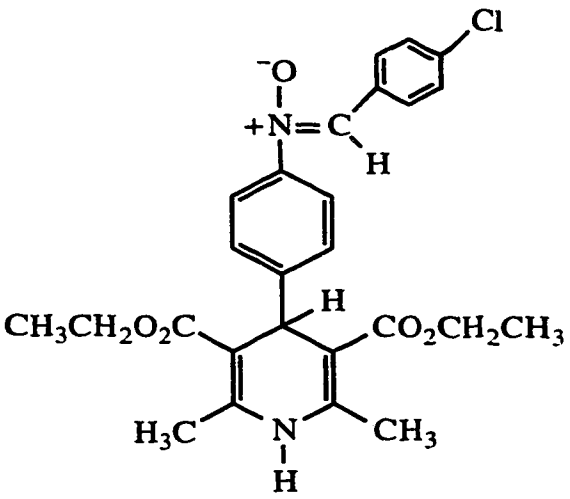
This compound was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **53g**, [mp 175-177°C, R_f 0.59 (ethyl acetate/hexane, 2:1, v/v), 42% yield].

IR (KBr): 3336 (NH), 1698 (CO_2), 1220 (NO).

^1H nmr ($CDCl_3$): δ 8.53 (d, $J_{2,3} = J_{5,6}$ = 9.0 Hz, 2H, C-phenyl H-3 and H-5), 8.31 (d, $J_{2,3} = J_{5,6}$ = 8.7 Hz, 2H, C-4 phenyl H-3 and H-5), 8.00 (s, 1H, nitrone =CH), 7.62 (d, $J_{2,3} = J_{5,6}$ = 8.7 Hz, 2H, C-4 phenyl H-2 and H-6), 7.43 (d, $J_{2,3} = J_{5,6}$ = 9.0 Hz, C-phenyl H-2 and H-6), 5.66 (s, 1H, NH), 5.07 (s, 1H, 1,4-DHP H-4), 4.11 (q, J = 7.1 Hz, 4H, CO_2CH_2Me), 2.36 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.24 (t, J = 7.1 Hz, 6H, CO_2CH_2Me).

Anal. Calcd. for $C_{26}H_{27}N_3O_7$: C, 63.28; H, 5.51; N, 8.51. Found: C, 63.68; H, 5.67; N, 8.10.

Diethyl 1,4-dihydro-2,6-dimethyl-4-{4-[(*Z*)-N-oxo-N-(4-chlorophenylmethylene)-λ^5-azanyl]phenyl}-3,5-pyridine-dicarboxylate (53h).



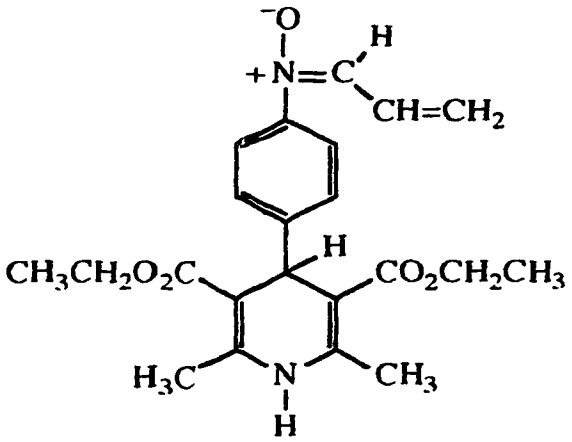
The title compound was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (2:1, v/v) as eluent to yield **53h**, as a yellow semi-solid which was difficult to recrystallize.

R_f 0.57 (ethyl acetate/hexane, 2:1, v/v), 51% yield.

IR (KBr): 3340 (NH), 1692 (CO_2), 1227 (NO), 1096 (C-Cl) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.34 (d, $J_{2,3} = J_{5,6}$ = 8.6 Hz, 2H, C-4 phenyl H-3 and H-5), 7.87 (s, 1H, nitrone =CH), 7.59 (d, $J_{2,3} = J_{5,6}$ = 8.6 Hz, 2H, C-phenyl H-3 and H-5), 7.40-7.52 (m, 4H, C-phenyl H-2 and H-6, and N-phenyl H-2 and H-6), 6.54 (s, 1H, NH), 5.03 (s, 1H, 1,4-DHP H-4), 4.10 (q, J = 7.1 Hz, 4H, CO_2CH_2Me), 2.28 (s, 6H, 1,4-DHP C-2 and C-6 Me), 1.23 (t, J = 7.1 Hz, CO_2CH_2Me).

Diethyl 1,4-dihydro-2,6-dimethyl-4-{4-[(*E*)-N-oxo-N-(vinylmethylene)-λ^5-azanyl]phenyl}-3,5-pyridinedicarboxylate (53i).



This compound was purified by silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane (2:1, v/v) as eluent to afford **53i**, as a yellow semi-solid which was difficult to recrystallize.

R_f 0.61 (ethyl acetate/hexane, 2:1, v/v), 48% yield.

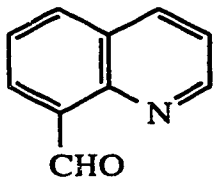
IR (KBr): 3339 (NH), 1698 (CO_2), 1226 (NO) cm^{-1}.

^1H nmr ($CDCl_3$): δ 7.18 (d $J_{2,3} = J_{5,6}$ = 8.6 Hz, 2H, C-4 phenyl H-3 and H-5), 6.91 (d, $J_{2,3} = J_{5,6}$ = 8.6 Hz, 2H, C-4 phenyl H-2 and H-6), 5.91 (s, 1H, NH), 5.69 (d, $J_{CH,CH}$ = 4.6 Hz, 1H, nitrone =CHCH=CH_2), 4.03 (q, J = 7.1 Hz, 4H, CO_2CH_2Me), 3.62-3.71 (m, 1H, vinyl CHCH=CH_2), 3.67 and 2.45 (two m, 1H each, vinyl CH=CH_2), 2.31 (s, 6H, 1,4-DHP C-2 and C-6 CH_3), 1.22 (t, J = 7.1 Hz, 6H, CO_2CH_2Me).

4.4.11.0 Synthesis of 8-Quinolinecarboxaldehyde (60).

Tc 5 g (35 mmol) of 8-methylquinoline was added 5.83 g (53 mmol) of freshly prepared selenium dioxide (SeO_2). The mixture was heated at 120°C for three hours. The dark, viscous mixture was allowed to cool to room temperature, diluted with ethyl acetate (30 ml) and filtered to remove solid selenium and unreacted selenium dioxide. The solvent was removed *in vacuo* and the crude product was purified by silica gel column chromatography using ethyl acetate/hexane (1:3, v/v) as eluent. After recrystallization from ethyl acetate/hexane the target compound (**59**) was obtained. The spectral and physical data of the compound is given below.

8-Quinolinecarboxaldehyde (60).



The product was purified by silica gel column chromatography using ethyl acetate/hexane (1:3, v/v) as eluent and then recrystallization from ethyl acetate/hexane (1:2, v/v) to yield **60**, [R_f 0.61 (ethyl acetate/hexane, 1:3, v/v), mp 99-101°C, 35% yield].

IR (KBr): 2820, 2718 (aldehydic CH), 1704 (CO), 1385 (aldehydic CH).

^1H nmr ($CDCl_3$): δ 11.45 (s, 1H, CHO), 9.05 (dd, $J_{2,3}$ = 4.2 Hz, $J_{2,4}$ = 1.7 Hz, 1H, quinolyl H-2), 8.33 (dd, $J_{6,7}$ = 7.2

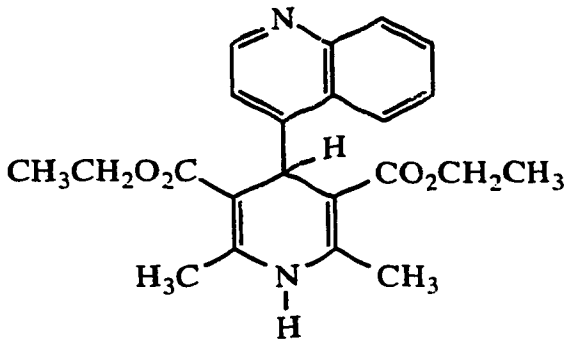
Hz, $J_{5,7}$ = 1.6 Hz, 1H, quinolyl H-7), 8.26 (dd, $J_{3,4}$ = 8.3 Hz, $J_{2,4}$ = 1.7 Hz, 1H, quinolyl H-4), 8.10 (dd, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.6 Hz, 1H, quinolyl H-5), 6.68 (t, $J_{5,6}$ = 8.0 Hz, $J_{6,7}$ = 7.2 Hz, 1H, quinolyl H-6), 7.52 (dd, $J_{3,4}$ = 8.3 Hz, $J_{2,3}$ = 4.2 Hz, 1H, quinolyl H-3).

4.4.12.0 Synthesis of Dialkyl 1,4-dihydro-2,6-dimethyl-4-(quinolinyl)-3,5-pyridinedicarboxylates (61a-61e).

General Procedure.

A mixture of the quinolinecarboxaldehyde (1 mmol, 157 mg), alkyl acetoacetate **49** (1 mmol) and alkyl 3-aminocrotonate **50** (1 mmol) were heated under reflux in ethanol (50 ml) for 18-48 hours. The solvent was removed *in vacuo* and the crude was product purified by a silica gel column and then preparative thin layer chromatography using ethyl acetate/hexane as eluent. Recrystallization from ethyl acetate/hexane gave the target compounds (**61a-61e**) in 28-78% yields. The spectral and physical data for these compounds are summarized below.

Diethyl 1,4-dihydro-2,6-dimethyl-4-(4-quinolyl)-3,5-pyridinedicarboxylate (61a).



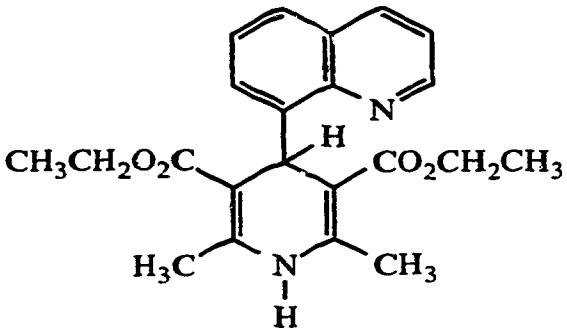
The title compound was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **61a**, [mp 203-204°C, R_f 0.45 (ethyl acetate/hexane, 2:1, v/v), 78% yield].

IR (KBr): 3182 (NH), 1691 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.80 (d $J_{2,3}$ = 4.8 Hz, 1H, quinolyl H-2), 8.64 (d, $J_{7,8}$ = 8.0 Hz, 1H, quinolyl H-8), 8.09 (dd, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H, quinolyl H-5), 7.70 (ddd, $J_{6,7}$ = $J_{7,8}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H, quinolyl H-7), 7.59 (ddd, $J_{5,6}$ = $J_{6,7}$ = 8.0 Hz, $J_{6,8}$ = 1.0 Hz, 1H, quinolyl H-6), 7.47 (d, $J_{2,3}$ = 4.8 Hz, 1H, quinolyl H-3), 6.47 (s, 1H, NH), 5.84 (s, 1H, 1,4-DHP H-4), 3.90 (q, J = 7.1 Hz, 4H, $CO_2\underline{CH_2}CH_3$), 2.38 (s, 6H, 1,4-DHP C-2 and C-6 $\underline{CH_3}$), 0.95 (t, J = 7.1 Hz, 6H, $CO_2CH_2\underline{CH_3}$).

Anal. Calcd. for $C_{22}H_{24}N_2O_5$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.24; H, 6.35; N, 7.41.

Diethyl 1,4-dihydro-2,6-dimethyl-4-(8-quinolyl)-3,5-pyridinedicarboxylate (61b).



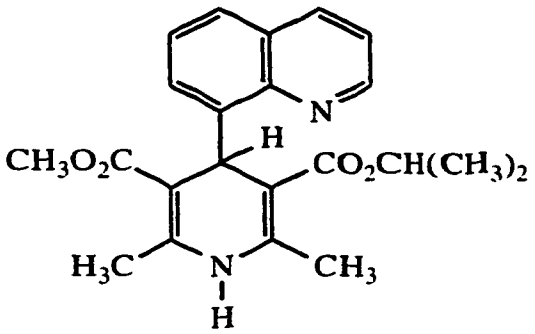
This product was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **61b**, [mp 160-161°C, Rf 0.48 (ethyl acetate/hexane, 2:1, v/v), 31% yield].

IR (KBr): 3188 (NH), 1698 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.93 (dd, $J_{2,3}$ = 5.0 Hz, $J_{2,4}$ = 1.5 Hz, 1H, quinolyl H-2), 8.13 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,4}$ = 1.5 Hz, 1H, quinolyl H-4), 7.56-7.67 (m, 2H, quinolyl H-5 and H-7), 7.40 (t, $J_{5,6} = J_{6,7}$ = 7.5 Hz, 1H, quinolyl H-6), 7.29 (dd, $J_{2,3}$ = 5.0 Hz, $J_{3,4}$ = 8.0 Hz, 1H, quinolyl H-3), 6.25 (s, 1H, NH), 5.72 (s, 1H, 1,4-DHP H-4), 3.88 (q, J = 7.1 Hz, 4H, $CO_2\underline{CH_2}CH_3$), 2.26 (s, 6H, 1,4-DHP C-2 and C-6 $\underline{CH_3}$), 0.8 (t, J = 7.1 Hz, 6H, $CO_2CH_2\underline{CH_3}$).

Anal. Calcd. for $C_{22}H_{24}N_2O_5$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.13; H, 6.60; N, 7.18.

3-Methyl 5-isopropyl-1,4-dihydro-2,6-dimethyl-4-(8-quinolyl)-3,5-pyridinedicarboxylate (61c).



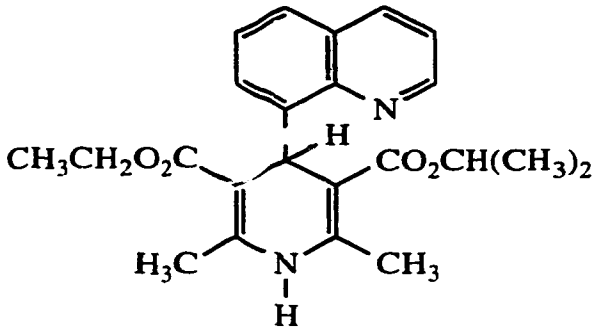
The title compound was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **61c**, [mp 192-193°C, R_f 0.52 (ethyl acetate/hexane, 2:1, v/v), 36% yield].

IR (KBr): 3183 (NH), 1704 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.95 (dd, $J_{2,3}$ = 5.0 Hz, $J_{2,4}$ = 1.5 Hz, 1H, quinolyl H-2), 8.04 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,4}$ = 1.5 Hz, 1H, quinolyl H-4), 7.56-7.66 (m, 2H, quinolyl H-5 and H-7), 7.41 (t, $J_{5,6}$ = $J_{6,7}$ = 8.0 Hz, 1H, quinolyl H-6), 7.30 (dd, $J_{2,3}$ = 5.0 Hz, $J_{3,4}$ = 8.0 Hz, 1H, quinolyl H-3), 6.23 (s, 1H, NH), 5.59 (s, 1H, 1,4-DHP H-4), 4.63 (septet, J = 6.2 Hz, 1H, $CHMe_2$), 3.38 (s, 3H, CO_2CH_3), 2.26-2.28 (two s, 3H each, 1,4-DHP C-2 and C-6 CH_3), 1.08 and 0.56 (two d, J = 6.2 Hz, 3H each, $CHMe_2$).

Anal. Calcd. for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N,7.36. Found: C, 69.12; H, 6.37; N, 7.25.

3-Ethyl 5-isopropyl-1,4-dihydro-2,6-dimethyl-4-(8-quinolyl)-3,5-pyridinedicarboxylate (61d).



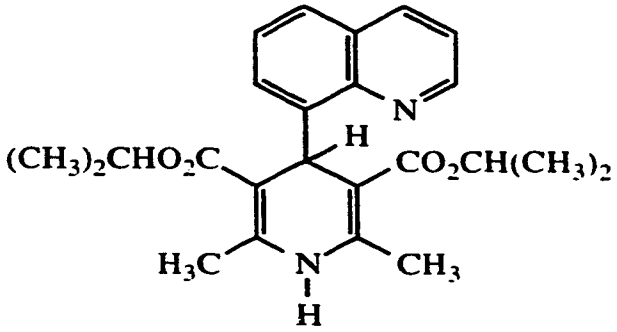
This product was purified by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to yield **61d**, [mp 96-97°C, R_f 0.51 (ethyl acetate/hexane, 2:1, v/v), 28% yield].

IR (KBr): 3217 (NH), 1696 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.91 (dd, $J_{2,3}$ = 4.3 Hz, $J_{2,4}$ = 1.7 Hz, 1H, quinolyl H-2), 8.20 (dd, $J_{3,4}$ = 8.3 Hz, $J_{2,4}$ = 1.7 Hz, 1H, quinolyl H-4), 7.85-7.95 (m, 1H, quinolyl H-6), 7.55-7.63 (m, 2H, quinolyl H-5 and H-7), 7.43 (dd, $J_{2,3}$ = 4.3 Hz, $J_{3,4}$ = 8.3 Hz, 1H, quinolyl H-3), 4.66 (septet, J = 6.1 Hz, 1H, $\underline{CH}Me_2$), 3.73 (q, J = 6.1 Hz, 2H, $CO_2\underline{CH_2}CH_3$), 2.78 (s, 6H, 1,4-DHP C-2 and C-6 $\underline{CH_3}$), 0.73 (d, J = 6.1 Hz, 3H, $CH\underline{CH_3}$), 0.50-0.53 (m, 6H, $COCH_2\underline{CH_3}$ and $CO_2CH\underline{CH_3}$).

Anal. Calcd. for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 70.10; H,6.31; N,7.00.

Diisopropyl 1,4-dihydro-2,6-dimethyl-4-(8-quinolyl)-3,5-pyridinedicarboxylate (61e).



The title compound was purified by silica gel column chromatography using ethyl acetate/hexane (1:4, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) to afford **61e**, [mp 162-163°C, R_f 0.47 (ethyl acetate/hexane, 1:3, v/v), 40% yield].

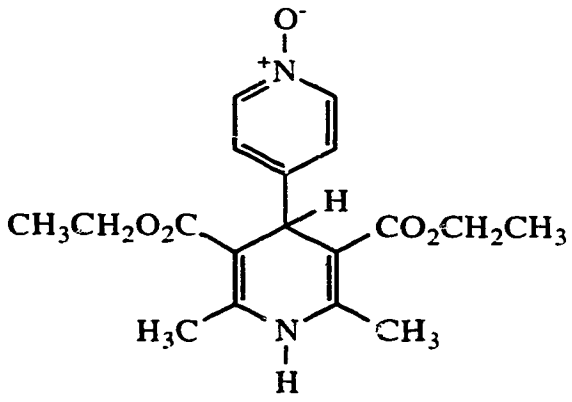
IR (KBr): 3201 (NH), 1698 (CO_2) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.90 (dd, $J_{2,3}$ = 4.2 Hz, $J_{2,4}$ = 1.8 Hz, 1H, quinolyl H-2), 8.18 (dd, $J_{3,4}$ = 8.3 Hz, $J_{2,4}$ = 1.8 Hz, 1H, quinolyl H-4), 7.88 (t, $J_{5,6}$ = $J_{6,7}$ = 8.1 Hz, 1H, quinolyl H-6), 7.56 (d, $J_{5,6}$ = $J_{6,7}$ = 8.1 Hz, 2H, quinolyl H-5 and H-7), 7.41 (d, $J_{2,3}$ = 4.2 Hz, 1H, quinolyl H-3), 4.64 (septet, J = 6.1 Hz, 2H, $\underline{CH}Me_2$), 2.71 (s, 6H, 1,4-DHP C-2 and C-6 $\underline{CH_3}$), 0.71 and 0.50 (two d, J = 6.1 Hz, 3H each, $CH\underline{Me_2}$).

Anal. Calcd. for $C_{24}H_{28}N_2O_4$: C, 70.57; H, 6.91; N,6.86. Found: C, 70.70; H,6.58; N, 6.77.

4.4.13.0 Synthesis of Diethyl 1,4-dihydro-2,6-dimethyl-4-(1-oxido-4-pyridyl)-3,5-pyridinedicarboxylate (62).

A mixture of 1-oxido-4-pyridinecarboxaldehyde (2 mmol, 246 mg), ethyl 3-aminocrotonate (2 mmol, 250 mg) and ethyl acetoacetate (2 mmol, 250 mg) was heated at reflux in ethanol for 24 hours. Removal of the solvent *in vacuo*, and purification of the product by silica gel column chromatography using ethyl acetate/hexane (2:1, v/v) as eluent prior to recrystallization from ethyl acetate/hexane (1:1, v/v) gave the target compound **61** [(600 mg, 86% yield), mp 218-220°C, R_f 0.43 (ethyl acetate/hexane, 2:1, v/v)].



IR (KBr): 3172 (NH), 1688 (CO_2), 1231 (NO) cm^{-1}.

^1H nmr ($CDCl_3$): δ 8.10 (dd, $J_{2,3} = J_{5,6} = 7.0$ Hz, $J_{2,6} = 1.5$ Hz, 2H, 1-oxido-4-pyridyl H-2 and H-6), 7.31 (d, $J_{2,3} = J_{5,6} = 7.0$ Hz, 2H, 1-oxido-4-pyridyl H-3 and H-5), 7.21 (s, 1H, NH), 5.02 (s, 1H, 1,4-DHP H-4), 4.12 (q, J = 7.1 Hz, 4H, $CO_2CH_2CH_3$), 2.38 (s, 6H, 1,4-DHP C-2 and C-6 CH_3), 1.23 (t, J = 7.1 Hz, 6H, $CO_2CH_2CH_3$).

Anal. Calcd. for $C_{18}H_{22}N_2O_5.1/4\ H_2O$: C, 61.62; H, 6.61; N,7.98. Found: C, 61.77; H, 6.35; N, 7.94

4.5.0.0. In vitro Calcium Channel Agonist and Antagonist Assays.

In all *in vitro* experiments, a HEPES buffered physiological solution (HPSS) of the following composition mM/L was used: NaCl 137.0, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.6, D-glucose 11.9, HEPES 9.0, and the pH of the solution was adjusted to 7.4 with sodium hydroxide. DMSO, which was used to dissolve the test compound, was purified by double distillation. The tissues used for the assays were isolated from Male Hartley strain guinea pigs (Charles River Canada Inc., St. Constant, Quebec).

Calcium channel antagonist activity was determined using guinea pig ileal longitudinal smooth muscle (GPILSM). The tissues were suspended in a water jacketed physiological tissue bath which was aerated with pure oxygen. The bath temperature was maintained at 37°C using a Haake Model E52 or Braun Thermomix II water circulator. Tissues were allowed to equilibrate for about 45 minutes, with a change of the HPSS solution every 15 minutes before any measurements were carried out. Solutions of the test compounds were prepared in DMSO-H_2O, where the ratio used was dependent on the solubility of the test compound. The molar concentration of the test compound required to produce a 50% decrease in the slow component or tonic contractile response (IC_{50} ± SEM) for

GPILSM by the muscarinic agonist carbachol (1.67×10^{-7} M), was determined graphically from the dose-response (prepared using 10 different concentrations) curve. A minimum of three different tissues were used (n = 3) for each experiment.

Calcium channel agonist activity was similarly determined using guinea pig left atria (GPLA) to determine the inotropic effect and guinea pig right atria (GPRA) to determine the chronotropic effect. The percentage change in the contractile response of GPLA, induced by the test compound, relative to its basal contractile force produced in the absence of the the test compound, was determined. At each concentration, the contractile force due to DMSO (previously determined prior to testing the compound) was subtracted from the overall response, in order to determine the response due solely to the test compound. The percentage change in chronotropic rate produced by the test compound on GPRA, relative to its rate in the absence of the test compound, was also determined. These calcium channel antagonist and agonist assays are similar to those previously reported.[163]

5.0.0.0 GENERAL CONCLUSIONS AND FUTURE TRENDS

The compounds investigated in this study have provided useful information on the synthetic chemistry, structure-activity correlations and pharmacological profiles of some classes of 1,4-dihydropyridine calcium channel modulators.

The use of a modified Hantzsch reaction involving the condensation of an oxazolinyl enamine (**32**) with a Knoevanegal derived adduct (**37**) afforded a superior method for preparing the target 1,4-DHP oxazolines (**38a-38f**). The use of a three component modified Hantzsch reaction also proved to be an excellent method for synthesizing dialkyl 1,4-dihydro-2,6-dimethyl-4-(nitrophenyl)-3,5-pyridinedicarboxylates (**52a-52g**), which were used as starting materials for the preparation of the target nitrones **53a-53i**. The conversion of **52a-52g** into their corresponding 1,4-DHP C(4)-phenylnitrones revealed some interesting chemistry. It was observed that larger alkyl ester groups at the C(3) and C(5) positions of the 1,4-DHP C-4 (nitrophenyl) compound, reacted less efficiently with benzylmagnesium chloride. Thus, the 1,4-DHP 3,5-dimethyl and 3,5-diethyl C-4 (nitrophenyl) diesters (**52b-52e**) reacted readily with benzylmagnesium chloride to yield the corresponding nitrones (**53a-53d**), whereas there was no reaction between benzylmagnesium chloride and the 3,5-diisopropyl analogs (**52f** and **52g**). Compounds **52f** and **52g** were converted to their corresponding nitrones (**53e** and **53f**), by first reducing the nitro group to

hydroxylamine (**54a**) and then reacting the hydroxylamine with benzaldehyde. It was also observed that the nitrones **53g-53i** could be prepared in better yields by first reducing the corresponding nitrophenyl compound (**52e**) to the hydroxylamine (**54b**) and then reacting the hydroxylamine with *para*-nitrobenzaldehyde, *para*-chlorobenzaldehyde or acrolein, respectively. The corresponding Grignard reagents did not react with **52e**. The use of hydrazine hydrate (65%) in the presence of 5% rhodium-on-charcoal catalyst, was an effective reagent for reducing **52f** and **52g** to their corresponding hydroxylamines. However, this reagent led to dimeric products when used to reduce **52e**. A milder reducing agent (zinc/ammonium chloride) was used to reduce **52e** to the corresponding hydroxylamine (**54b**). The modified three component Hantzsch reaction was also a good method for preparing compounds **61a-61e** and **62**.

All the compounds synthesized in this study were evaluated for their smooth muscle calcium channel antagonist effect using a GPILSM assay. The calcium channel activity for all compounds was generally less than that of the reference drug, nifedipine. Compounds **38a-38f**, **53a-53i**, **61a** and **62** were also evaluated for their cardiac calcium channel agonist effect using a GPLA assay. All compounds, except for **38b**, **53h** and **53i**, exhibited varying degrees of a positive inotropic effect on heart. Most of these compounds exhibited a substantial calcium channel agonist effect at their calcium channel antagonist IC_{50} value or at lower concentrations.

This dual smooth muscle calcium channel antagonist/ cardioselective calcium channel agonist effect would be very desirable for developing potential drugs for the treatment of congestive heart failure. Thus, compounds (**38a-38f** and **53a-53i**) could provide good "leads" for the development of a new generation of effective drugs for treating congestive heart failure.

Some useful structure-activity correlations were also obtained from this study. For **38a-38f**, it was observed that the C(4)-3-(pridinyl) compounds were generally more active than their corresponding C(4)-2-(pyridinyl) and C(4)-4-(pyridinyl) analogs with the same C(3) ester moiety. This indicated that the presence of a negative minima (nitrogen atom) at the 3-position of the C(4)-pyridinyl group was a major determinant of calcium channel agonist activity for this class of compounds. It was also observed that for compounds **53a-53f**, the C(4)-3-(phenylnitrones) were generally more active than the corresponding C(4)-4-(phenylnitrone) analogs. This again indicated that the presence of a negative minimum (nitrone moiety) at the 3-position of the C(4) phenyl ring enhanced calcium channel agonist activity. These observations agreed partially, with earlier report by Testa *et al*[162] and Knaus *et al*[161] that the presence of a negative minimum at or beyond the *meta*-position of the aryloxypropanolamines and quinolyloxypropanolamines, respectively, enhanced β_1-selective antagonist activity. For the C(4)-4-(phenylnitrone), it was observed that replacement

of the C-phenyl group of the nitrone moiety with a *para*-nitrophenyl group (**53g**) increased calcium channel agonist activity, when compared to that of the unsubstituted-phenyl compound (**53d**). This may be attributed to the additional negative minima introduced by the nitro group into the *para*-position of the C-4 phenyl ring. However, replacement of the C-phenyl group in compound **53d** with either a *para*-chlorophenyl (**53h**) or vinyl group (**53i**) led to a marked decrease in calcium channel agonist properties of the compounds. These compounds (**53h** and **53i**) exhibited a negative inotropic effect. Thus, the nature of the C-substituent of the nitrone moiety is an important determinant of calcium channel properties.

For the 1,4-DHP C(4)-quinolinyl compounds (**61a-61e**), it was observed that the size of the ester alkyl group at the C(3) and C(5) positions of the 1,4-DHP ring, was a major determinant of calcium channel antagonist activity for this class of compounds. The smaller the size of the ester alkyl group, the greater the calcium channel antagonist activity.

The use of Hyperchem Version 3 for Windows minimizer (MM^+) program from Autodesk (IBM PC Version) provided useful information on the conformation, local minimum energy and stereochemistry of compounds **38a-38f** and **53a-53i**, respectively. For compounds **38a-38f**, it was determined that the most stable conformation (lower energy) in each case, was the one with an ap C=O ester and an sp N=C oxazolinyl group. For compounds **53a-53i**, it was determined that the (*E*)-

stereoisomer (**53d**) was the most stable for the C(4)-(C-phenylnitrones), whereas for the C(4)-(C-vinylnitrone), **53i**, the (*Z*)-stereoisomer was the most stable. These results contrast with that reported in the literature,[172,175] which assigns (*Z*)-stereochemistry to the most stable phenylnitrone and (*E*)-stereochemistry to the vinylnitrone. Further studies would be necessary to resolve these differences.

From the results obtained in this study, both the oxazolinyl and nitrone moieties of 1,4-DHP compounds exhibit desirable dual smooth muscle calcium channel antagonist/ cardioselective calcium channel agonist properties. It would therefore be of interest to synthesize and pharmacologically evaluate additional analogs of compounds **38a-38f**, where the ester alkyl group, R = ethyl, phenethyl, substituted phenethyl, t-butyl etc. and the C(4) heteroaryl group, Het = quinolyl, indolyl, or substituted-phenyl etc. Also for compounds **53a-53i**, additional *para*-substituted C-phenyl analogs (especially with nucleophilic substituents) ought to be investigated. These investigations may lead to the development of b[illegible] drugs for the treatment of congestive heart failure. It would also be of interest to synthesize the 1,4-DHP C(4)-quinolinyl analog of compounds **61a-61e**, where R = R^1 = Me, and evaluate its calcium channel antagonist activity.

6.0.0.0. REFERENCES

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