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Anabilim Dalı: **Farmasötik Kimya**

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Öğrenci Adı-Soyadı: **Ahmed Elkhouly**

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ÖZET

Elkhouly, A. S. Kondanse 1,4-dihidropiridin türevlerinin sentezi ve kalsiyum kanal modülatör ve antioksidan aktivitelerinin araştırılması, Hacettepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Programı Doktora Tezi, Ankara, 2014. Bu çalışmada, kalsiyum kanal blokörü ligandlar temel alınarak LigandScout ile bir farmakofor model oluşturulmuş ve ondokuz yeni 2-(metakriloiloksi)etil 4-aril-2,6,6-trimetil-5-okso-1,4,5,6,7,8-hekzahidrokinolin-3-karboksilat türevi, çok bileşenli Hantzsch reaksiyonu ile 4,4-dimetilsiklohekzan-1,3-dion, 2-(metakriloiloksi) asetoasetat, sübstitüe benzaldehit ve amonyum asetat ile metanol içinde mikrodalga irradyasyonu ile kısa sürede sentezlenmiştir. Sentezlenen bileşiklerin yapıları, IR, ^{1}H-NMR, ^{13}C-NMR ve kütle spektroskopisi ile aydınlatılmış ve elemental analiz ile doğrulanmıştır. Bileşiklerin kalsiyum modülatör aktivitesi nifedipin standart olarak kullanılarak sıçan gastrik fundus izole düz kas şeritlerinde miyorelaksan aktivitesi test edilerek tayin edilmiştir. Bileşiklerin antioksidan aktivitesi, diferansiyel pulse voltametri tekniği uygulanarak tayin edilmiştir. Sonuçlar, bileşiklerin mide fundus düz kas şeritleri üzerinde konsantrasyon-bağımlı gevşeme cevapları oluşturduğunu fakat bileşiklerin aktivitesi nifedipinden daha düşük olduğunu, bazı bileşiklerin iyi antioksidan aktiviteye sahip olduğunu göstermiştir.

Anahtar Kelimeler: Hekzahidrokinolin; Dihidropiridin; Farmakofor; LigandScout; Mikrodalga sentezi; Kalsiyum kanal.

ABSTRACT

Elkhouly, A. S. Synthesis of condensed 1,4-dihydropyridine derivatives and evaluation of their calcium channel modulating and antioxidant activities. Hacettepe University Institute of Health Sciences, Ph.D. Thesis in Pharmaceutical Chemistry, Ankara, 2014. In this study, a pharmacophore model based on calcium channel blocker ligands was generated by LigandScout and nineteen 2-(methacryloyloxy)ethyl 4-aryl-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives were synthesized via multicomponent one pot Hantzsch reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, substituted benzaldehyde and ammonium acetate in methanol under microwave irradiation in a short time. The structures of the synthesized compounds were identified by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy; and were proved by elemental analysis. The calcium modulating activity of the compounds were determined by testing their myorelaxant effect on isolated strips of rabbit gastric fundus smooth muscle by using nifedipine as standard and the antioxidant activity was determined by applying differential pulse voltammetry technique. The results showed the compounds exerted concentration-dependent relaxation responses on gastric fundus smooth muscle strips but the compounds were less potent than nifedipine; and some of the compounds exhibited good antioxidant activity.

Keywords: Hexahydroquinoline; Dihydropyridine; Pharmacophore; LigandScout; Microwave synthesis; Calcium channel.

CONTENTS

ABBREVIATIONS

ATP	Adenosine triphosphate
CAL-B	Candida antarctica lipase-B
CAN	Ceric ammonium nitrate
cAMP	Cyclic adenosine monophosphate
CCBs	Calcium channel blockers
cGMP	Cyclic guanosine monophosphate
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
COX	Cyclooxygenase
CRC	Concentration-response curve
CSA	Cellulose sulfuric acid
DHP	Dihydropyridine
DMSO-$_{d6}$	Deuterated dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC_{50}	The half maximal effective concentration
ECF	Extracellular fluid
ED_{50}	Median effective dose
ETX	Ethosuximide
GABA	Gamma-Aminobutyric acid
HIV-1	Human immunodeficiency virus
^{1}H-NMR	Hydrogen-1 nuclear magnetic resonance
HVA	High-voltage-activated
IBD	Iodobenzenediacetate
IC_{50}	The half maximal inhibitory concentration
ICF	Intracellular fluid
I_{or}	The oxygen current
I_{res}	The residual current
I_{red}	The electrochemical reduction current
IP_3	Inositol trisphosphate
IR	Infrared

IVA	Intermediate-voltage-activated
K	Coefficients of antioxidant activity
KHS	Krebs'-Henseleit solution
LC-MS	Liquid chromatography–mass spectrometry
L-NAME	Nω-nitro-L-arginine methyl ester
LVA	Low-voltage-activated
MFE	Mercury film electrode
MLCK	Myosin light-chain kinase
MMFF	Merck molecular force field
MWI	Microwave irradiation
NADH	Nicotinamide adenine dinucleotide
PAF	Platelet aggregation factor
PB	Phenobarbital
PD2	The half-maximal response
PGE	Pencil graphite electrode
PPh_3	Triphenyl phosphine
PTZ	Pentylenetetrazol
QSAR	Quantitative structure activity relationship
RDF	Radial distribution function
SAR	Structure activity relationship
TBPA	Tris(p-bromophenyl)amine
TLC	Thin layer chromatography
TMSI	Trimethylsilyl iodide
UV	Ultraviolet
VDCCs	Voltage-dependent calcium channels
VPA	Valproate magnesium

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1. INTRODUCTION AND AIM

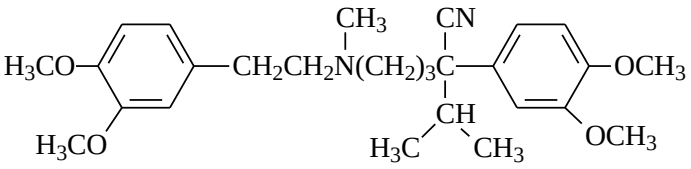
Calcium is involved in several vital processes inside the cell including contractile, secretory and neural activities. It performs these tasks either as a free cation or more often after combination with some macromolecules such as calmodulin. Therefore, the interference with the calcium function was shown to have important pharmacological applications.

Calcium anatgonists, a drug that interfere with the cellular function of calcium by inhibiting its entry and/or release or by interfering with one of its cellular actions.

Calcium channels are specific channels that are resposible for the influx calcium ions across the cell membrane into the cell, leading to the regulation of cytoplasmic concentration of Ca^{+2} and altering the cell function. The diversity of calcium channel types and also their wide distribution within the tissues resulted in variety in their physiological acitivites as well as their pharmacological applications (1).

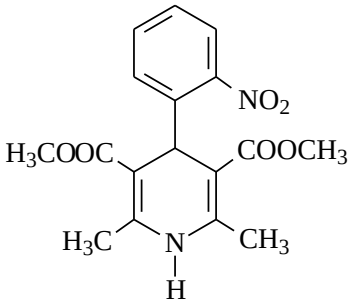
Calcium channel blockers (CCBs) represent a chemically and pharmacologically diverse group of drugs that are widely used for the treatment of the cardiovacular system complications such as hypertension and angina. Also CCBs are used for the treatment of atherosclerosis, atrial fibrillation, cardioplegia, cerebral insufficiency, cerebral ischemia, hypertrophic cardiomyopathy, migraine, myocardial ischaemia, peripheral vascular disease, Raynaud's syndrome, subarachnoid haemorrhage, ventricular tachycardia and venous insufficiency (2-5). Furthermore, the scope of their pharmacological applications has extended to other complications outside the cardiovascular system.

Verapamil, was the first drug introduced as calcium channel blocker, it was already used in the 1960s for the treatment of angina pectoris (2-9). Then, several drugs have ben introduced to serve as calcium channel blockers.



Verapamil

The 1,4-dihydropyridine (1,4-DHP) drug family, nowadays is one of the most commonly used CCB drugs for the treatment of cardiovascular diseases. Although the first synthesis of these compounds have been reported in 1882 by Arthur Hantzsch, the prototype of this family, nifedipine, was commercially introduced to market by Bayer in Germany under the trade name "Adalat" in 1975.



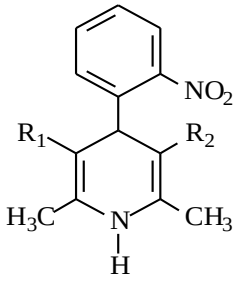
Nifedipine

1,4-DHP derivative drugs are shown to alter the entry of calcium ions by modulating voltage dependent calcium channels in cardiac and vascular cells.

The importance of this group of compounds is not only due to the pharmacological effects, but also to explore the properties of the calcium channels.

Following the clinical success of these drugs, many efforts have been done to synthesize new derivatives that have a better bioavailability and tissue selectivity (10, 11).

The ester groups were the most involved in the structural modifications to alter the activity of 1,4-DHP derivatives, either by modifing the alkyl chain attached or even by transforming to acyl or carbamoyl moiety (12, 13).



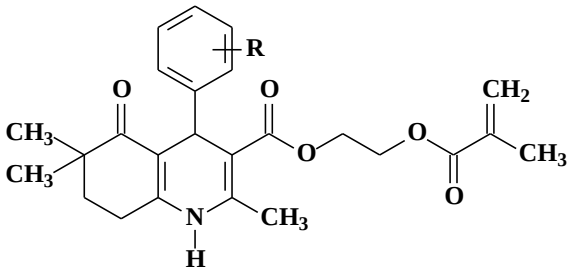
R_1, R_2: COOR, COR, $CONR_2$, SO_2R

The introduction of the DHP ring in a fused ring system, such as hexahydroquinolines, is one of those modification trials to alter the activity of 1,4-DHPs, and it was shown to maintain the Ca^{+2} channel modulating activity (14-16).

Increasing recognition of the clinical importance of 1,4-DHP calcium channel blockers stimulated us to conduct this study that aims to:

- Generating a ligand-based pharmacophore model that may provide additional information about the structural requirements of DHP calcium channel blockers.
- Synthesize of hexahydroquionline derivatives in the light of our pharmacophore modeling study, the previous structure-activity relationship studies and literature data. These newly synthesized compounds are suggested to act as calcium channel blockers. The synthesized compounds are shown in Table 1.1.
- Application of microwave irradiation as a recent promising technique in the synthesis of our compounds by an easy and time consuming method.
- Determination of the myorelaxant activity and the antioxidant properties for the newly synthesized compounds.

Table 1.1. Structure of the synthesized compounds.



Compound	R	Compound	R
4a	2-Cl	**4k**	2,4-diF
4b	2-F	**4l**	2,5-diF
4c	2-NO_2	**4m**	4-Cl
4d	3-Cl	**4n**	4-F
4e	3-F	**4o**	4-NO_2
4f	3-NO_2	**4p**	4-CN
4g	3-CN	**4q**	2-Cl,3-CF_3
4h	2,3-diCl	**4r**	2-Cl,5-CF_3
4i	2,4-diCl	**4s**	2-F,5-CF_3
4j	2,5-diCl		

2. GENERAL DESCRIPTIONS

2.1. Resting membrane potential and distribution of ions

An electrical potential difference which can be recorded across the plasma membrane of living cells, caused by a slightly unbalanced distribution of ions between the intracellular fluid (ICF) and extracellular fluid (ECF) and amounts to -50 to -100 mV (cell interior is negative).

The main factor involved in establishing the membrane potential is the maintenance of unequal distribution of ions which can be achieved by different mechanisms.

Mechanisms involved in maintenance of unequal distribution of ions

- The Na^+-K^+-ATPase continuously and actively pumps Na^+ out of the cell and K^+ into it, consuming ATP as a source of energy. As a result, the intracellular K^+ concentration is around 35 times higher and the intracellular Na^+ concentration is roughly 20 times lower than the extracellular concentration.
- The membrane of a resting cell is only very slightly permeable to Na^+ and Ca^{2+} while it is relatively easy for K^+ ions to diffuse across the cell membrane.

All living cells have a (resting) membrane potential, but only excitable cells such as nerve and muscle cells are able to greatly change the ion conductance of their membrane in response to a stimulus, as in an action potential.

The ion transport is achieved by membrane ion channels; these channels are specific for different ion species (Na^+, Ca^{2+}, K^+, Cl^-….etc.) (1, 5).

Channel open-probability is controlled by three main factors:

- **Membrane potential**, especially in Na^+, Ca^{2+} and K^+ channels in nerve and muscle fibres.
- **External ligands**, includes acetylcholine, glutamate (interfere with cation channels), and glycine or GABA (interfere with Cl^- channels).
- **Intracellular messenger substances**, such as: cAMP, cGMP, IP_3, tyrosine kinases and Ca^{2+}.

2.2. Role of Ca^{2+} in cell regulation

The cytosolic Ca^{2+} concentration (0.1 to 0.01μmol/L) is several decimal powers lower than the extracellular Ca^{2+} concentration (1.3 mmol/L). This is because Ca^{2+} is continuously actively pumped from the cytosol into intracellular Ca^{2+} stores such as the endoplasmic and sarcoplasmic reticulum, vesicules, mitochondria and nuclei by Ca^{2+}-ATPases or is actively transported out of the cell through $Ca^{2+}/3Na^{+}$ exchanger (17).

The increase the cytosolic Ca^{2+} concentration can be obtained by two mechanisms: rapid but transient Ca^{2+} release from intracellular Ca^{2+} stores and rather slow Ca^{2+} influx from the extracellular space. Evidence has shown that Ca^{2+} influx through Ca^{2+} channels from the extracellular space is especially important for regulation of cytosolic Ca^{2+} concentration; and the use of great number of Ca^{2+} antagonists clinically supported this evidence (18).

The frequency of Ca^{2+}channel opening in the cell membrane is increased by depolarization of the cell membrane (e.g., nerve and muscle cells), ligands (e.g., via G proteins), intracellular messengers (e.g., IP_3 and cAMP), stretching or heating of the cell membrane. While the Ca^{2+} channels of the endoplasmic and sarcoplasmic reticulum open more frequently in response to signals such as arise in cytosolic Ca^{2+}concentration or IP_3.

Arising of cytosolic Ca^{2+} concentration is a signal for many important cell functions, including myocyte contraction, excitation of certain sensory cells, exocytosis of neurotransmitters in presynaptic nerve endings, endocrine and exocrine hormone secretion, opening of other types of ion channels, thrombocyte activation and the migration of leukocytes and tumour cells, in addition to other activities are mediated by calmodulin such as smooth muscle contraction.

A calmodulin molecule can bind up to four Ca^{2+} ions when the cytosolic Ca^{2+} concentration rises. The Ca^{2+}-calmodulin complexes activate a number of different enzymes, including calmodulin-dependent protein kinase II (Ca M-kinase II) and myosin light chain kinase (MLCK), which is involved in smooth muscle contraction.

2.3. Calcium channels

Calcium channels are members of a gene superfamily of trans-membrane ion channel proteins that also include voltage-gated potassium and sodium channels. In the 1970s and 80s physiologists used the term “current” rather than “channel”.

Generally they are classified into major groups: voltage-dependent and ligand-gated calcium channels.

Voltage-dependent calcium channels (VDCCs) serve as an important mechanism for Ca^{2+} influx into cells, leading to the regulation of cytosolic Ca^{2+} concentration and cell function.

VDCCs are a group of ion channels that found in the membrane of excitable cells (*e.g.*, muscle, neurons, etc.) (19). VDCCs are normally closed at resting membrane potential. They are activated (*i.e.*, opened) at depolarized membrane potentials and so they were called "voltage-dependent".

They were initially divided into two classes: high-voltage-activated (HVA) and low-voltage activated (LVA) Ca^{2+} channels. The activation threshold of HVA Ca^{2+} channels occurs at -40 ~ -10 mV, while the threshold activation of LVA channels occurs at lower membrane potentials of -60 ~ -70 mV. HVA Ca^{2+} channels are further divided into L-type, N-type and P/Q-type. While LVA Ca^{2+} channels consists of only T type channels. R-type is occasionally classified as intermediate voltage-activated (IVA) channels.

More recently classification of calcium channels was based on analysis of α_1 subunit genes. L (long-lasting)-type of Ca^{2+} channels is inhibited by dihydropyridine (DHP) derivatives. This group is called Cav1 includes muscle *1S*, cardiac *1C*, neuronal *1D*, and photoreceptor *1F* channels (Table 2.1) (20).

L-type is also sensitive to snake toxin, calciseptine. The part of long-lasting current, which is not blocked by dihydropyridines (DHPs) was termed N (neuronal)-type (21).

Channels that are found on Purkinje cells (a class of GABAergic neurons located in the cerebellum) described and termed as P-type. P-type channels are blocked by low concentration of ω-agatoxin IVA, and the part of this type which is blocked by only high concentration of ω-agatoxin IVA have been termed as residual

(or resistant) Ca^{2+} channel or R-type. T-type (transient, in opposite to long-lasting current) which is a low-voltage-activated (LVA) channel is inhibited by submicro molar concentrations of mibefradil (22-28).

Table 2.1. Types of Ca^{2+} channels.

Type	α_1 Subunit gene	Voltage	Current properties	Found in
L-type "Long-lasting" "DHP receptors"	$Ca_v1.1$ "*IS*", $Ca_v1.2$ "*1C*", $Ca_v1.3$ "*1D*", $Ca_v1.4$ "*1F*"	HVA	Long, large, high threshold	Cardiac, smooth, skeletal muscles, neuron, endocrine cells, bone
N-type "Neural"	$Ca_v2.2$	HVA	Short, high threshold	Neurons, sperms
P/Q type "Purkinje'	$Ca_v2.1$	HVA	Long, high threshold	Purkinje neurons in the cerebellum
R-type "Residual"	$Ca_v2.3$	IVA	Intermediate threshold	Neurons, sperms
T-type "Transient"	$Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$	LVA	Short, small, low threshold	Neurons, heart, pacemaker cells

L-type calcium channels

They are widely distributed in all types of cells except for platelets, especially found in high concentration in skeletal muscles. In sensory neurons, L-type Ca^{2+} channels show a slight inactivation during application of 200 msec depolarization pulses, the decay time constant being higher than 500 msec so they were termed (Long-lasting) or L-type (29, 30).

Biochemically they are complex proteins composed of four or five distinct subunits having different molecular masses (Figure 2.1). The α_1 subunit which forms the ion channel and contains Ca^{2+} antagonist binding sites; the α_2 subunit which is associated with α_1 and does not contain any high-affinity binding site; and three low

molecular-weight subunits, ß, γ and δ. The α_1 and ß subunits contain phosphorylation sites for cAMP-dependent protein kinase (31-36).

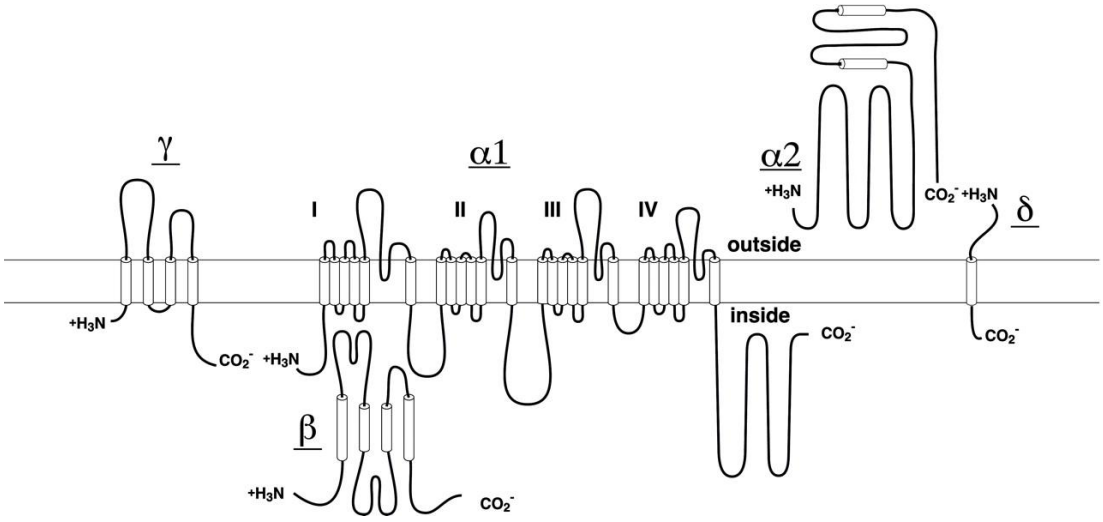


Figure 2.1. Subunits of skeletal muscle Ca_v1 Ca^{2+} channel.

The α_1 subunit is the largest one, and it incorporates the conduction pore, the voltage sensor and gating apparatus. Also, it contains most of the known sites of channel regulation by second messengers, drugs, and toxins.

Like the subunits of Na^+ channels, α subunit of voltage-gated Ca^{2+} channels is organized in four domains (I-IV), with six trans-membrane segments (S_1-S_6) in each (Figure 2.2). Domains are homologous in Na^+ channels while those of Ca^{2+} channels are heterogeneous.

The S_4 segment serves as the voltage sensor. The pore loop between trans-membrane segments S_5 and S_6 in each domain determines ion conductance and selectivity, and changes of only three amino acids in the pore loops in domains I, III, and IV will convert a sodium channel to calcium selectivity.

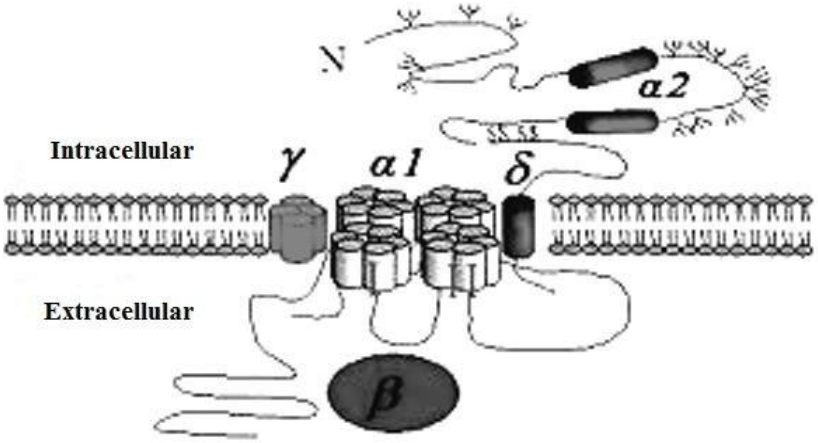


Figure 2.2. 3D structure of Ca^{2+} channel.

Although these auxiliary subunits modulate the properties of the channel complex, the pharmacological and electrophysiological diversity of calcium channels arises primarily from the existence of multiple α_1 subunits (37).

L-type Ca^{2+} channel blockers

It has been shown that L-type calcium channel antagonists interact in an allosteric manner with the channel protein and have been classified broadly into the following three groups: DHPs, phenylalkylamines and benzothiazepines. These compounds are quite different chemically, biochemically, and pharmacologically. At least six binding sites are believed to exist on the α_1 subunit for various kinds of L-type Ca^{2+} channel antagonists and the binding sites of the three major types of Ca^{2+} channel antagonist are different (38-40). These Ca^{2+} channel antagonists have different inhibitory effects on the cardiovascular system (Table 2.2).

Table 2.2. Inhibitory effects of three types of Ca^{2+} channel antagonists on the cardiovascular system.

Type	Vasodilation	Inhibition of cardiac conduction	Prolongation of atrial-venticular
Phenylalkylamines (Verapamil)	++	+++	+++
Benzothiazepines (Diltiazem)	++	++	++
Dihydropyridines (Nifedipine)	+++	+	0

While DHPs (e.g. nifedipine) has a greater inhibitory effect on vessels than on the heart, phenylalkylamines (e.g. verapamil) has a greater inhibitory effect on the heart. Benzothiazepines (e.g. diltiazem) (Figure 2.3) has an intermediate selectivity for vascular calcium channels. That is why nifedipine is used as an antihypertensive drug and verapamil is used for treatment of arrhythmias.

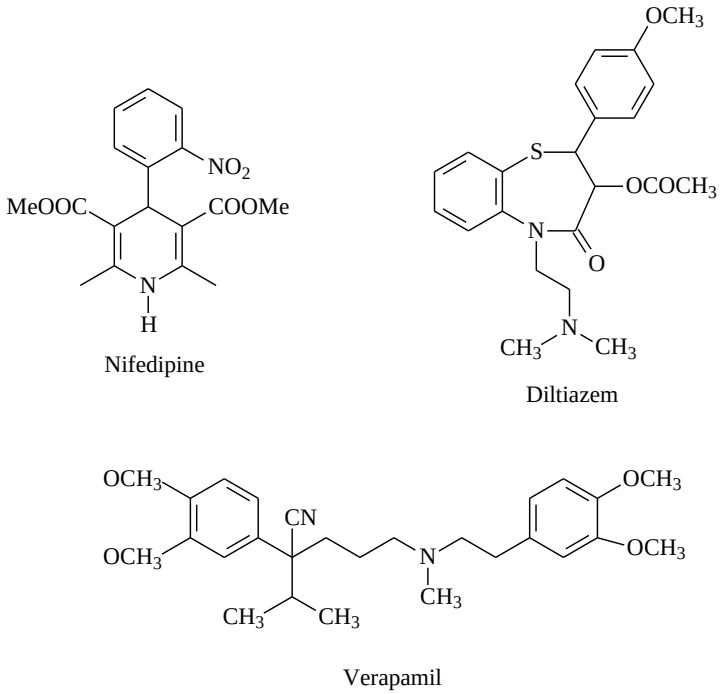


Figure 2.3. Prototypes of the three groups of Ca^{2+} channel blockers.

There are two possibilities for the selectivity of DHPs on vessels. First, the sensetivity of the α_1 subunit for DHPs is higher in vascular smooth muscle than in the cardiac ventricle. Second, the DHPs have a high affinity for the inactivated state of the Ca^{2+} channel, and vascular smooth muscle (resting membrane potential: -50 ~ -60 mV) has more inactivated channels than do ventricular myocytes (resting membrane potential: -90 mV).

2.4. 1,4-DHPs as Ca^{2+} channel modulators

DHPs were early recognized as Ca^{2+} channel modulators; nifedipine was early introduced as a member of the first generation of Ca^{2+} channel blockers as well as verapamil and diltiazem and logged into clinical medicine as antihypertensive agent. DHPs act streoselectively at distinct binding sites and their action is voltage-dependent (41, 42).

It is worth mentioning that some DHPs can act as calcium channel activators by making small molecular changes (Figure 2.4), so it is very advisable to classify DHPs as calcium channel modulators i.e. can serve as blockers and openers, rather than restrict their activity on calcium channel as blockers (43).

Although all membranes isolated from heart, vascular smooth muscle, neuron as well as skeletal muscle cells possess Ca^{2+} channels carrying binding sites specific to DHPs; DHPs exhibit a noticed selectivity in their effect on different tissues, and subsequently this selectivity controls their clinical applications.

Skeletal muscles appear to be resistant to pharmacological intervention by using Ca^{2+} channel blockers because the intracellular stores provide sufficient quantities of calcium that guarantee full activation of contractile system, so it is insensitive to changes in trans-membrane calcium conductivity.

On the other hand, the myocardial and smooth muscles are found to be much more susceptible to variation in environmental calcium or pharmacological agents that affect the trans-membrane calcium conductivity. Because their intracellular calcium stores have limited capacity, they have to be rapidly refilled from extracellular source via Ca^{2+} channels during the contraction process, so the myocardial and vascular muscles are susceptible to pharmacological effects of DHPs calcium channel modulators while skeletal muscles are not (44).

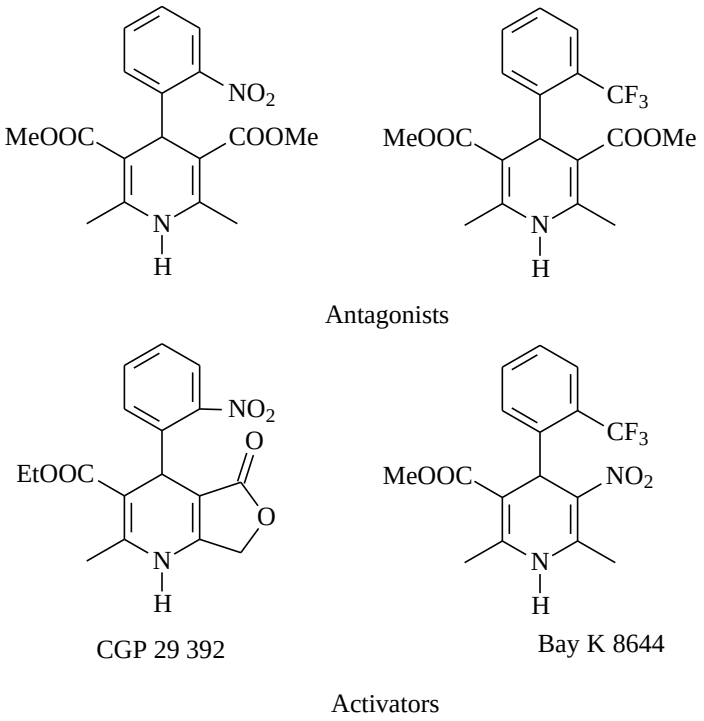


Figure 2.4. Example of DHPs that act as calcium channel antagonists and others act as activators.

Ligand binding studies using radio labelled DHPs such as [^{3}H]nimodipine and [^{3}H]nitrendipine were used to provide information about the binding of DHP analogues to different tissue cell membranes in heart, kidney, lung and brain.

In these studies, the receptor binding sites have been characterized and were shown to be highly specific and stereoselective. Also, according to these studies, the binding affinity of membranes of vascular muscles, cardiac muscles and neurons appeared to be similar high affinity while their sensitivity to that DHPs drugs differs greatly. The vascular smooth muscle membranes have the great sensitivity while the cardiac muscle membranes appear to be less sensitive and neuron membranes are very insensitive (45-48).

Furthermore, these results showed the high affinity of DHP-sensitive binding sites of Ca^{2+} channels that are in an inactivated state and offered a simple explanations of why low concentration of DHPs affects the contractility of smooth muscle but, so far as is known, has no effect on normal heart or brain function. This resistance of heart cells is due to that it is very likely that any region of heart reaches

a depolarized membrane for long enough to permit significant DHP binding. Though heart Ca^{2+} channels certainly become inactivated during action potential plateau, the time a channel spends in the inactivated state (> 1 sec.) is much less than the minute it takes of DHPs to bind and interact with channels (49-53).

Structure activity relationships (SAR) of DHP Ca^{2+} channel modulators

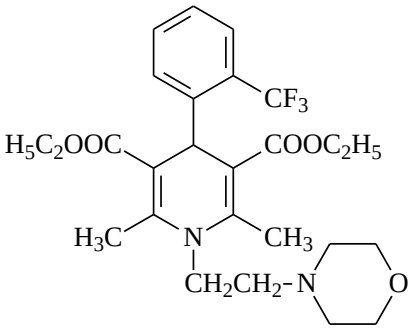
Numerous studies have been examined to reveal DHP's SAR and each position of the ring have been separately examined to estimate the effects of changes at this position on activity. SAR studies show the following:

a) Amendments to the 1,4-dihydropyridine ring

1,4-Dihydropyridine ring is essential for activity. Any alteration of ring by either oxidation to pyridine or reduction to piperidine abolishes the activity (54). It has been shown that DHPs exist in a boat conformation (55).

b) Amendments to N-1

The N-1 of the 1,4-DHP ring must be unsubstituted for optimum activity. Any substitution at N-1 decreases or even abolishes the activity (43, 56). Fluordipine is the only known exception of this rule (57).



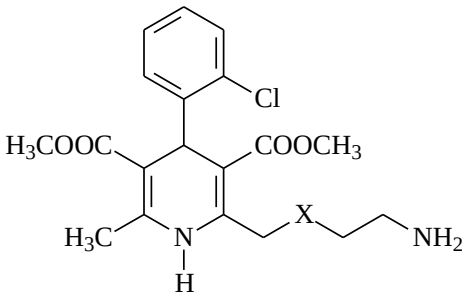
Fluordipine

c) Amendments to C-2 and C-6

The calcium agonistic activity of some DHP derivatives that do not have any substituents have been reported (27).

Although the presence of lower alkyl groups at C-2 and C-6 of 1,4- DHP ring is required for the optimal calcium antagonist activity, it is recognized that changes in substituents at these positions can be tolerated by DHP receptors and an increase in activity can be observed (56, 58). Substitution of lower akyl group by alkoxymethyl, hydroxymethyl, cyano, or other alkyl substituents was observed to have a positive contribution to the activity, and only one amino group is tolerated (59-61).

Lipophilic substituents at these positions affect the pharmacokinetic properties of DHP derivatives such as their duration of action (62, 63).



X: O (Amlodipin), S, CH_2

Substituents at C-2 and C-6 of 1,4-DHP has been reported to inhibit binding to cytochrome P_{450} enzyme which is responsible for the oxidation of the DHP ring. Therefore, 2-chloro-1,4-DHP derivatives exhibited an increased vasodilatory effect and extended duration of action (64-66).

d) Amendments to C-3 and C-5

C-3 and C-5 substituents modulate activity and tissue selectivity (67, 68). Ester groups at C-3 and C-5 of the 1,4-DHP ring are optimum, and replacement of ester group by acetyl or cyano greatly reduces activity. It has been proposed that carbonyl oxygen participates in hydrogen bonding with the receptor (69).

Based on the orientation of the individual carbonyl groups of the C-3 and C-5 ester substituents with respect to the DHP ring double bond, there are three different conformations are: trans/trans, cis/cis, and enantiomeric cis/trans and trans/cis arrangements (Figure 2.5) (70). Cis/cis arrangement or at least one cis arranged ester group is optimum for activity (71,72).

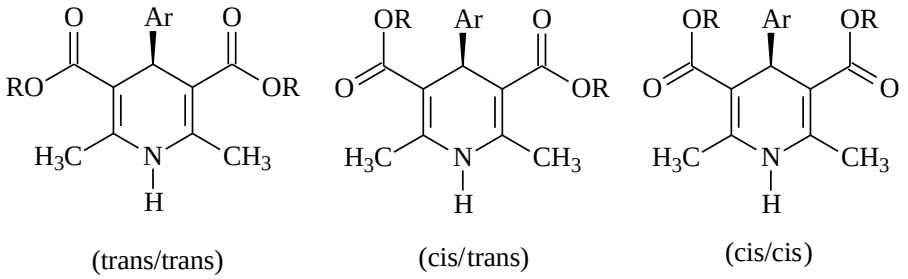


Figure 2.5. Ester carbonyl orientation with respect to the 1,4-DHP ring double bond.

Ester groups larger than methyl ester generally maintain or even increase the activity, suggesting a region of bulk tolerance in the 1,4-DHP binding site. Also, insertion of tertiary amino group to one of the ester side chain has been reported to prolong the duration of the antihypertensive action (73).

The replacement of one of the lipophilic ester groups by a polar nitro group changes the activity from negative inotropic hypotensive to positive inotropic hypertensive due to the change of the binding mode to binding site (43).

Introducing of non-identical ester groups at C-3 and C-5, the C-4 carbon becomes chiral, and stereoselectivity between the enantiomers is observed. It was proposed that Ca^{2+} channel modulating activity is dependent on the absolute configuration at C-4, whereby the orientation of the 4-aryl group acts as a "molecular switch" between antagonistic and agonistic activity, as we may find an enantiomer acts as antagonist while the other behaves as agonist (74, 75).

e) Amendments to C-4

The nature of C-4 aryl ring as well as nature and location of its substituents affect the Ca^{2+} channel blocking activity. Phenyl ring is preferred because animal

studies have proved the toxicity of compounds that have heteroaromatic ring as aryl group (56).

Compounds with ortho and meta substituted phenyl derivatives have a higher activity compared to para analogues.

The pseudoaxial conformation of C-4 aryl ring is also important. In case of ortho- or meta- substituted phenyl group at C-4, the synperiplanar conformer is favourable (76).

QSAR studies have applied the Hansch analysis method to a series of 4-phenyl-substituted DHPs to discuss the effect of phenyl groups substituted by different groups, differ in their lipophilic, electronic and steric properties on DHPs activity. These studies showed that the biological activity of DHPs is dependent on the lipophilic, electronic and steric properties of the substituents on 4-phenyl-1,4-DHP derivatives (77).

Other studies have found that bulky and lipophilic groups at the ortho-position and bulky groups with high Hammet electronic constant (i.e. electron withdrawing groups) at the meta-position of the 4-phenyl ring increase the 1,4-DHPs' activity. They also concluded that the potency of DHPs decreases with the increase in minimum width or length of substituents at the para-position (71).

3D QSAR study of 4-phenyl-substituted 1,4-DHPs indicates unfavourable steric interactions for bulky moieties in the para-position of the phenyl ring, while that bulky substituents are favourable in ortho- and meta-positions.

In addition the study concluded that the best combination is obtained when the bulky substituents at ortho or meta produce negatively charged electrostatic potential ; and the potential of electron-deficient 4-aryl moieties behave as electron acceptors in charge transfer mechanism (78).

The general requirements for DHP to act as Ca^{2+} channel modulator are summarized in Figure 2.6.

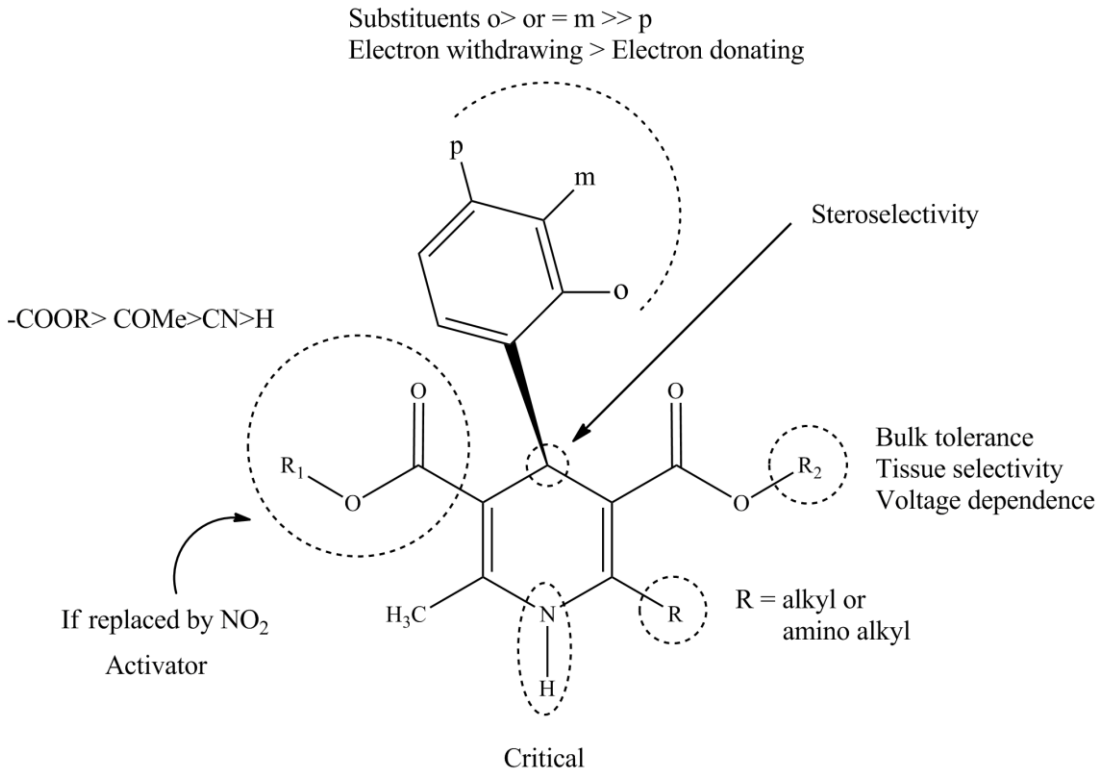


Figure 2.6. General requirements for Ca^{2+} channel modulator.

2.5. Geometry of 1,4-DHPs

A study based on computational chemistry calculations excluded the possibility of existence of 1,4-DHP derivatives in envelope or chair conformations because of the strong distortion of the double bonds in the chair arrangement. It also shows that the boat form is very likely because this form is an extremely flexible system, which may easily undergo defolding to the planar conformation along the line connecting the atoms N-1 and C-4. The general preference of a highly flexible boat conformation of the 1,4-DHP ring is also maintained in 3,5-dicarboxy- and 4-phenyl-substituted derivatives, respectively, which are more similar to the biologically active calcium channel blockers 1,4-DHPs.

Furthermore, X-ray studies for numerous derivatives indicate both boat and planar arrangements of the 1,4-DHP ring system (79). The 1,4-DHP boat form has 4-aryl ring at the axial position and orthogonal to the plane of dihydropyridine ring.

The position of substituent on the 4-aryl ring is very important. Ortho and meta substitution favours the synperiplanar arrangement (.i.e. the substituent is directed to the same side of the hydrogen at C-4) (Figure 2.7) which might be a common feature of DHP calcium channel antagonists.

Also, the orientation of C-3 and C-5 ester groups with respect to the DHP ring double bond also should be considered as the cis-cis arrangement ensures the optimum activity. X-ray structural investigations and theoretical calculations of fused 1,4-DHPs (compounds that one of ester groups is enclosed in rigid structure such as the lactone group) indicate that at least one ester must be in the cis arrangement, which is necessary for hydrogen bonding to the receptor.

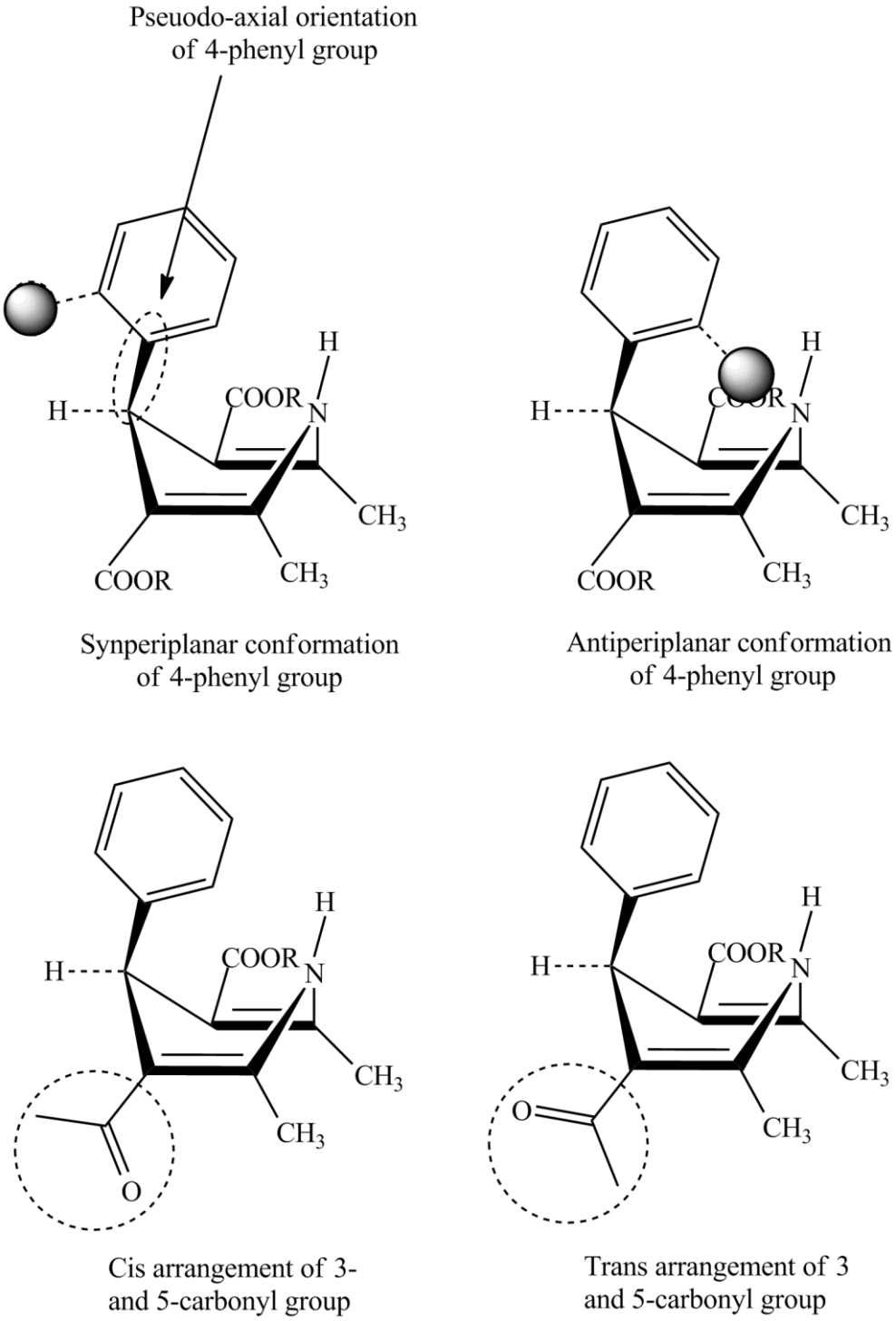


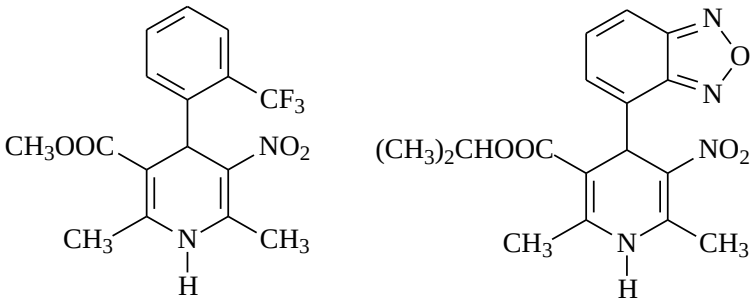
Figure 2.7. Geometry of 1,4-DHPs.

2.6. Stereochemistry of 1,4-DHPs

It was discussed before that the introducing of non-identical ester groups at C-3 and C-5, creates a chiral centre at C-4; and consequently two enantiomers are generated which was noticed in many occasions that they are opposing in their effect on calcium channels i.e. Ca^{2+} channel modulating activity is dependent on the absolute configuration at C-4. Thus makes the configuration of the 4-aryl group acts as a "molecular switch" between antagonist and agonist activity.

The strength of binding of both enantiomers to binding site as well as the potency of their opposing effects is not the same, thus makes their racemates exhibit the biological response of the predominant potent enantiomer. Therefore, some DHP racemates showed calcium channel activation effect such as Bay K 8644 and PN 202-791 (Figure 2.8) which are considered the most well-known calcium channel agonists or openers (65, 80).

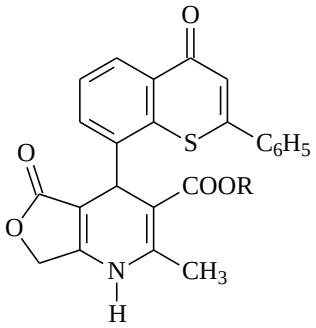
The results indicate that racemic mixture of Bay K 8644 acts as a calcium-channel agonist on both smooth and cardiac muscle; the (-)-(S)-enantiomer is approximately tenfold more potent as an agonist than the antagonist (+)-(R)-enantiomer (43).



Bay K 8644 PN 202-791

Figure 2.8. Structure of some 1,4-DHP Ca^{2+} channel agonists.

Similar findings were shown by some lactone fused DHP derivatives; the (S) antagonist enantiomer binds 50 fold stronger than the (R) agonist enantiomer (81).



2.7. Dual acting 1,4-DHPs

It was discussed that derivatives of DHP containing 3,5-dicarboxylic acid esters exhibit a spectrum of activity toward Ca^{2+} channels and their effect varying between being antagonist and agonist (82). However, it was interesting to study the behaviour of AK-2-38 (Figure 2.9), which is a C-4 2-pyridinyl DHP analogue. Although it exhibited twice the potency of nifedipine on smooth muscle, its dosage range that inhibited smooth muscle contraction (i.e. antagonistic activity) was shown to exhibit partial agonism on cardiac muscle. This high differential activity could be therapeutically beneficial as an antihypertensive agent, especially in the treatment of congestive heart failure. Therefore, the term "dual-acting agents" was known, a term that describes "dual, cardio-selective Ca^{2+} channel agonist and smooth muscle-selective Ca^{2+} channel antagonists" (83).

Structure-activity relationship (SAR) studies of these compounds have revealed that although C-4 2-pyridinyl isomer acts as a dual-acting agent, the 3-pyridinyl and 4-pyridinyl isomers act as agonists on both heart and smooth muscle. Therefore, the position of the pyridyl nitrogen-free electron pair might be important determinants of calcium-channel agonist-antagonist modulatory effects (67, 84, 85).

Other studies indicate that dual-acting derivatives can be obtained by introducing the DHP structure into condensed ring systems.

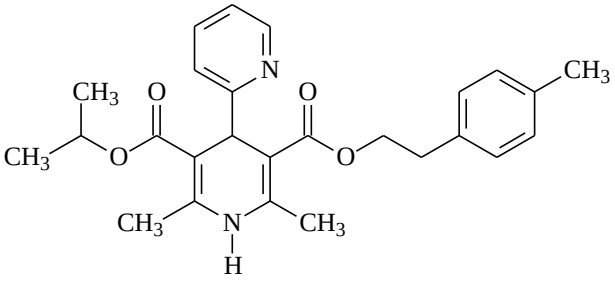


Figure 2.9. Structure of AK-2-38, an example of "dual-acting" 1,4-DHPs.

2.8. Fused 1,4-DHPs

Many studies showed the introduction of DHP in fused ring systems such as hexahydroquinolines, fluoroquinolines and indenopyridines (Figure 2.10) maintain the Ca^{2+} channel modulating activity. Racemic condensed DHP derivatives exhibit antagonistic effects on smooth muscle while show positive inotropic activity on electrically stimulated guinea pigs' atria (86). The lactone derivatives also show agonist-antagonist properties (80).

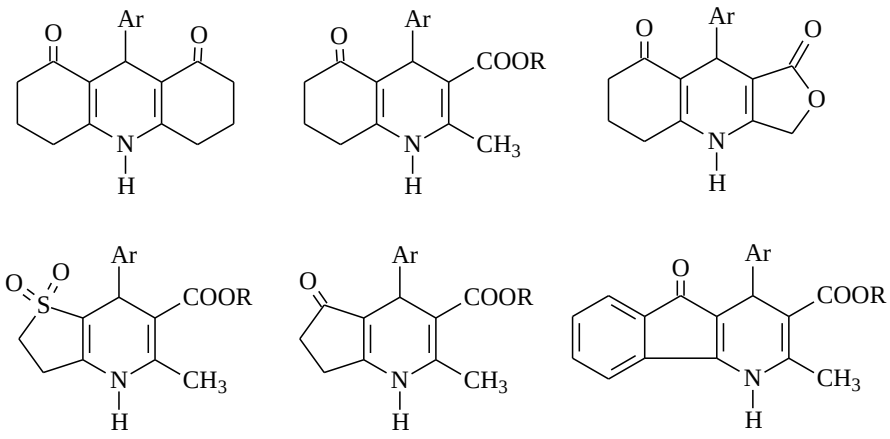


Figure 2.10. Structure of some fused 1,4-DHPs.

2.9. 1,4-DHPs as antihypertensive drugs

Ca^{2+} channel blocker 1,4-DHPs reduce peripheral resistance and blood pressure. The mechanism of action in hypertension is inhibition of Ca^{2+} influx into arterial smooth muscle cells. DHPs are more selective as vasodilators and have less cardiac depressant effect than verapamil and diltiazem. Reflex sympathetic activation with slight tachycardia maintains or increases cardiac output in most patients given DHPs as a compensatory mechanism.

Some epidemiologic studies reported an increased risk of myocardial infarction or mortality in patients receiving short-acting nifedipine for hypertension. Therefore, it is recommended that short-acting oral DHPs not be used for hypertension.

More than 30 years after the introduction of nifedipine, many DHP analogues have been synthesized. DHPs do continue to have a significant clinical role in the treatment of hypertension, particularly hypertension associated with complicating disorders of diabetes and in coronary artery disease and where other agents are contraindicated.

Amlodipine dominates the share of calcium channel blockers in the world market which reaches about $6 billion. Sucssesive efforts resulted in introduction of numerous second generation commercial products to the market with superior bioavailability and a slower onset and longer duration of action. Such compounds include amlodipine, nimodipine, felodipine, nisoldipine, mebudipine, clevidipine, azelnidipine, nicardipine and cilnidipine (Figure 2.11) (87). For example, amlodipine is a DHP with slow absorption and prolonged effect.

Felodipine seems to have even greater vascular specificity than either nifedipine or amlodipine, at concentrations that produce vasodilation; there is no negative inotropic effect.

Nisoldipine is more than 1000 times as potent in preventing contraction of human cardiac muscle *in vitro*, suggesting a very high degree of vascular selectivity.

Nimodipine was developed as an agent to relax the cerebral vasculature because of its high lipid solubility, (88).

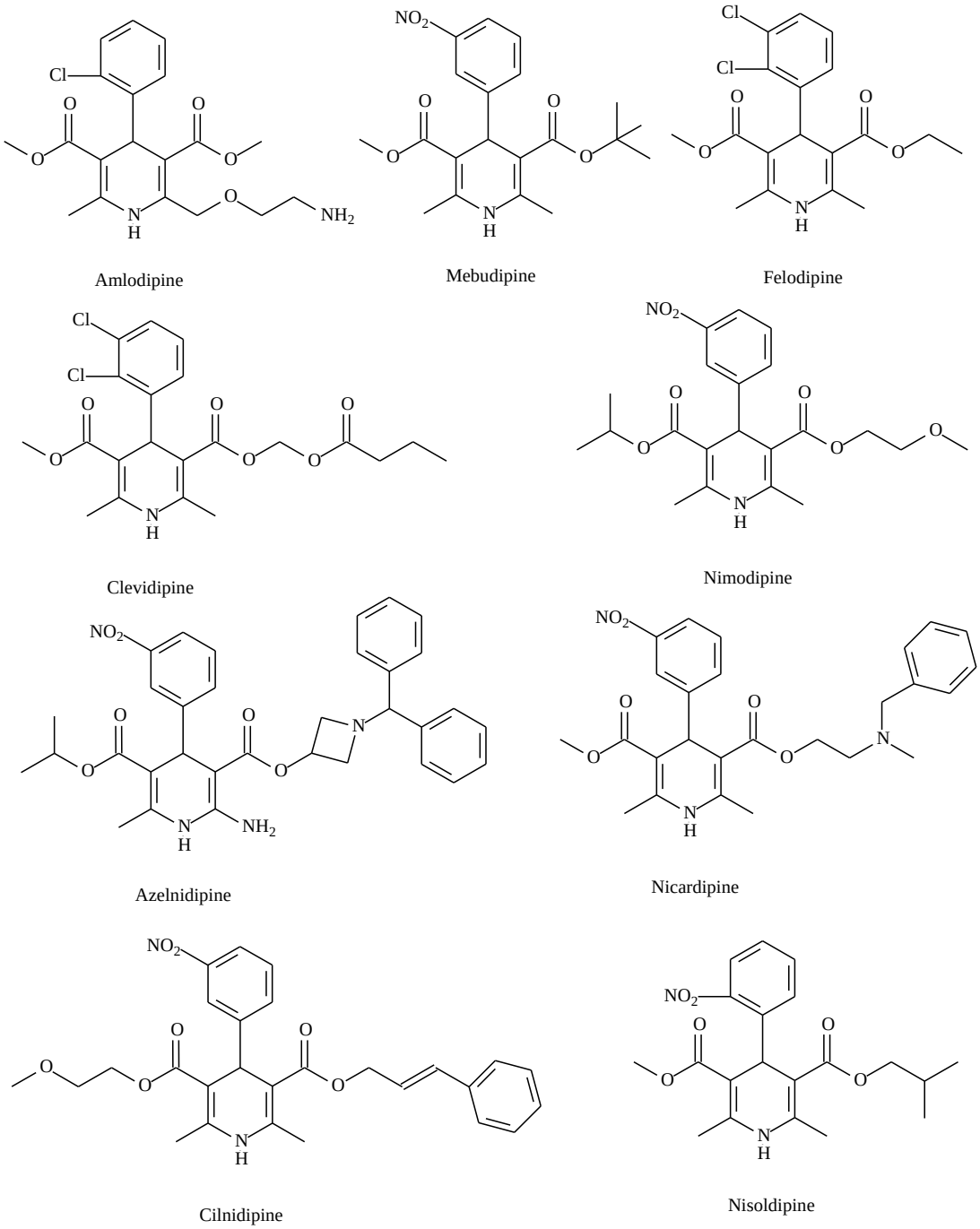


Figure 2.11. Structures of some Ca^{2+} channel blocker 1,4-DHPs.

The mechanism of calcium channel blockers in the smooth muscle relaxation is illustrated in Figure 2.12. The smooth muscle contraction is triggered by influx of Ca^{2+} (which can be blocked by Ca^{2+} channel blockers) through trans-membrane Ca^{2+} channels. The Ca^{2+} combines with calmodulin to form a complex that converts the enzyme myosin light-chain kinase (MLCK) to its active that phosphorylates the myosin light chains, thereby initiating the interaction of myosin with actin.

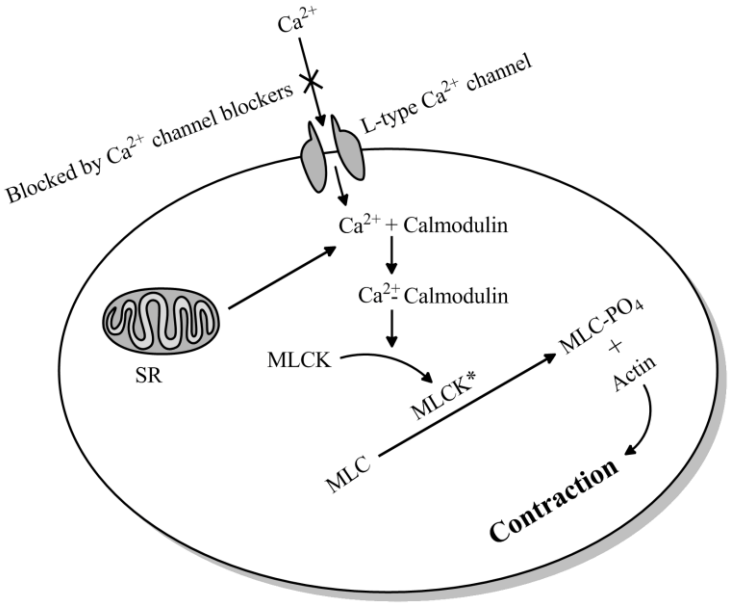
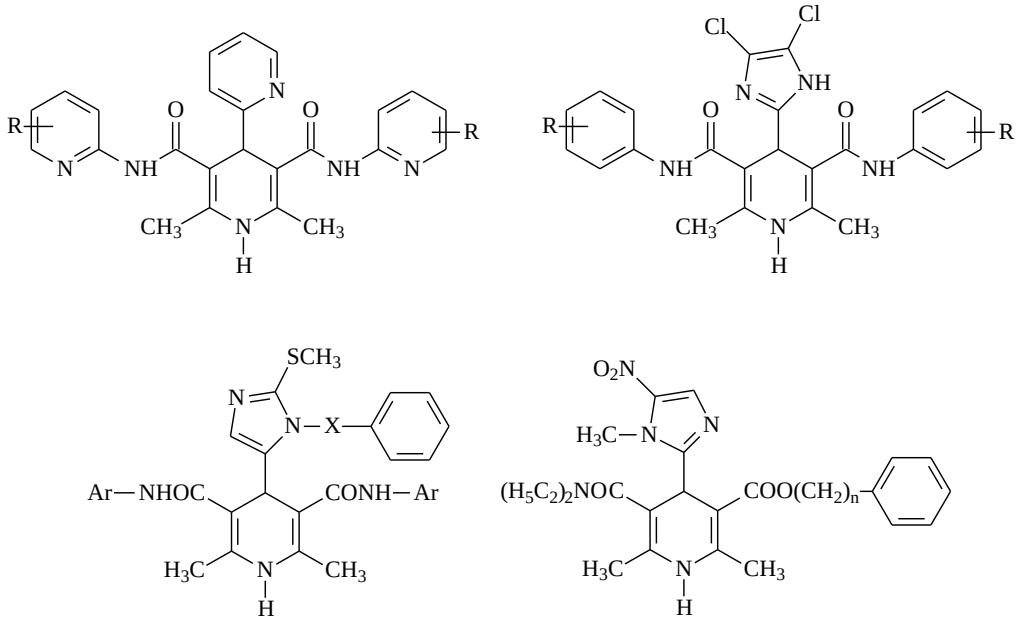


Figure 2.12. Mechanism of Ca^{2+} channel blockers in the smooth muscle relaxation.

2.10. Other pharmacological activities of DHPs

Antitubercular activity

Some DHPs, which do not obey the calcium channel blockers' SAR requirements, were studied and showed significant antitubercular activity. In these compounds, the classical phenyl group at C-4 may be replaced by heteroaryl group such as substituted pyridine or imidazole ring, and the ester groups at C-3 and C-5 was replaced by aryl or heteroaryl amides.

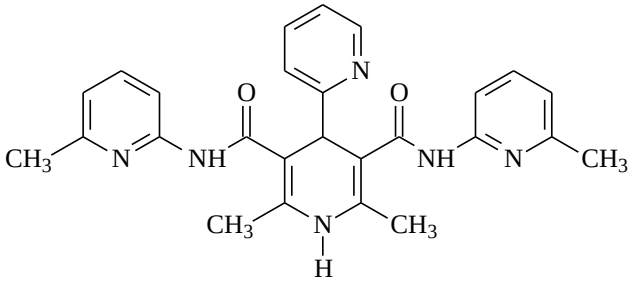


Ar: substituted phenyl or pyridyl n: 1-5

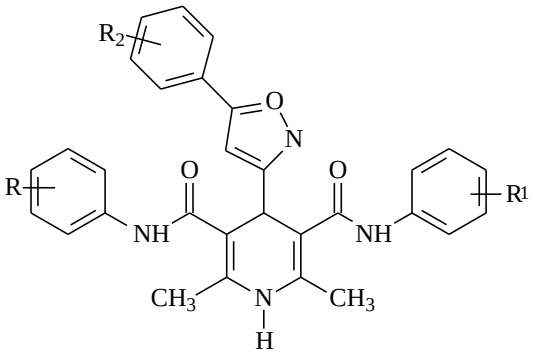
X: CH_2, NH R = alkyl or halid

Some of these compounds were more potent or almost equipotent to pyrazinamide when have been studied for antituberculosis activity against *M. tuberculosis* H37 Rv.

4-(2-Pyridyl)-2,6-dimethyl-3,5-bis-N-(6-methylpyridin-2-yl)-carbamoyl-1,4-dihydropyridine with half maximal inhibitory concentration (IC_{50}) = 12.5 μg/mL was more potent than control pyrazinamide with IC_{50} = 32 μg/mL) (89, 90).

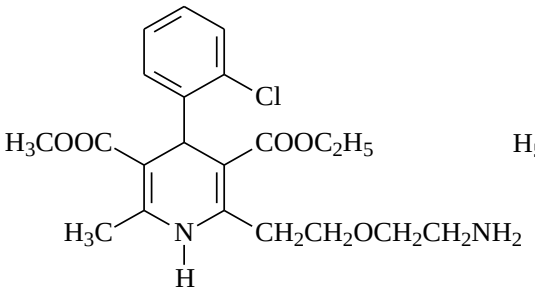


Other studies concluded that a series of N^3, N^5-Diaryl-4-(5-arylisoxazol-3-yl)-1,4-dihydropyridine-3,5-dicarboxamide exhibited antitubercular activity (91).

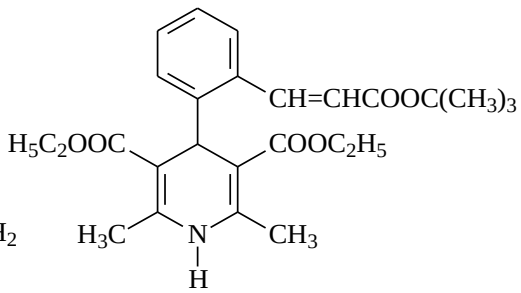


Antimicrobial, antifungal and antiparasitic activity

Antihypertensive 1,4-DHPs such as amlodipine and lacidipine showed activity against visceral leishmaniasis (92).

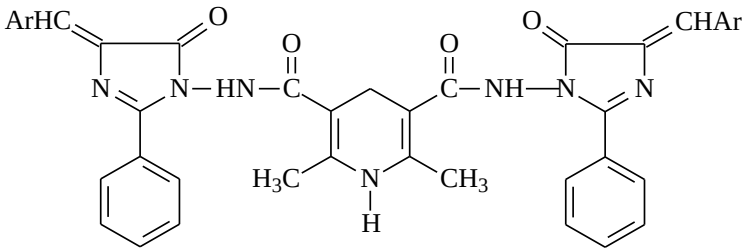


Amlodipin



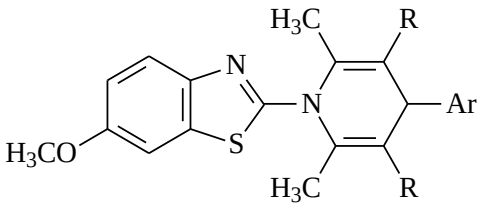
Lacidipine

Other diimidazolin-5-one carboxamide derivatives of 1,4-DHPs showed antifungal and insecticidal activity (93).



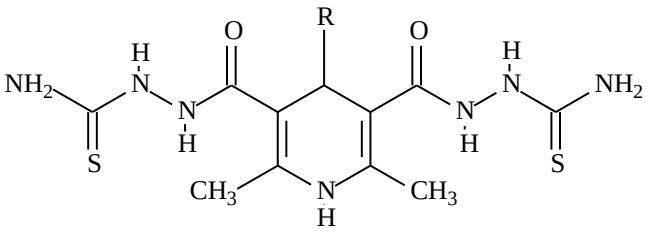
Some other 4-substituted-3-(4,4-dimethyloxazolin-2-yl)-1,4-dihydropyridylacetic acid derivatives have been screened against Gram-positive bacteria and only few analogues show moderate antibacterial activity (94).

A series of 2,6-dimethyl-N-(6-methoxylbenzothiazol-2-yl)- 2, 3, 5, 6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines exhibited activity against various bacterial strains like Lactobacillus, *Pseudomonas aeruginosa*, *Micrococcus lutius* and *Kocuria rosea*, also showed activity against fungi such as *Aspergillus niger* and *A. candidus* (95).

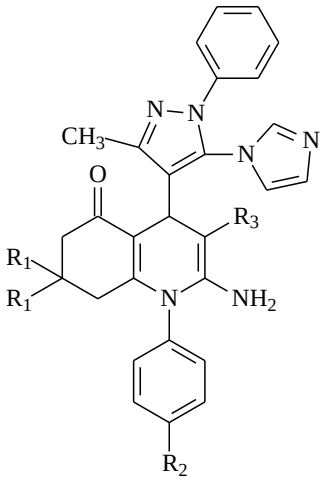


Ar: Substituted phenyl R: $COOC_2H_5$, $COCH_3$

A series of 3,5-bis[2-(aminothioxomethyl)hydrazidinocarbonyl]-1,4-dihydropyridines were screened for their antimicrobial activity, where they showed higher antimicrobial activity than ciprofloxacin against *Staphylococcus aureus,* while 4-(4-hydroxyphenyl) derivative showed higher antifungal activity than clotrimazole against *Candida albicans* (96).



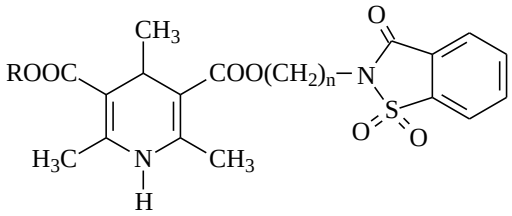
A new category of 4-(5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazolyl)-hexahydroquinoline derivatives were synthesized and their antimicrobial activity was tested. Some of them exhibited excellent antibacterial activity and moderate antituberculosis activity (97).



R_1: H, CH_3 R_2: H, OH R_3: CN, $CONH_2$ or COOEt

Antithrombotic activity

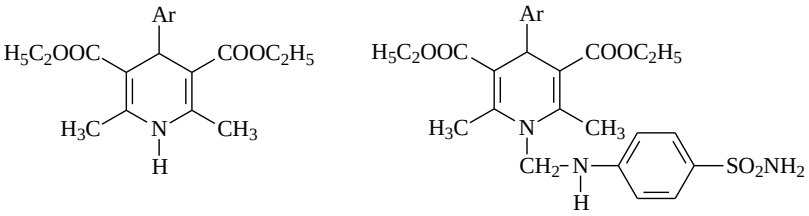
Some DHPs showed significant *in vivo* antithrombotic activity by inhibiting platelet aggregation that induced by platelet aggregation factor (PAF) or thrombin (98, 99).



n: 1, 2

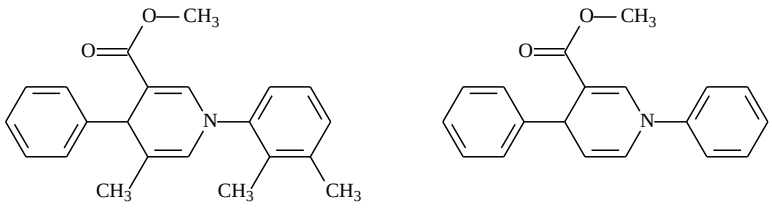
Antiulcer activity

The effect of calcium channel blockers on gastric acid secretion was suggested because of the role of calcium ion which plays in gastric acid secretion from parietal cells stimulated by acetylcholine, gastrin and histamine. Some DHPs showed antiulcer activity, that activity was enhanced by conjunction with carbonic anhydrase inhibitor sulphanilamide. The antiulcer activity was evaluated by estimating volume of gastric acid, PH, free and total acidity and ulcer index and compared them with that of omeprazole (100, 101).



Antidyslipidemic activity

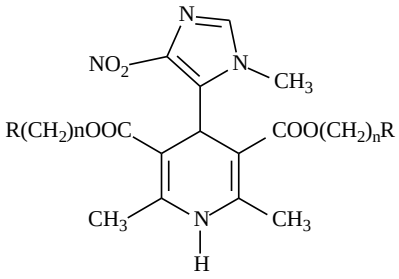
A series of N-aryl-1,4-dihydropyridines were screened for their lipid and triglycerides lowering activity *in vivo* and *in vitro* and compounds that have methyl and ester groups exhibited promising antidyslipidemic activity (102).



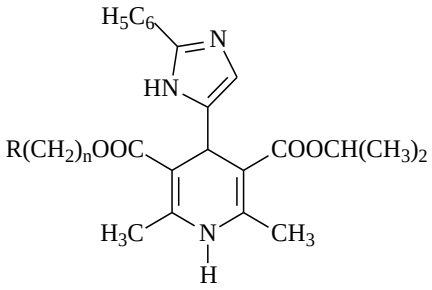
Anticonvulsant activity

Amlodipine (at 2.5 mg/kg) combined with ethosuximide (ETX), valproate magnesium (VPA) or phenobarbital (PB) significantly reduced their median effective dose (ED_{50}) values against pentylenetetrazol (PTZ)-induced clonic seizures (103, 104).

A new series of 4-(1-methyl-4-nitro-5-imidazolyl)-1,4-dihydropyridines exhibited anticonvulsant activity on maximal electroshock, PTZ or strychnine-induced convulsions in rats (105).

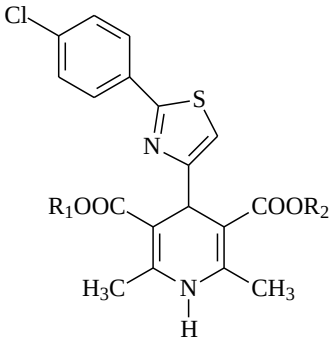


Other C-4 2-phenyl-4-imidazolyl substituted DHPs with three and five carbon chain ester group shown to be a positive contribution to PTZ-induced seizures in guinea pigs (106).



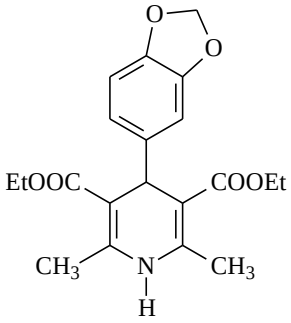
n: 1-5

Alkyl/aryl 2,6-dimethyl-4-[2-(4-chlorophenyl)-4-thiazolyl]-1,4-dihydropyridine-3,5-dicarboxylate derivatives which showed a low Ca^{2+} channel antagonist activity, expressed protection against PTZ-induced seizures (107).

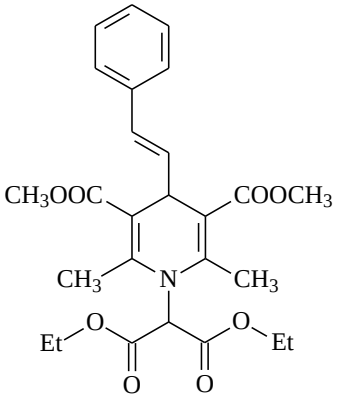


R_1, R_2: Alkyl or aryl

Dialkyl 4-(benzo[*d*][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylate derivatives were synthesized and tested for anticonvulsant activity using maximal electroshock and subcutaneous pentylenetetrazole induced seizure methods. 1,4-DHP compounds, that possess free N-H and diethyl ester functionality, showed significant anticonvulsant and antioxidant activities (108).

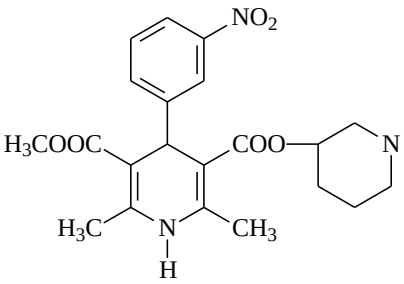


N-diethylmalonyl-1,4-dihydropyridine derivatives were synthesized and showed significant anticonvulsant activities (109).

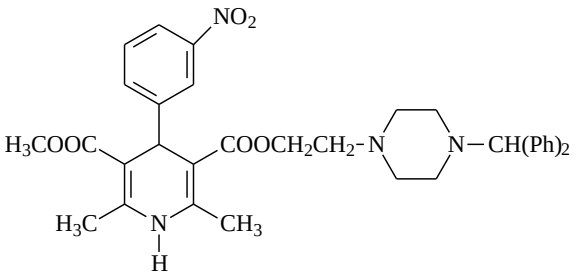


Cardio-protective activity

A study showed that orally administered benidipine and manidipine protect the myocardium from ischemia /reperfusion injury (110, 111).



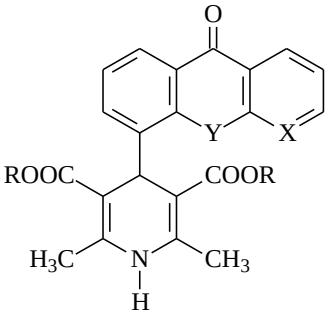
Benidipine



Manidipine

Antiarrhythmic activity

A series of 4-heterotricyclic substituted 1,4-dihydropyridines which are fairly good as calcium antagonists exhibited a potent selective bradycardic effects. These compounds also showed dose-dependently inhibition of atrial fibrillation, atrio-ventricular block and various types of arrhythmias (112-114).



R: Alkyl X: CH, N Y: O, S

Anti multidrug resistance (anti-MDR) activity

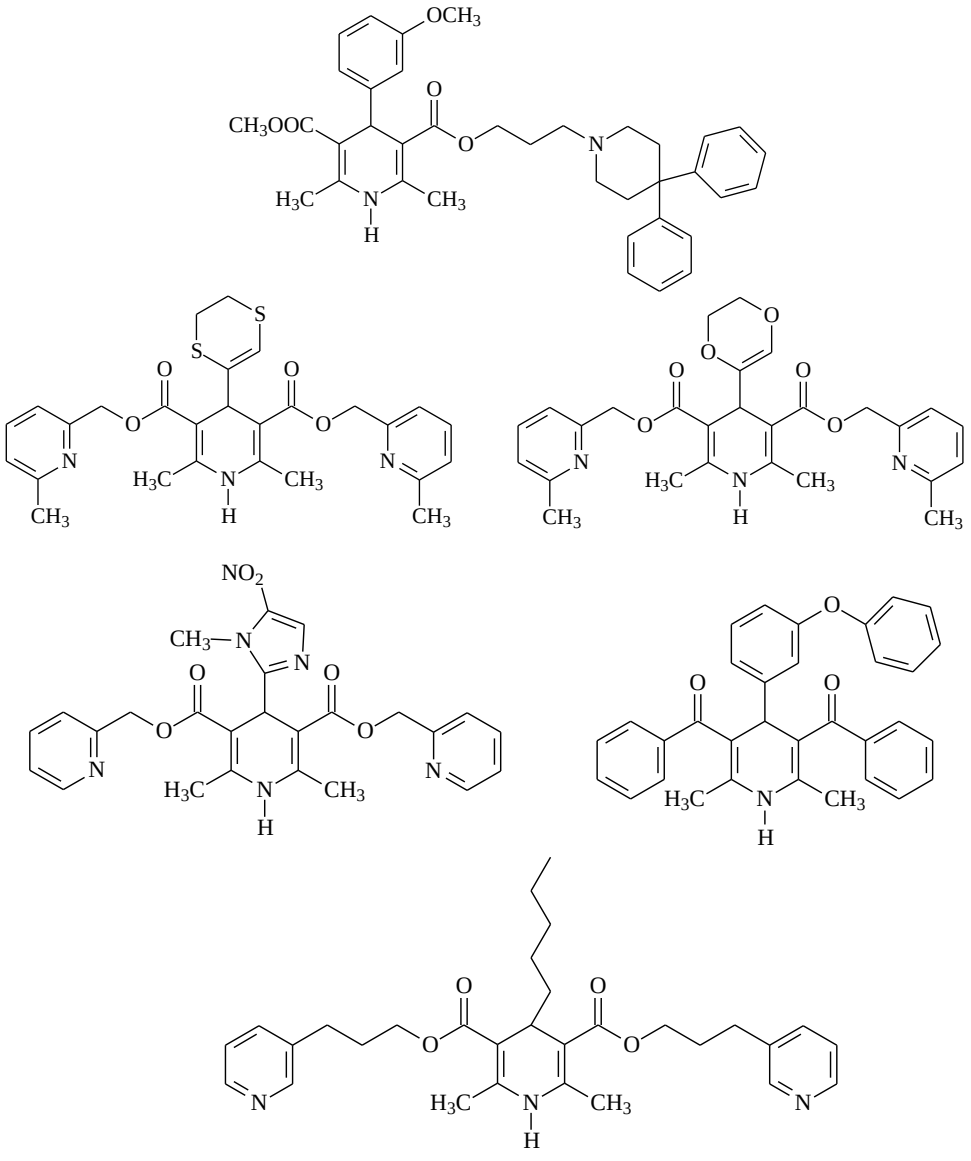
Multidrug resistance (MDR) is defined as resistance of tumor cells to the cytostatic and cytotoxic action of multiple structurally dissimilar chemotherapeutic agents. Such resistance is considered to be one of the major reasons of failure of chemotherapy for the majority of cancer patients (115).

Although Tsuruo, Philip and their coworkers showed that DHPs have reversing effects on MDR in cell lines, nifedipine failed clinically due to very poor outcome (6% response, 1 patient out of 15); and its dose-limitting cardiotoxicity (116, 117). These limitations encouraged the development of a new generation of anti-MDR

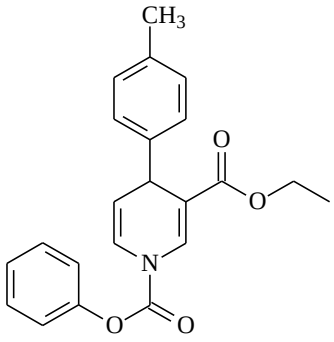
dihydropyridines, with high MDR reversing effect and significantly low Ca^{2+} channel blocking activity. In this way, some lead compounds were introduced, which were effective in classical MDR.

Successive studies findings can be helpful to derive a suitable anti-MDR DHP pharmacophore (118). It is suggested to synthesize asymmetrical derivatives of DHPs with ester groups on C-3 and C-5 positions that carry pyridyl group or 4,4-diphenyl piperidine group (119-122).

Conversion of ester group into acyl group can significantly reduce cardiovascular side effects. Also, replacement of C-4 phenyl group with heteroaromatic ring, such as nitroimidazole, or aliphatic groups is effective both in increasing MDR reversing activity and in decreasing Ca^{2+} channel blocking activity.

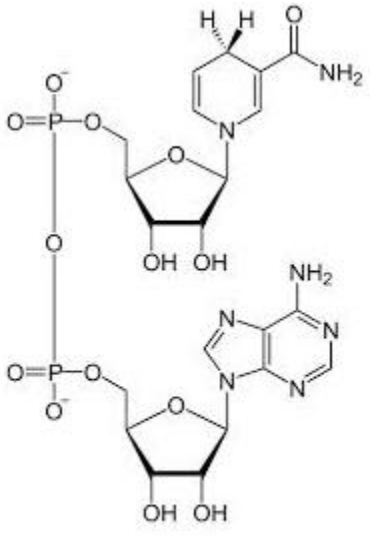


Removal of one methyl group also has positive effects on reducing Ca^{2+} channel blocking activity. Voigt and coworkers showed that protection of DHP nitrogen by transforming it into N-acyloxy caused better effects in reversing MDR and decrease in Ca^{2+} channel blocking activity (123).

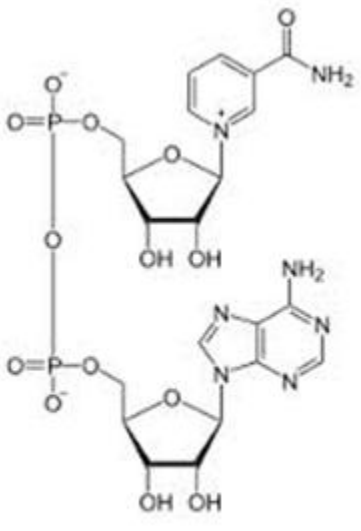


Antioxidant activity

The NAD^{+}-NADH biological redox systems refered to the important role that DHP nucleus plays role in biological systems as it conducts oxidative aromatization reactions place in presence of certain enzymes.



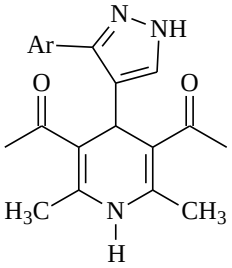
NADH



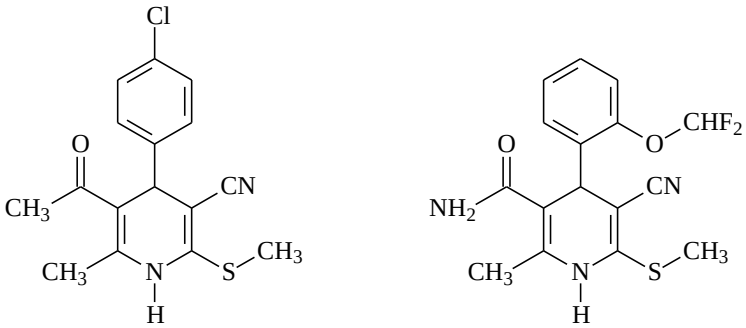
NAD^{+}

This “Hydrogen Transferring Coenzyme” system has a reduced 1,4-dihydropyidine form of nicotinamide adenine dinucleotide (NADH) while the oxidized pyridinium form is known as (NAD^+). Therefore, successive studies investigated the antioxidant activity of 1,4-DHP derivatives.

A series of substituted alkyl 1H-pyrazol-4-yl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate was investigated by measuring the diphenylpicrylhydrazyl (DPPH) radical scavenging assay and showed significant antioxidant activity (124, 125).



A study was conducted to investigate the effects of 3-acetyl/ carbamoyl-6-methylthio-1,4-dihydropyridine-5-carbonitriles on rat liver mitochondrial function and the compounds showed a mitochondrial protective action against lipid peroxidation (126).

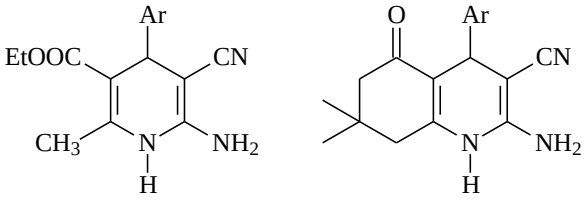


4-Nitrophenyl-1,4-DHP derivatives, is believed to inhibit the oxidative stress associated with hepatic transformation of drugs (127).

Other studies concluded that N-aryl-1,4-dihydropyridines that have t-butyl ester derivarives showing potent antioxidant activities (102).

Neuroprotective activity

6-amino-5-cyano-1,4-dihydropyridine derivatives induced a remarkable neuroprotective effect against toxicity caused by high [K^+]-elicited, [Ca^{+2}]-overload and against H_2O_2-generated free radicals in SH-SY5Y cells (128).

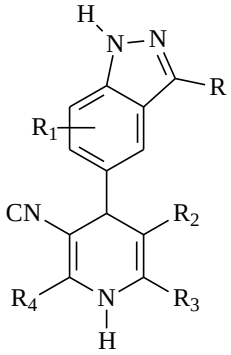


Ar: substituted phenyl or pyridyl

A study was conducted to examine the neuroprotective effect of isradipine on dopaminergic neurons, revealed that isradipine produced a dose-dependent sparing of dopamine fibres and cell bodies at concentrations achievable in humans, suggesting that isradipine is a potentially viable neuroprotective agent for Parkinson's disease (129).

Tyrosine kinase inhibiting activity

Novel 4-(indazolyl)-1,4-dihydropyridine showed protein tyrosine kinase inhibitory activity that can be used for the treatment cancer and other proliferative disorders (130).



Miscellaneous pharmacological activities of 1,4-DHPs

Several DHP derivatives have been found to have bronchodilatory and hepatoprotective activity (131). They have also shown analgesic and anti-inflammatory properties as it potentiated the hypothermic effect of opiates, also some DHPs were shown to be exhibit inhibition of GABA and adenosine receptors (132-135).

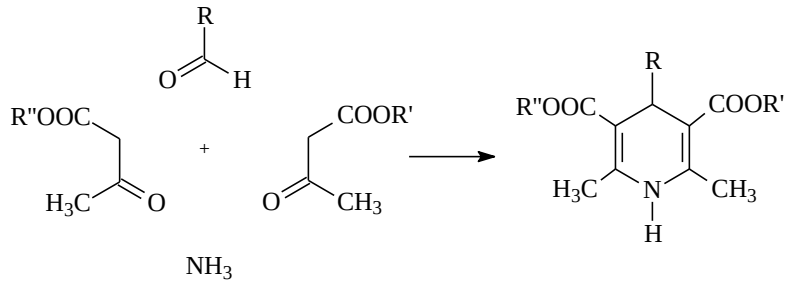
Recent studies have demonstrated that DHP Ca^{+2} channel blockers are able to inhibit aldosterone-induced activation of mineralocorticoid receptor (136).

Furthermore, other studies concluded that efodipine, a DHP Ca^{2+} channel blocker that blocks both L and T-type channels, has an inhibitory effect on aldosteron secretion in human adenocarcinoma cells (137, 138). Also DHPs can be used in treatment and diagnosis of diseases associated with accumulations of Alzheimer's amyloid (139, 140). Dimeric 4-aryl-1,4-dihydropyridine exhibited HIV-1 protease inhibiting activity (141, 142).

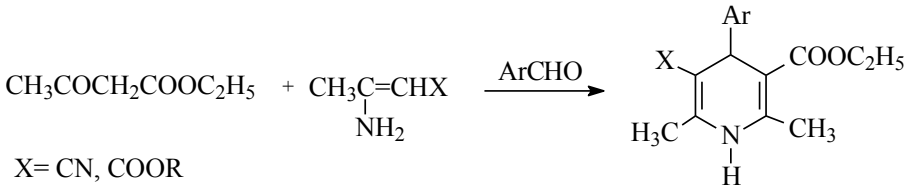
2.11. Synthesis of 1,4-DHPs

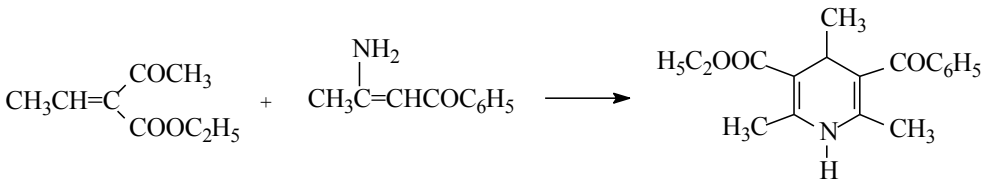
Classical Hantzsch synthesis of 1,4-DHPs

In 1882, Hantzsch reported the first synthesis of symmetrical substituted 1,4-dihydropyridines via multi-component one pot condensation of aldehyde, alkyl acetoacetate and ammonia in acetic acid or refluxing alcohol. (143-146).

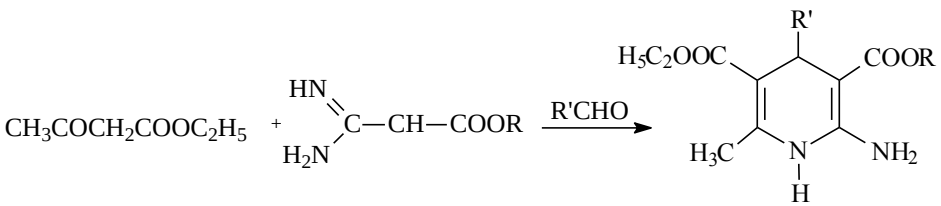


Active methylene compound can be used directly in Hantzsch reaction or can be incorporated in enamine or β-aminocrotonate form (147-150).

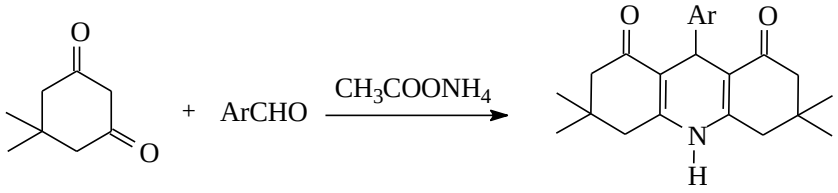




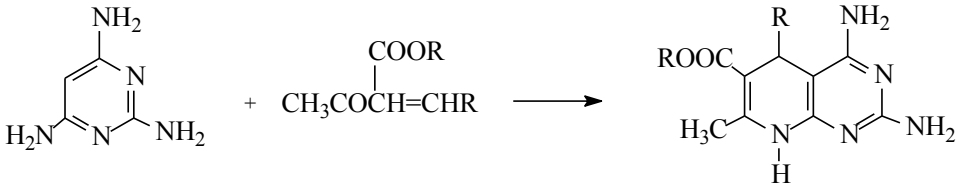
Instead of enamine compounds, amidin derivatives may be used in the preparation of 1,4-DHPs (134).



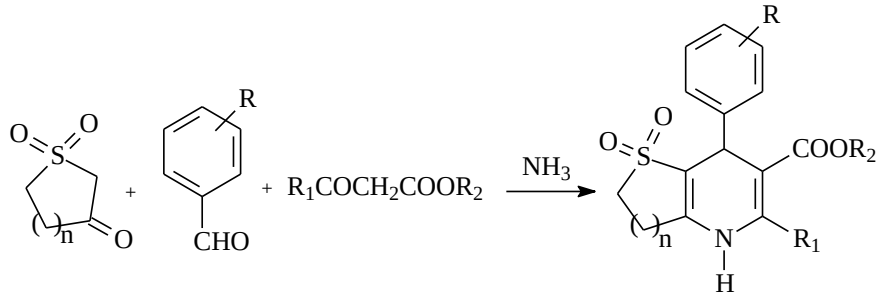
In classical Hantzsch reaction, ammonia was used as a nitrogen source. However, ammonium acetate, ammonium carbonate, formamide, and aldehyde-ammonia solid derivatives may also be used (151, 152).



Condensed derivatives of 1,4-DHP were obtained by the reaction of 2,4,6-triaminopyrimidine with α, β-unsaturated ketones (134).

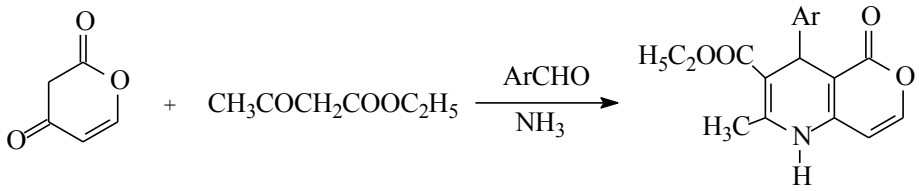


Condensed 1,4-DHP derivatives bearing five or six membered cyclic sulfone ring were obtained by reaction of tetrahydrothiophene-3-one-1,1-dioxide or dihydro-2H-thiopyran-3-one-1,1-dioxide, substituted benzaldehyde, appropriate acetoacetate and ammonia (153).

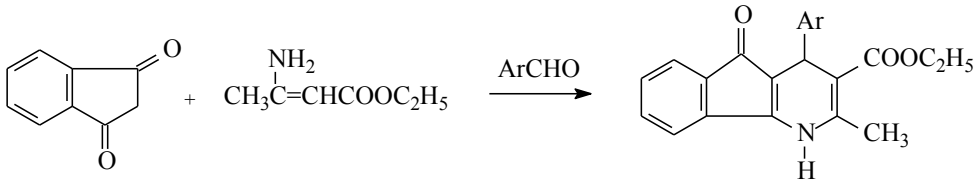


n: 1, 2

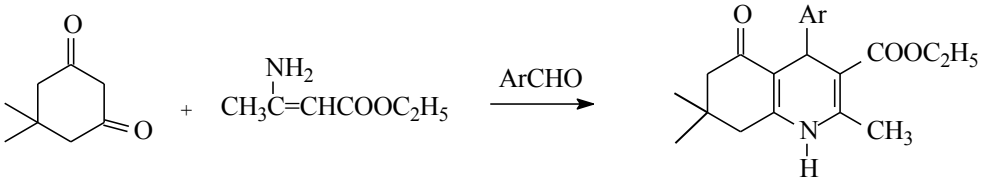
Pyrano-1,4-DHP derivatives can be obtained by using of 3H-pyran-2,4-dione in Hantzsch reaction (134).



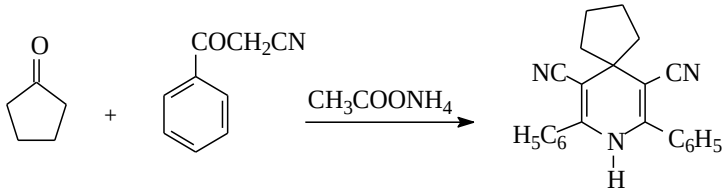
Reaction of 1,3-indandione, ethyl 3-aminocrotonoate and substituted aromatic aldehydes afforded indeno[l,2-b]-1,4-dihydropyridine derivatives (154).



Condensed 1,4-DHP derivatives can be obtained by Hantzsch reaction such as hexahydroqinoline that can be resulted from the reaction of dimedon, ethyl β-aminocrotonoate and substituted aromatic aldehydes (155-159).



Spiro1,4-dihydropyridine derivatives can be obtained by the reaction of cyclopentanone with two moles of 2'-cyanoacetophenone in presence of ammonium acetate (151).

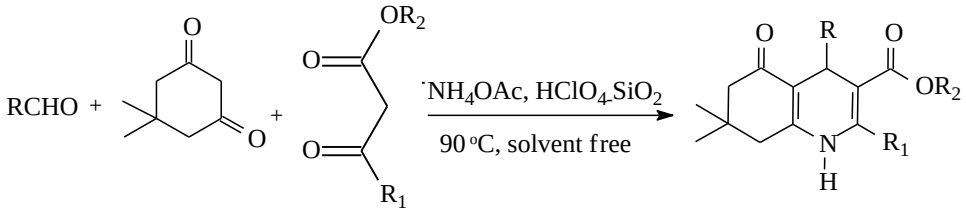


Catalysed Hantzsch synthesis of 1,4-DHPs

The aforementioned uncatalyzed Hantzsch 1,4-DHP synthesis methodologies have been associated with several shortcomings such as requirement of long reaction times, harsh reaction conditions, low product yields and occurrence of several side products. Therefore, successive efforts have been done to develop other methodologies that can overcome some of these disadvantages by using catalysts.

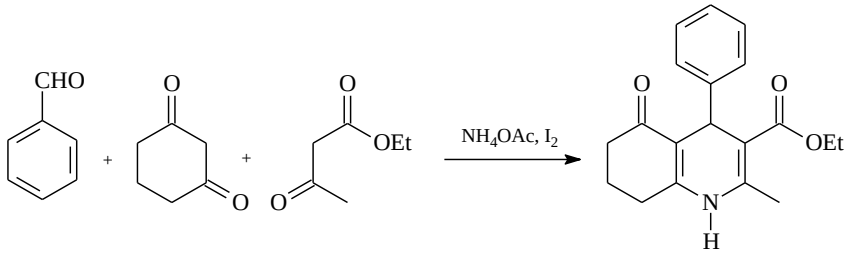
Hantzsch reaction of aldehydes, dimedone, ethyl acetoacetate and ammonium acetate in the presence of $HClO_4$-SiO_2 under solvent-free conditions affords

condensed 1,4-DHP derivatives in short reaction times and with high yields (80-90%) (160).



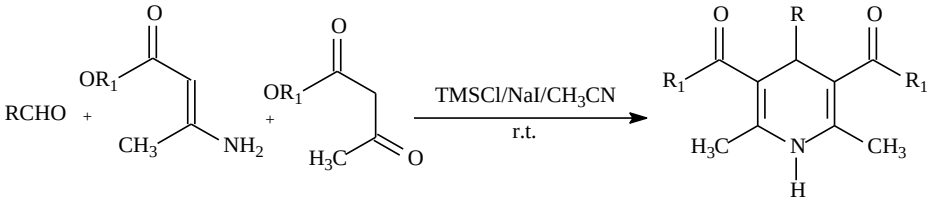
R= phenyl, substituted phenyl, thienyl, furyl and pyridyl R_1, R_2= alkyl

Other studies described the synthesis of 1,4-DHP derivatives at room temperature using catalytic amount of iodine with excellent product yields. Although reactions occurred both in the presence and in the absence of molecular iodine, but iodine catalyzed the reaction efficiently and also improved the reactions yields (161, 162).

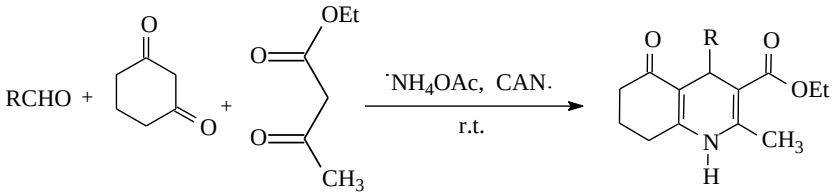


Iodine (mol%)	Time (h)	Yield (%)
0	4	56
15	4	99
30	2.5	99
50	1.5	70

Substituted Hantzsch 1,4-DHPs have been achieved using the classical Hantzsch procedure at room temperature in the presence of trimethylsilyl iodide (TMSI) that is generated in situ in acetonitrile. The reaction was complete in six h. at room temperature and the product was isolated by usual work-up, in 80% yield, with high purity (163).

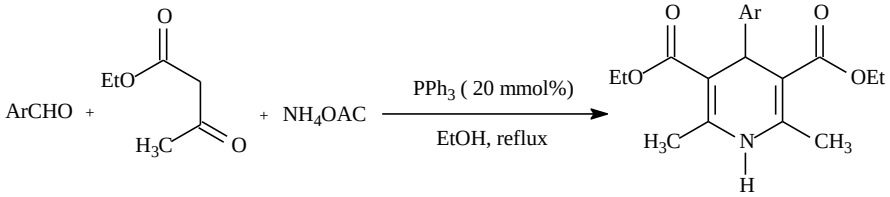


An efficient one-pot synthesis of high yields of polyhydroquinoline derivatives at ambient temperature using ceric ammonium nitrate (CAN) as catalyst via the Hantzsch reaction was reported (164).

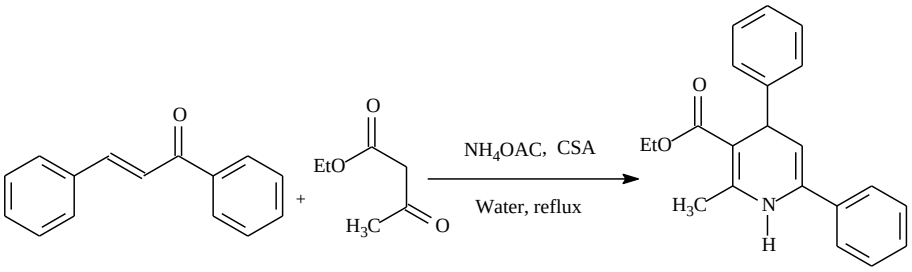


CAN (equiv)	Time (h)	Yield (%)
0	4	56
0.02	2	93
0.05	1.5	98
0.1	1	65

Other study described synthesis of 1,4-DHPs in good yields via the triphenylphosphine-catalyzed Hantzsch three-component reaction of an aromatic aldehyde, ethyl acetoacetate and ammonium acetate (165).

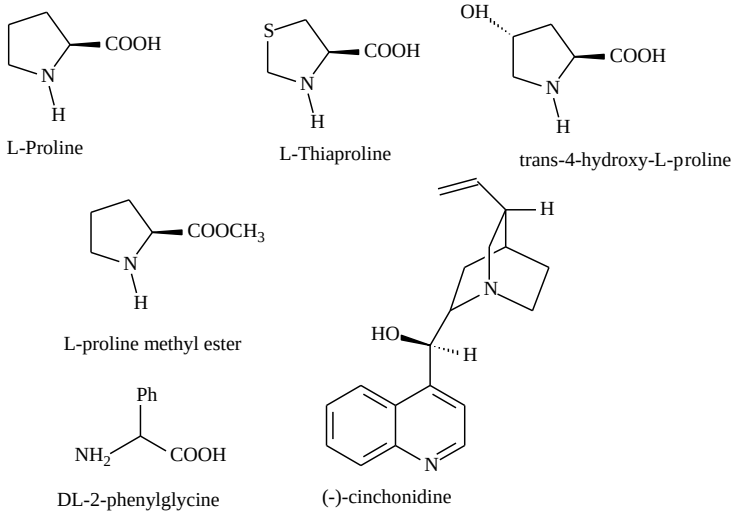


C5-unsubstituted 1,4-DHPs were obtained in good to excellent yields by proceeding through a simple, mild and efficient procedure by reaction of ethyl acetoacetate, chalcone derivatives and ammonium acetate in presence of cellulose sulfuric acid (CSA) as a catalyst. The catalyst can be easily separated from reaction mixture and reused several times in subsequent reactions (166).

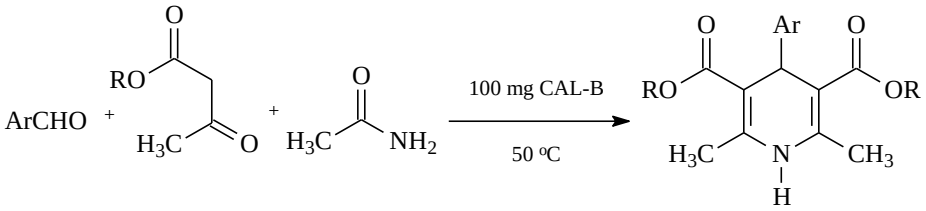


CSA (g)	Time (h)	Yield (%)
0	6	35
0.02	3	90
0.05	1.5	98
0.1	1	75
0.12	1	74

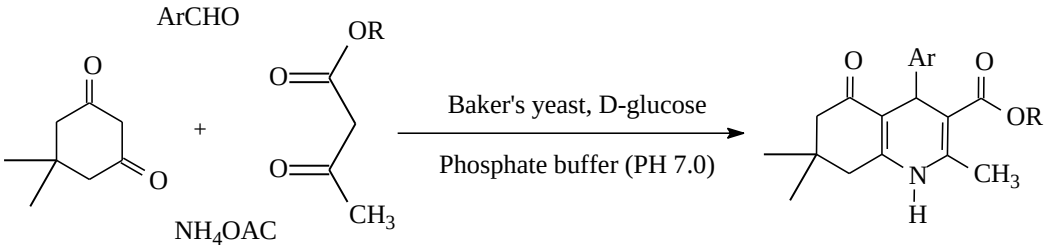
The catalytic efficiency of various small organocatalysts such as L-proline, trans-4-hydroxy-L-proline, L-proline methyl ester, L-thiaproline, DL-phenylglycine, and (-)-cinchonidine in the synthesis of polyhydroquinoline derivatives was reported (167).



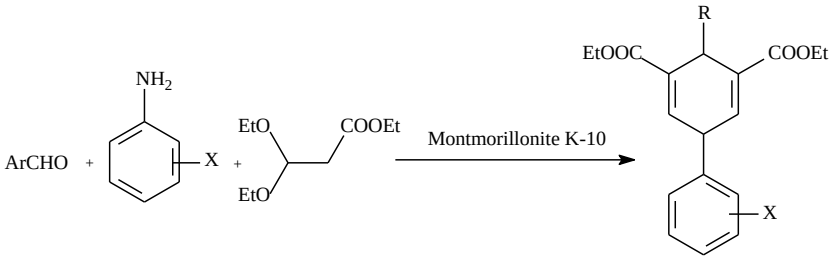
Enzyme catalysed biotransformation methodologies have been applied in Hantzsch 1,4-DHP synthesis. Candida antarctica lipase-B (CAL-B)-catalyzed three-component Hantzsch type reaction of aldehyde with 1,3-dicarbonyl compounds and acetamide in non-aqueous solvent has been developed. Acetamide was utilized as a novel ammonia source in the Hantzsch-type reaction (168).



Also, Bakers' yeast efficiently catalyzes the unsymmetrical Hantzsch reaction through a four-component coupling of aldehydes, β-ketoesters, dimedone and ammonium acetate to form polyhydroquinoline derivatives in good to excellent yields (169).



A green methodology for multiple component reaction of anilines, arylaldehydes and ethyl-3,3-diethoxypropionate in water using montmorillonite K-10 as catalyst afforded 2,6-unsubstituted dihydropyridines depending on the nature of anilines employed (170).



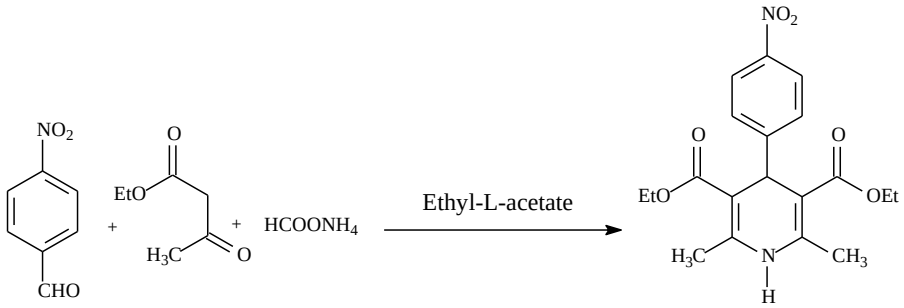
Another study described the synthesis of multi-substituted symmetric and asymmetric 1,4-DHPs in moderate to good yields via one-pot multicomponent reactions of β-dicarbonyl compounds, aldehydes and amines at room temperature on montmorillonite K-10 (171).

Other procedures comprise the use of ionic liquids, zinc chloride, Indium(III) chloride, $SiO_2/NaHSO_4$, tetrabutylammonium hydrogen sulfate, metal triflates, Iron(III) fluoride and tetrabutylammonium hexatungstate $[TBA]_2[W_6O_{19}]$ (172-179).

Also nanotechnolgoy took a part in development of new catalysts for Hantzsch reaction such as titanium dioxide, tin dioxide, zinc oxide, and SBA-15/SO_3H nanoparticles (180-183).

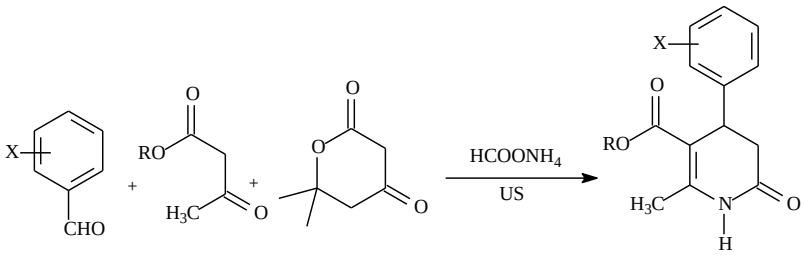
Visible light, ultrasound and microwave assisted synthesis of 1,4-DHPs

A mixture substituted benzaldehyde, ethyl acetoacetate and ammonium formate was irradiated with tungsten lamp at room temperature in ethyl-L-lactate water solution at room temperature to afford symmetrical and unsymmetrical 1,4-DHPs by highly efficient one-pot green methodology (184, 185).



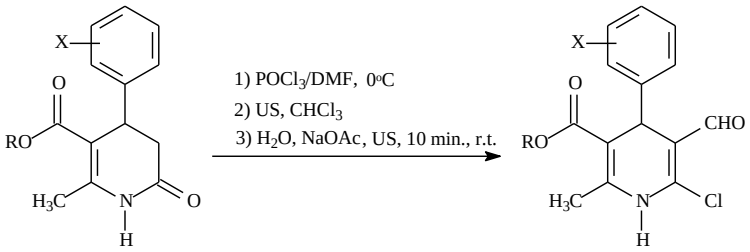
Reaction conditions	Temp. (°C)	Time	Yield (%)
Thermal	27	72 h	-
Visible light (150 W tungsten lamp)	27	150 min.	92
Visible light (150 W tungsten lamp)	27	90 min.	95
Visible light (150 W tungsten lamp)	27	120 min.	88
Visible light (150 W tungsten lamp)	27	135 min.	85

6-Chloro-5-formyl-1,4-dihydropyridine derivatives can be obtained in two steps. First step was done by the condensation of Meldrum's acid, aromatic aldehydes, alkyl acetoacetates and ammonium acetate in glacial acetic acid under ultrasound irradiation at room temperature to give 3,4-dihydropyridone derivatives, that can be converted to the desired product in the second step via Vilsmeier-Haack chloroformylation under ultrasound irradiation (186, 187).

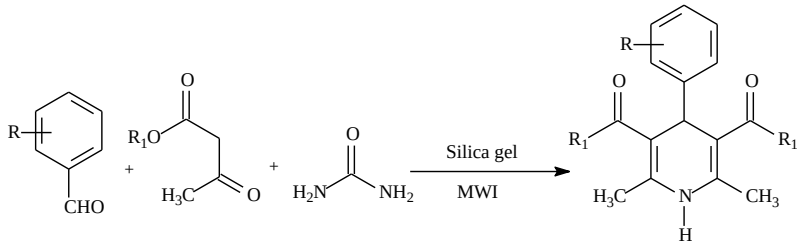


R= CH_3 and C_2H_5. X= H, 2-Cl, 4-Cl, 2-NO_2, 3-NO_2, 4-NO_2, 4-CN, 4-$COOCH_3$

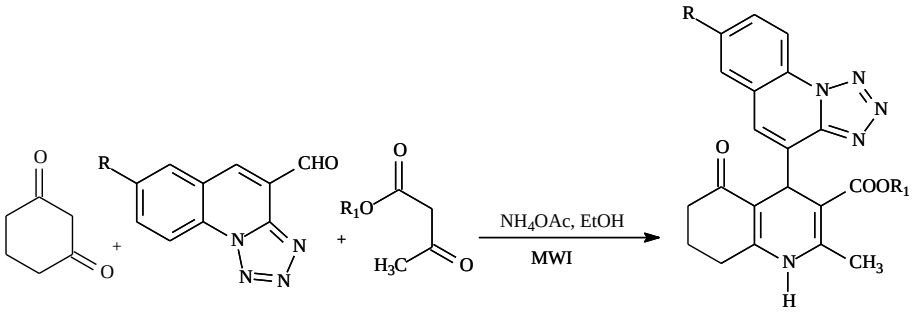
Solvent	Time (min.)	Yield (%)
$CHCl_3$	15	85
AcOH	10	95
DMF	10	88
EtOH	20	85



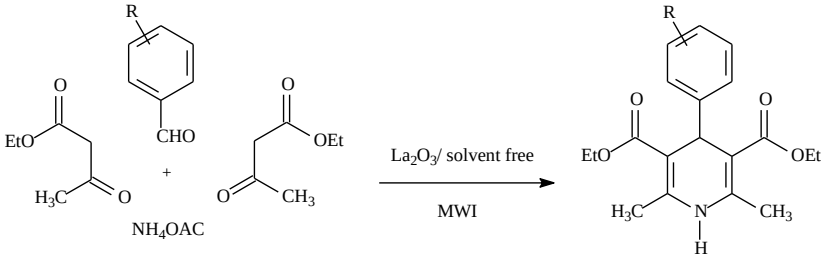
The efficiency of microwave irradiation (MWI) in promoting organic synthesis and the success of their application in these heterocyclic synthesis extended to the synthesis of 1,4-DHPs. A mixture of aromatic aldehyde, two moles of ethyl/methyl acetoacetate and urea was thoroughly mixed with silica gel, and then the mixture was taken in a beaker, placed in an alumina bath and subjected to microwave irradiation. The reaction afforded 4-aryl-1,4-DHPs in a very short time (188).



Other microwave assisted efficient Hantzsch reaction via one pot coupling reactions of tetrazolo[1,5-a]quinoline-4-carbaldehyde, cyclohexane-1,3-dione, alkyl acetoacetate and ammonium acetate was reported to synthesize of tetrazolo[1,5-a]quinoline derivatives of 1,4-DHPs, acridine-1,8-diones and polyhydroquinolines. The process was shown to be high yielded rapid method (189).



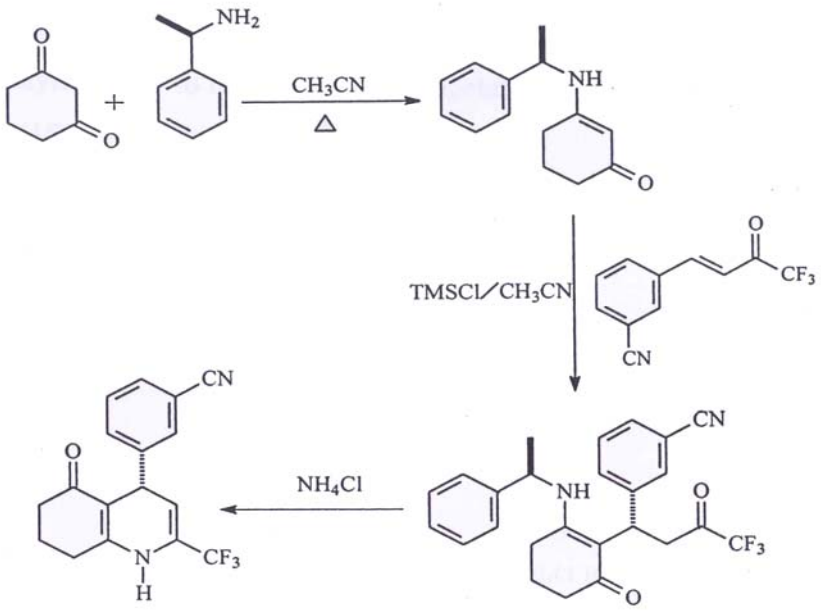
An efficient high yielded synthesis procedure of 1,4-dihydropyridines via lanthanum oxide catalysed Hanzch reaction of aldehydes, β-ketoester and ammonium acetate without solvent under the irradiation of microwave was described (190).



Stereoselective synthesis of 1,4-DHPs

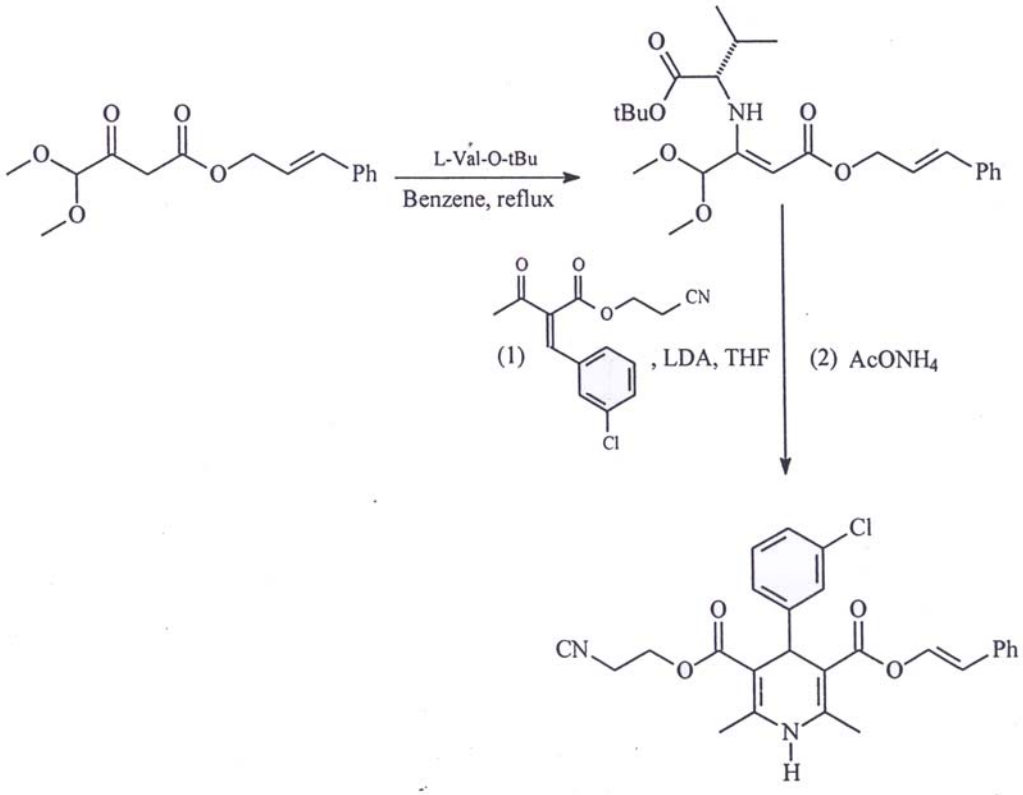
Several methods for stereoselective synthesis of 4-substituted 1,4-DHPs have been reported, such as: classical resolution, diastereoselective addition of aryllithium to the 4-position of chiral pyridines, 3-5 chiral acetoacetate esters in Hantzsch synthesis, enantioselective Hantzsch synthesis using a chiral auxiliary, and chemo-enzymatic methods (191-194).

An asymmetric synthesis of ZD0947, a potassium channel opener dihydropyridine, was developed by synthesis of (R)-3-(phenylethylamino)-2-cyclohexen-1-one in situ by heating (R)-α-methylbenzylamine with 1,3-cyclohexanedione in acetonitrile, then by heating with α,β-unsaturated ketone in the presence of trimethylsilylchloride and then treated with aqueous ammonia to give the desired compound in 29% overall yield in 95% enantiomeric excess (195).

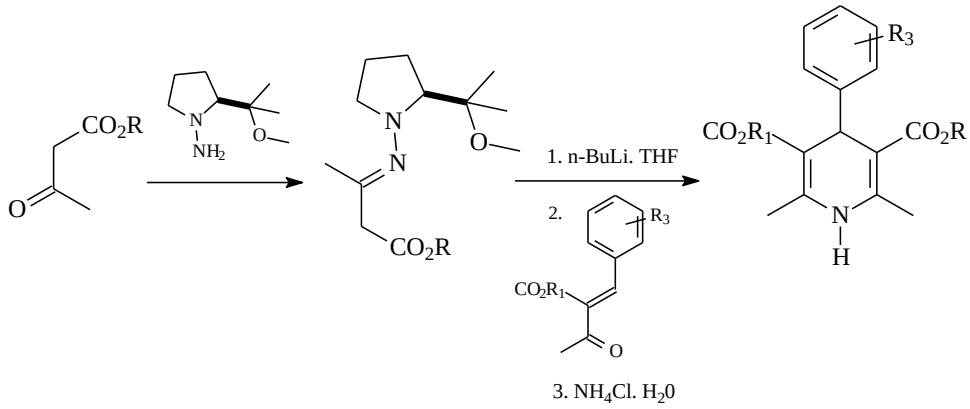


ZD0947

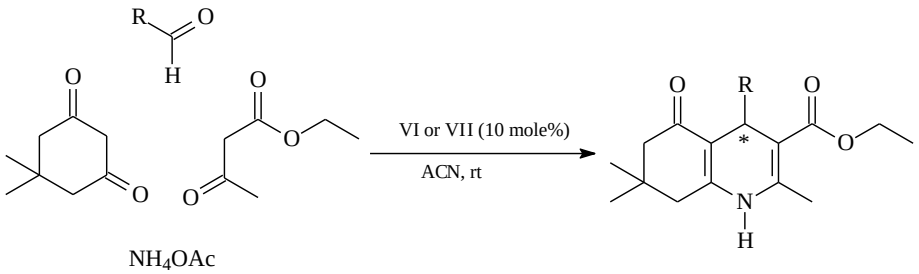
A similar strategy was applied using t-butyl ester of L-valine as a chiral auxiliary that act as a driving force for the synthesis of dihydropyridines in highly enantiomeric purity and moderate yields (196).

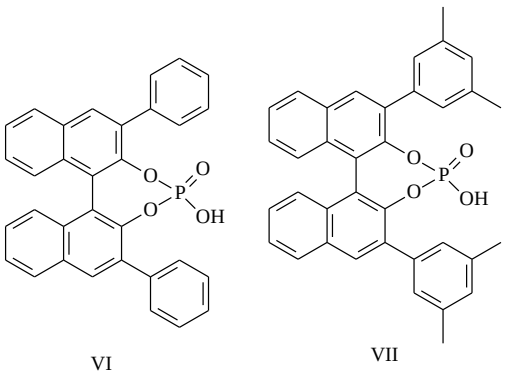


Other asymmetric approach was conducted by the reaction of chiral alkyl acetoacetate hydrazones to benzylidine acetoacetate derivatives in the presence of n-butyllithium (197).



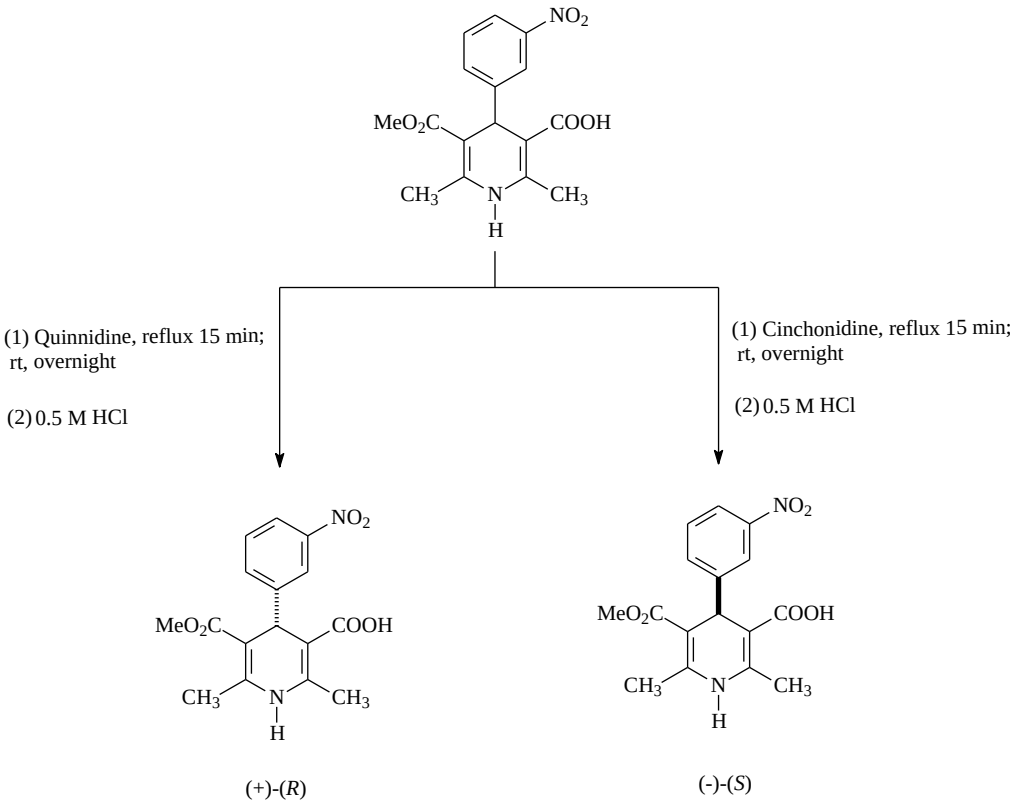
Recent procedure described that Hantzsch reaction was catalysed by chiral 1,1'-bi-2-naphthol-phosphoric acid derivatives (VI and VII) provided good yields (84-85%) with 98% enantiomeric excess (193).





VI VII

Other strategy was conducted by synthesis of classic racemate of alkyl 1,4-dihydropyridine-3,5-dicarboxylate derivative which can be hydrolysed to its 1,4-dihydropyridine-3-carboxylic acid analogue when treated with sodium hydroxide. Then, the resolution protocol using chiral organic bases such as quinidine and cinchonidine is conducted to obtain the single enantiomers (198).

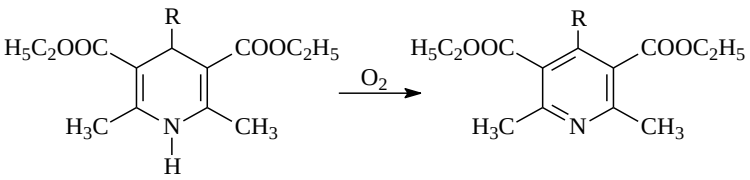


2.12. Chemical reactions of 1,4-DHPs

Oxidation

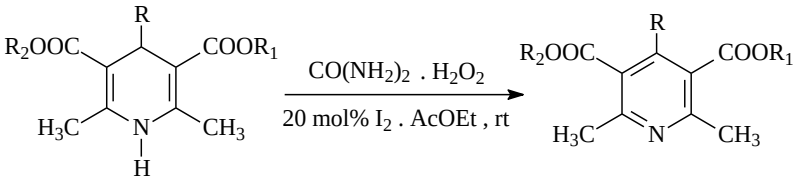
1,4-DHP derivatives can be oxidized to their pyridine analogues with various oxidants (151).

Nitrous acid, dilute nitric acid and chromic acid are the most commonly used reagents in the dehydrogenation of DHPs. Hydrogen peroxide, potassium permanganate, silver nitrate, platinum/acetic acid, mercury(II) acetate, iodine, iron and nickel compounds, also oxygen or air can be used for oxidation (199-202).

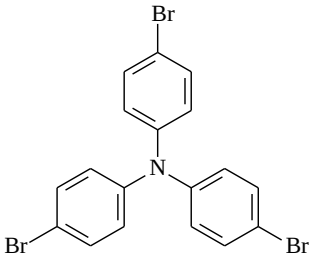


R: H, CH_3, C_2H_5, C_6H_5

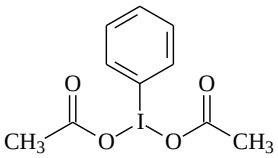
An efficient and metal-free method for aromatization of 1,4-dihydropyridines by using urea-hydrogen peroxide adduct as oxidant in presence of 20 mol % of molecular iodine was reported (203). The reaction was carried out in ethyl acetate at room temperature and the products were isolated in high to excellent yields.



Aromatization of 1,4-dihydropyridines was achieved also by using tris(p-bromophenyl)amine (TBPA) radical cation as an efficient catalyst to prompt the aerobic oxidation (204). Other study showed the potential of iodobenzenediacetate (IBD) for the oxidative aromatization of Hantzsch-1,4-dihydropyridines under ultrasonic irradiation (205).



TBPA



IBD

Reduction

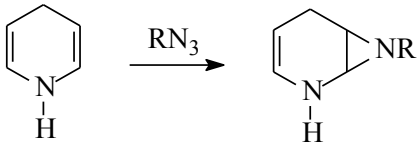
Catalytic hydrogenation of DHPs results in transformation to their tetrahydro or hexahydro analogues (206, 207).

The presence of bulky groups at the fourth position of the 1,4-DHP nucleus was found to hinder their hydrogenation. Also 1,4-DHPs were found to be more resistant to the borohydride reduction (151).

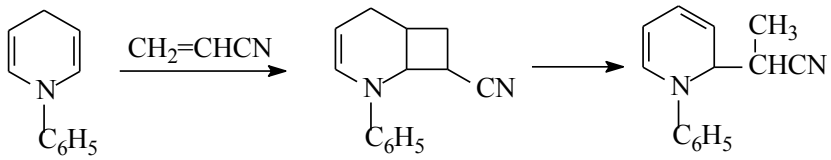
1,2-DHPs are obtained in case of reduction of the pyridinium salt using $NaBH_4$ (208).

Addition reactions

Bicyclic tetrahydropyridine derivatives are obtained by addition reaction of azides to 1,4-DHPs (144).

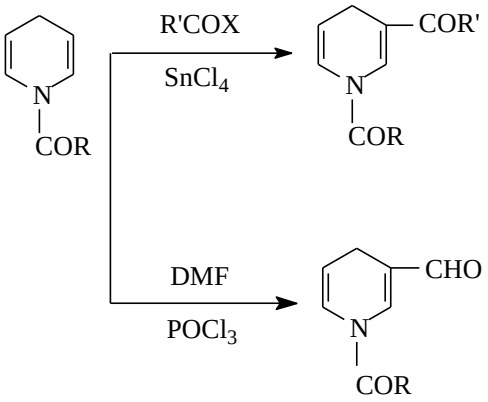


The reaction of N-substituted 1,4-DHPs with acrylonitrile resulted in formation of cyclobuta-tetrahydropyridine which undergoes rearrangement to form 2-substituted-1,2-DHP derivatives (152).

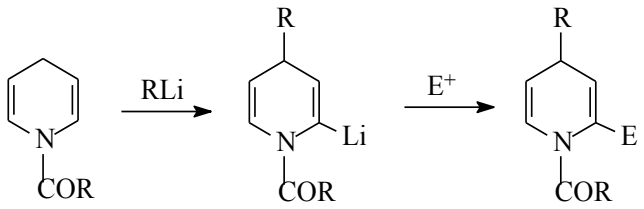


Substitution reactions

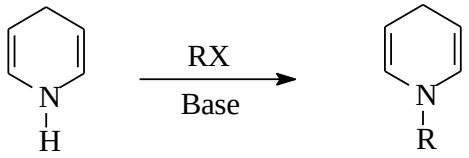
1-Acyl-1,4-DHPs undergo Friedel-Crafts acylation and Vilsmeier-Haack formylation to yield 3-substituted derivatives (144).



While 2-substituted DHP derivatives can be obtained by the reaction of 1-acyl-1,4-DHPs with alkyl lithium produces 2-lithio derivatives that can be easily converted to the desired derivatives by the reaction with appropriate electrophile (144).



1-Alkyl-1,4-DHP derivatives can be obtained by reaction of DHPs with alkyl halides in presence of strong bases such as sodium hydride or sodium hydroxide (152).

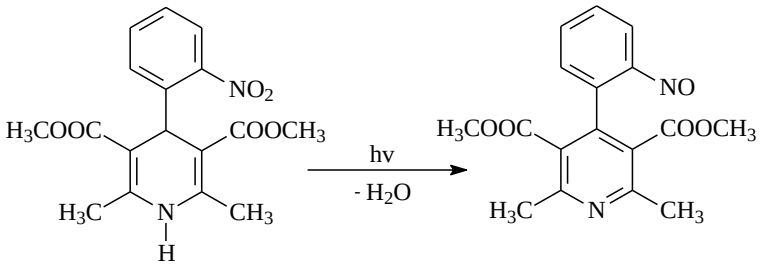


Photochemical reactions

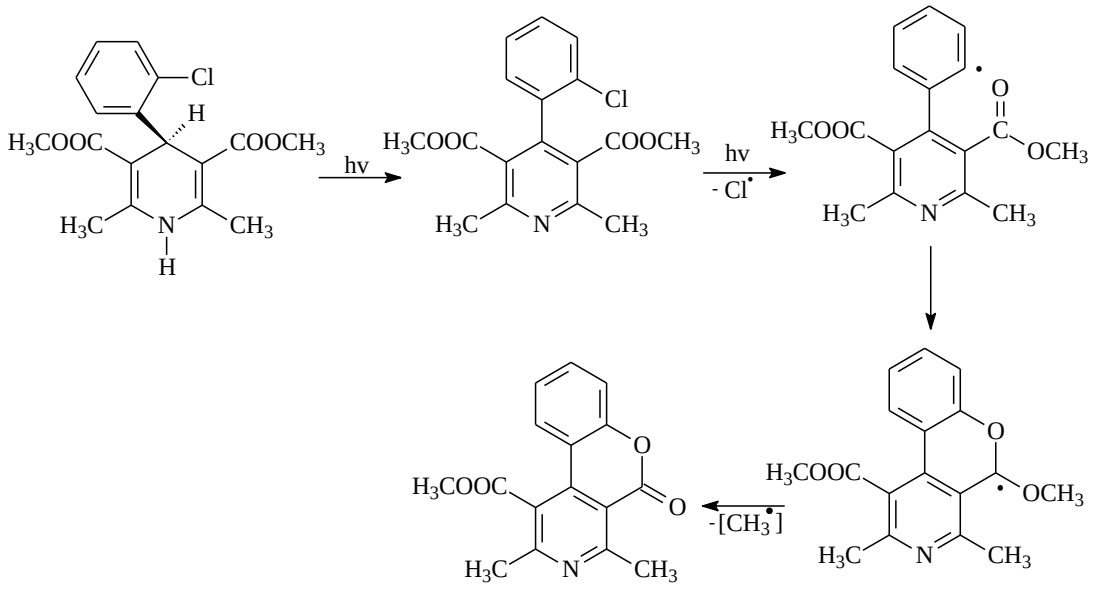
1,4-DHPs are very sensitive to light (209). Photostability studies tested in aerobic and anaerobic conditions showed that the presence of oxygen leads to increase the rate of photodegradation and those resulting degradation products were found to cause toxic effects in cells (210).

Photosensitivity of 4-aryl-1,4-dihydropyridine-3,5-dicarboxylate compounds is considered a serious problem has to be taken in consideration.

4-(2-Nitrophenyl)-1,4-dihydropyridine derivatives such as nifedipine and nisoldipine, when photo-chemically examined, have been shown to be converted to 4-(2-nitrosophenyl)-pyridine derivatives in solutions as well as in solid state.



While the 4-(2-chlorophenyl)-1,4-dihydropyridine derivatives showed homolytic breakage of the carbon-chlorine bond that undergoes intermolecular lactonization with the ester group (211).



Acid-base properties

1,4-DHPs can act as both weak acids and weak bases. Although their basicity insufficient for N-alkylation, they can react with alkyl halides in presence of strong bases (151).

2.13. Spectroscopic properties of 1,4-DHPs

Ultraviolet spectra

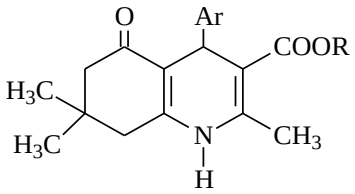
Because of the conjugated structure of 1,4-DHPs, they have a maximum wavelength near 240 nm and show two absorption bands near 200-240 nm and 300-400 nm (151).

Infrared spectra

1,4-DHPs show characteristic peaks in the infrared spectrum. C=C at 1430-1600 cm^{-1}, stretching band of C-N appears at 1140-1190 cm^{-1} while the bending band of N-H is observed at 1600 cm^{-1} (212).

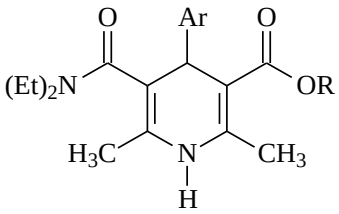
The IR spectra of alkyl 2,7,7-trimethyl-4-aryl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives show N-H stretching bands at 3270-

3340 cm^{-1}, ester carbonyl stretching bands at 1685-1705 cm^{-1}, ketone carbonyl stretching bands near 1650 cm^{-1} (213).



R: CH_3, C_2H_5

While in IR spectra of alkyl/aryl-5-(diethylcarbamoyl)-2,6-dimethyl-4-aryl-1,4-dihydropyridine-3-carboxylate derivatives, N-H stretching bands at 3240-3317 cm^{-1}, ester group C=O stretching bands near 1690 $cm^{-1}$and the amide group C=O stretching bands at 1610-1650 cm^{-1} were observed (214)



Nuclear magnetic resonance spectra

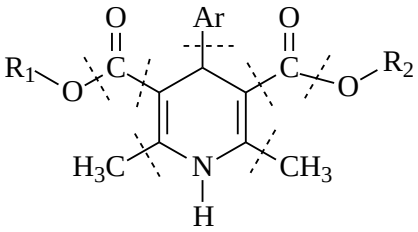
1,4-DHPs' ^{1}H-NMR spectra in DMSO-*d6* shows NH singlet protons at 8.76-9.19 ppm, while the same protons are observed in the range 6.55-7.97 ppm when $CDCl_3$ is used as solvent.

C-4 characteristic singlet proton appears at 4.75-5.02 ppm, while protons of the methyl groups at C-2 and C-6 appear as singlet at 2.20 ppm. The protons of phenyl group attached to C-4 appear at the normal range where the aromatic protons should be observed at 7.0-8.5 ppm (215).

Mass spectra

The most known fragmentation in the mass spectra of derivatives of 1,4-DHPs is that the formation of the pyridinium cation by either removal of hydrogen or loss 4-aryl group as aromatic radical (151, 216).

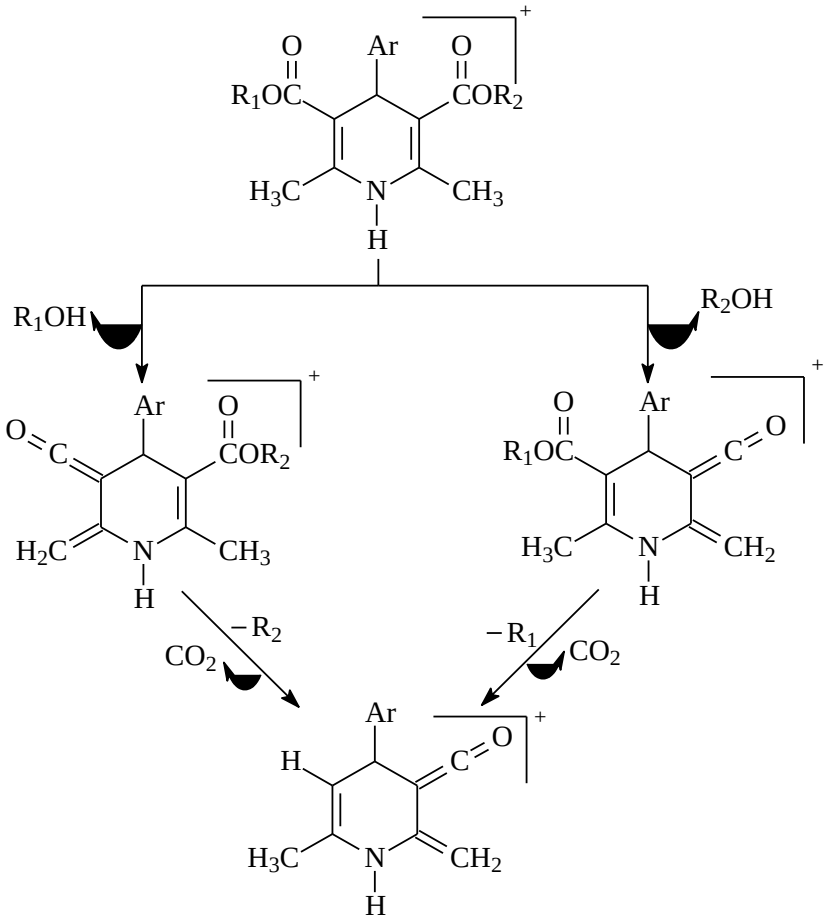
It was observed that fragmentations that follow the formation of the pyridinium cation vary according to the nature and structure of DHPs. Loss of the substituents at C-3 and C-5 and 1,4-DHP ring opening can be observed (217).



Formation of base peak after loss of 4-aryl group was observed in the mass spectra of 4-aryl-1,4-dihydropyridine that were obtained by electron ionization technique (218).

In the mass spectrum of methyl 2,7,7-trimethyl-4-aryl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives, formation of degradations related to $[M - COOCH_3]^+$, $[M - OCH_3]^+$ peaks and cyclohexane ring opening was reported (219). Also, formation of ions resulted from the loss of 4-aryl group and cyclohexane ring rupture were observed (220).

For 1,4-DHPs that have 4-aryl and long alkyl chain ester groups, when their mass spectra was done by electron spray ionization (ESI) technique, unlike electron impact (EI) technique, it was found that the 4-aryl group was not cleaved while alkyl chain of ester group was lost as alcohols or olefins. Then, the rest of the ester group is lost while the other ester is transformed to ketene group (221).

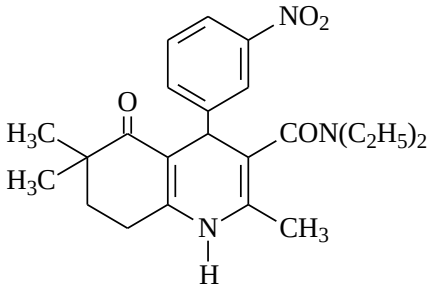


X-ray crystallography

X-ray analysis, is one of the commonly used methods in structure elucidation of 1,4-DHP derivatives (222-224). Spatial conformation of 1,4-DHP derivatives of the ring varies according to the presence and absence of substituents at C-4.

4-Aryl or 4-heteroaryl DHPs show boat conformation and the substituents-free derivatives are planar, while non-planar conformation is exhibited by spiro-DHPs (225).

The examination of N,N-Diethyl-2,6,6-trimethyl-4-(3-nitrophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide by X-ray studies showed that 3-nitrophenyl group exhibits synperiplanar to the DHP ring plane (226).



The crystal structure of ethyl 2,7,7-trimethyl-4-(1-methyl-1H-indol-3-yl)-5-oxo-1,4,5,6,7,8 hexahydroquinoline-3-carboxylate showed that the cyclohexene ring is in a sofa conformation while the 1,4-DHP ring is in a slight boat conformation. A weak intramolecular C-H···O hydrogen bond also was observed (Figure 2.13) (227).

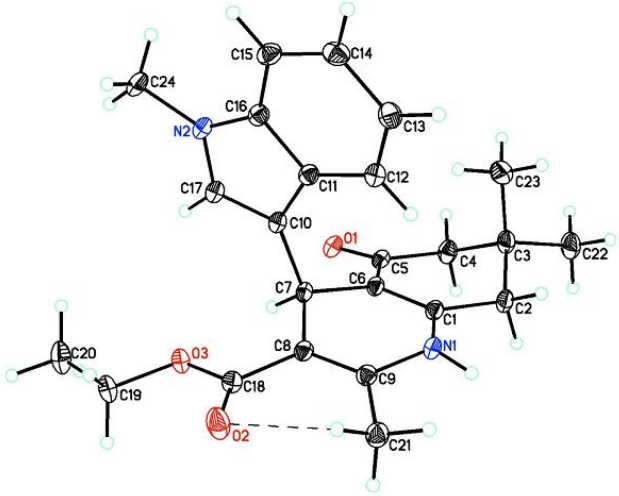


Figure 2.13. Crystal structure of 4-indolyl-1,4-DHP.
The dashed line indicates the intramolecular C-H···O interaction

2.14. Biotransformation of 1,4-DHP derivatives

It is known that 1,4-DHP derivatives undergo first pass metabolism in the liver and are metabolized by hepatic cytochrome P_{450} oxidative system that is mostly localized in the endoplasmic reticulum. Cytochrome P_{450NF} is determined as the main enzyme which is responsible in the process of oxidation of nifedipine in human body (228, 229).

1,4-DHPs are converted to their inactive pyridine analogues as a result of oxidation (230,231). Hydrolysis of the ester group, 2-methyl group hydroxylation and lactonization are among other known pathways of biotransformation.

For 4-substituted-1,4-DHP-3,5-dicarboxylate derivatives, the rate of oxidation of 4-alkyl-1,4-DHPs was reported to be higher than that of the 4-aryl analogues (232, 233).

In vitro biotransformation studies of 2,6,6-trimethyl-4-(2-bromophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives showed that the molecule is subjected to lactonization to be converted to furoquinoline analogues (155).

2.15. Pharmacophore

A pharmacophore is an ensemble of chemical features that characterizes a specific mode of action of a ligand in the active site of the macromolecule in three-dimensional (3D) space, it explains how structurally diverse ligands can bind to a common receptor site (234).

A pharmacophore model can be established by two techniques:

- Ligand-based pharmacophore modeling: by superposing a set of active molecules and extracting common chemical features that are essential for their bioactivity.
- Structure-based pharmacophore modeling: by probing possible interaction points between the receptor and ligands.

Ligand-based pharmacophore modeling has become a key computational strategy for facilitating drug discovery in the absence of a macromolecular target

structure. It is usually carried out by extracting common chemical features from 3D structures of a set of known ligands representative of essential interactions between the ligands and a specific macromolecular target. In general, pharmacophore generation from multiple ligands (usually called training set ligands) involves two main steps: creating the conformational space for each ligand in the training set to represent conformational flexibility of ligands, and aligning the multiple ligands in the training set and determining thee ssential common chemical features to construct pharmacophore models.

Pharmacophore features arepharmacophoric descriptors include H-bond donors, H-bond acceptors, hydrophobic, aromatic, positive ionizable and negative ionizable groups; they represent chemical feature complementarity to the receptor in the 3D space (234).

Avogadro 1.1.0

An open source advanced molecular builder, editor and visualizer designed for cross-platform use in computational chemistry, molecular modeling and related areas (235).

Merck molecular force field (MMFF)

A force field refers to the form and parameters of mathematical functions used to describe the potential energy of a system of particles (typically molecules and atoms). **MMFF** is a family of force fields developed by *Merck Research Laboratories*. They are based on the MM3 force field and perform well for a wide range of organic chemistry calculations (236).

LigandScout 3.1

LigandScout is a fully integrated platform for accurate virtual screening based on 3D chemical feature pharmacophore models. It offers starting both from ligand- and structure-based pharmacophore modeling, and includes novel high performance alignment algorithms for excellent prediction quality with unprecedented screening speed. The algorithms are scientifically published and based on several years of experience in pharmacophore creation (237-239).

2.16. General microwave physics

The electromagnetic field mainly causes heating of polar organic-solvent systems via dipolar polarization mechanism. For a substance to be heated when irradiated by microwaves it must have a dipole moment, dipole moments are sensitive to external applied electric field and will attempt to align themselves with the field by rotation. The applied field provides the energy for this rotation, so the molecules gain energy and sample is heated i.e. microwave energy is not transferred primarily by convection or by conduction, as with conventional heating (240-243). Thus, the heating rate is affected by the dielectric properties of a sample.

Only a specific wavelength within the microwave band, ranging from 1 cm. to 1 mm., is utilized for organic reactions.

The extent of heating of microwave energy to a sample is mainly affected by a factor (tan δ) that is often named the dissipation factor or the dielectric loss tangent. The word ‘loss’ refers to the input microwave energy that is lost to the sample by being dissipated as heat.

Polar solvents have high tan δ values and are preferable for microwave-promoted reactions.

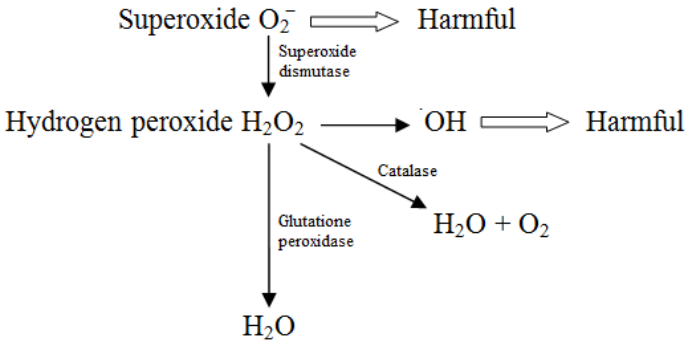
Microwave irradiation also produces superheating (i.e. temperatures much above conventional reflux temperatures) at atmospheric pressure and can be rapidly generated in closed vessel systems.

The efficiency of microwave in dramatically reducing reaction times (reduced from days and hours to minutes and seconds) has recently been proven in several different fields of organic chemistry. As a consequence, the amount of articles describing efficient rapid microwave-assisted synthesis has grown quickly in the last 20 years.

2.17. Antioxidant activity

Recent studies confirmed that oxygen is generally metabolized in human by a two-electron reduction that results in formation of water molecules. Superoxide (O_2□) is formed as a by-product of oxygen metabolism by one-electron reduction; superoxide can be further reduced to form hydrogen peroxide (244).

Superoxide and hydrogen peroxide are produced in small quantities during normal biological processes. Natural cell defense mechanisms, such as superoxide dismutase, effectively remove superoxide and other active oxygen species. However, under certain conditions, such as intake of drugs, UV-radiation or metabolic dysfunction, these reactive oxygen species can be generated in sufficient quantity to exceed the normal defense capabilities of the body.



It is obvious that superoxide is highly reactive and toxic; and it can cause oxidation of biomacromolecules as well as initiating radical-chain processes that cause hazardous effects on tissues, cell destruction and incurable diseases (245).

The antioxidants interrupt these radical-chain processes and form low activity radicals, which are easily removed from the organism. This improves general health, helps cell rejuvenation and prevents cancer; this explains the wide need and use of antioxidants in different fields of food-processing industries, cosmetics, and medicine.

Determination of antioxidant activity by differential pulse voltammetry (DPV)

The concept of determination is the ability of antioxidants to react with the superoxide and decrease its concentration at the electrode. Thus, electrochemical reduction current of oxygen decreases and therefore it can be used as a comparative value of the antioxidant activity in the solution being analyzed.

The technique of this method is very simple (Figure 2.14). An electrochemical cell (V=10 ml) is connected to the analyzer and consists of a working mercury film electrode (MFE) or glassy carbon electrode, a silver-silver chloride reference electrode with saturated KCl (Ag-AgCl, KCl sat.) and a nitrogen (or inert gas) supply tube.

The technique involves initial scanning of the oxygen reduction voltammogram in the supporting electrolyte without antioxidant to obtain the original limiting value of the oxygen current (I_{or}), which corresponds to oxygen solubility in this electrolyte.

Then the supporting electrolyte is bubbled for 3-5 min with pressurized nitrogen through the gas tube to remove oxygen from the electrolyte, and the oxygen reduction voltammogram in the supporting electrolyte is scanned to obtain the residual current (I_{res}) value, which corresponds to voltammogram in the supporting electrolyte without oxygen.

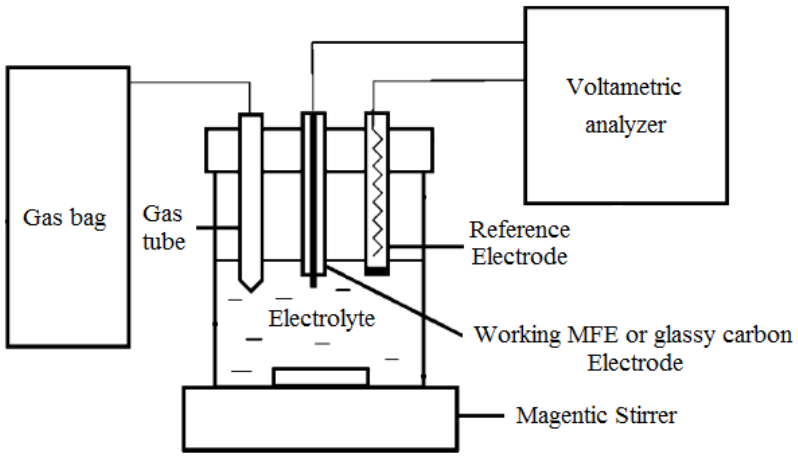


Figure 2.14. Scheme of DPV equipment.

Then antioxidant solution with a known concentration is added to the renewed portion of the supporting electrolyte under the same conditions and the voltammogram is scanned, the proportional decrease of the oxygen reduction current corresponding to the concentration of the added antioxidant at constant potential was observed (Figure 2.15) (246).

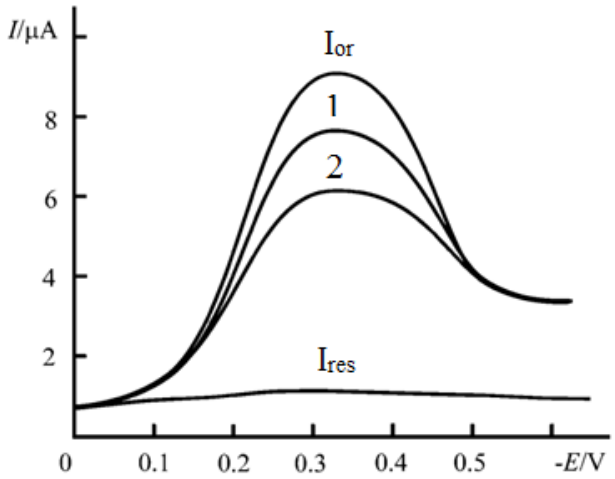


Figure 2.15. Voltammograms of the oxygen reduction current. (I_{res}) The residual current. (I_{or}) The oxygen reduction current. (1) and (2) are the oxygen reduction current with different antioxidant concentrations.

3. MATERIAL AND METHODS

3.1. Pharmacophore modeling

3.1.1. Method and software

Data set collection and compounds preparation

After nifedipine was introduced into market, many 1,4-DHP analogues have been synthesized. Most of trials to optimize the activity of 1,4-DHPs were focused on modifying the ester groups of 1,4-DHP.

When the commercially available 1,4-DHP Ca^{2+} channel blocker ligands were examined according to structure diversity of ester groups attached to 1,4-DHP ring, it was found that those ligands can be empirically classified into two main groups;

- The first group: Ligands have lower aliphatic chain esters (Figure3.1)
- The second group: Ligands have long chain esters terminated with aromatic hydrophobic group (Figure 3.2)

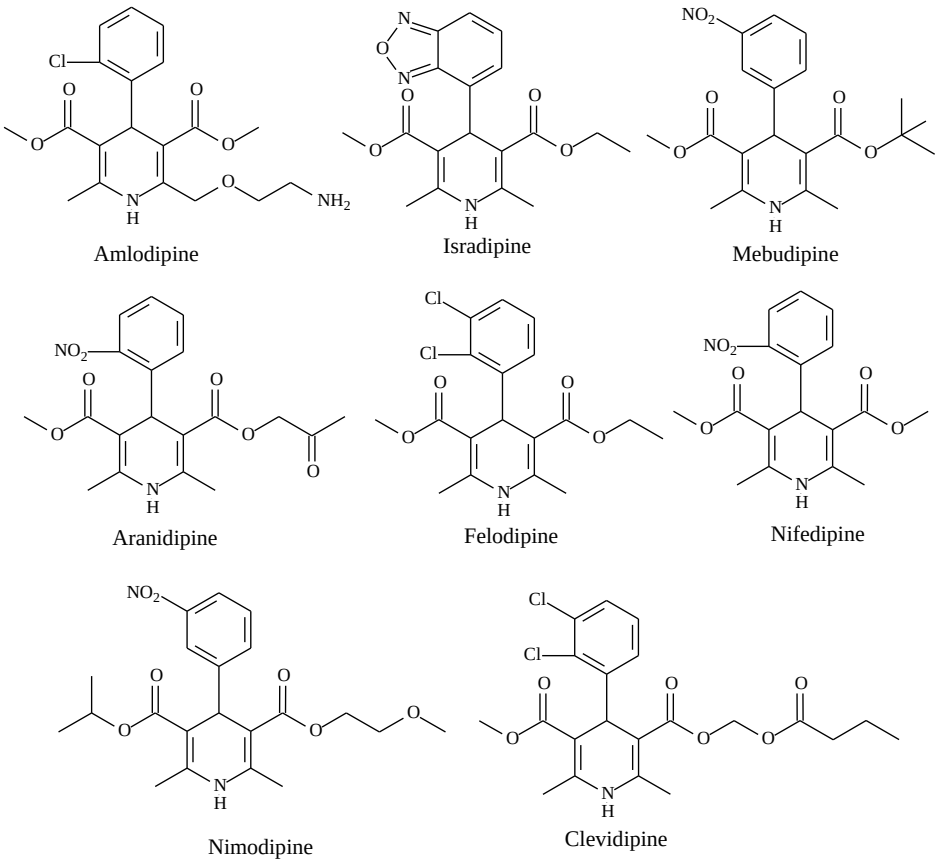


Figure 3.1. The first group: Ligands have lower aliphatic chain esters.

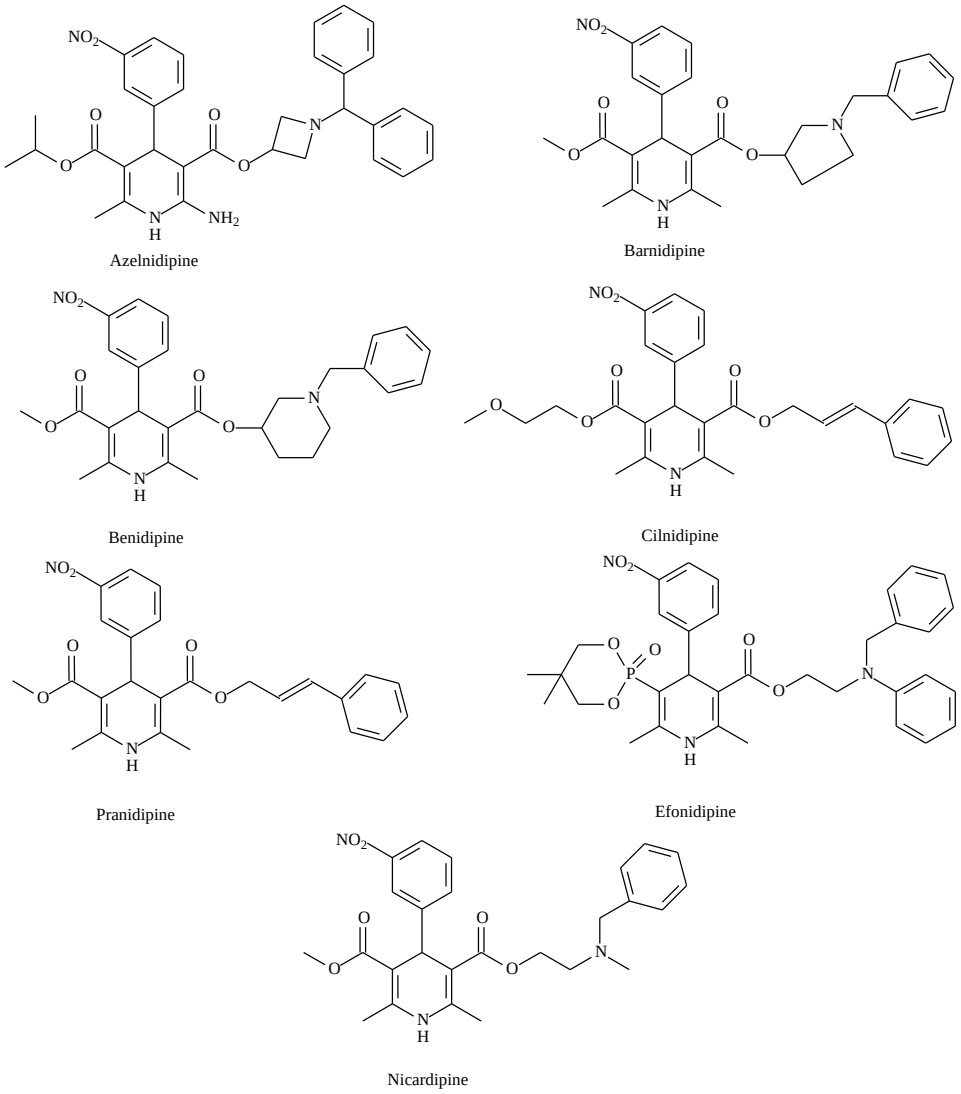


Figure 3.2. The second group: Ligands have long chain esters terminated with aromatic hydrophobic group.

Some ligands of the second group were selected to be used in the pharmacophore generation. The two-dimensional chemical structures of the selected ligands were sketched using ACD/ChemSketch (Freeware), and saved in MDL Molfiles (*.mol) format (247). Subsequently, they were imported into Avogadro 1.1.0 and their geometry were optimized and energy were minimized to a “rms” gradient of 0.0001 kcal/mol/A on the MMFF94 using Steepest Descent algorithm (Figure 3.3).

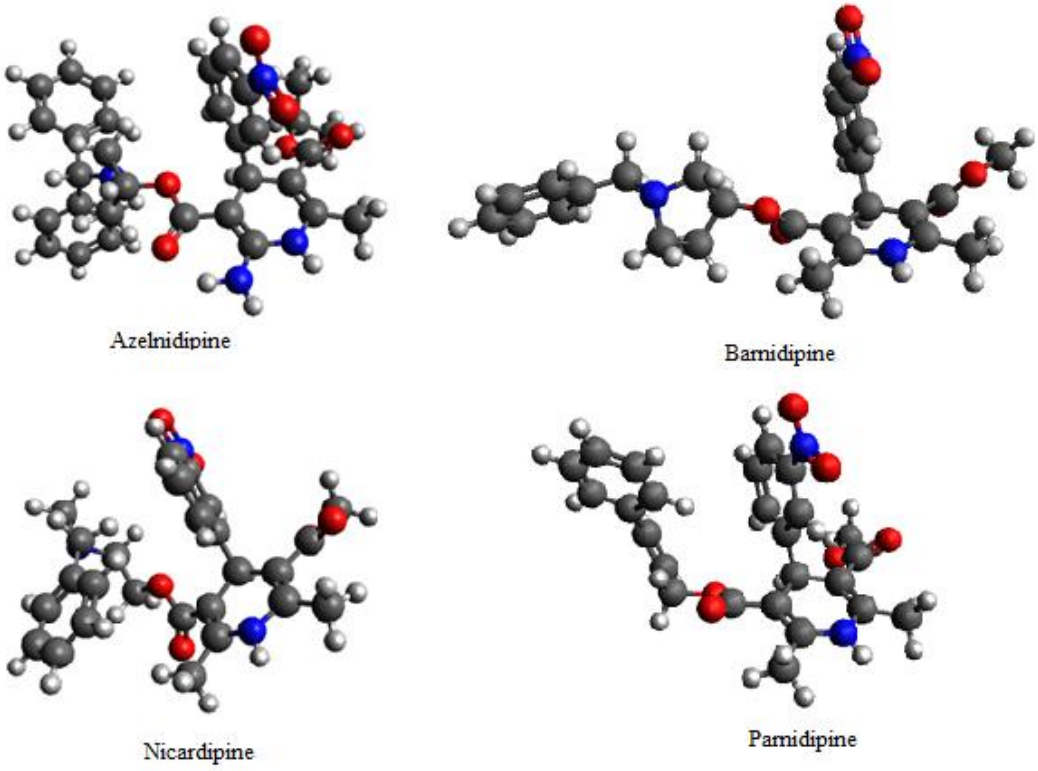


Figure 3.3. 3D structure of some optimized DHP ligands on MMFF94 by Avogadro

Pharmacophore generation

Pharmacophore creation consists of three main processes: conformer generation, clustering of ligands and then pharmacophore generation; each process has special settings that were adjusted to give the optimum results.

The optimized ligands were imported by LigandScout and five ligands (barnidipine, benidipine, parnidipine, nicardipine and cilnidipine) were identified as a training set to generate a pharmacophores and two ligands (azelnidipine and efonidipine) were identified as a test set to verify the resulting pharmacophores.

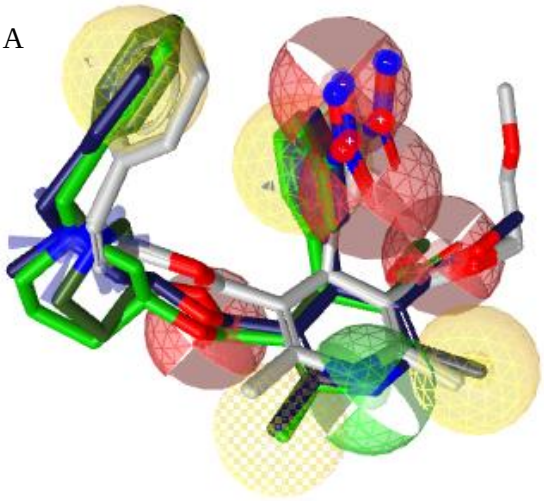
Ligands first were subjected to conformer generation by applying the best settings that increases maximum number of conformations for each molecule to 500.

Then clustering was done by using 25 conformations of each ligand and the similarity of the pharmacophore radial distribution function (RDF) was set as a similarity measure.

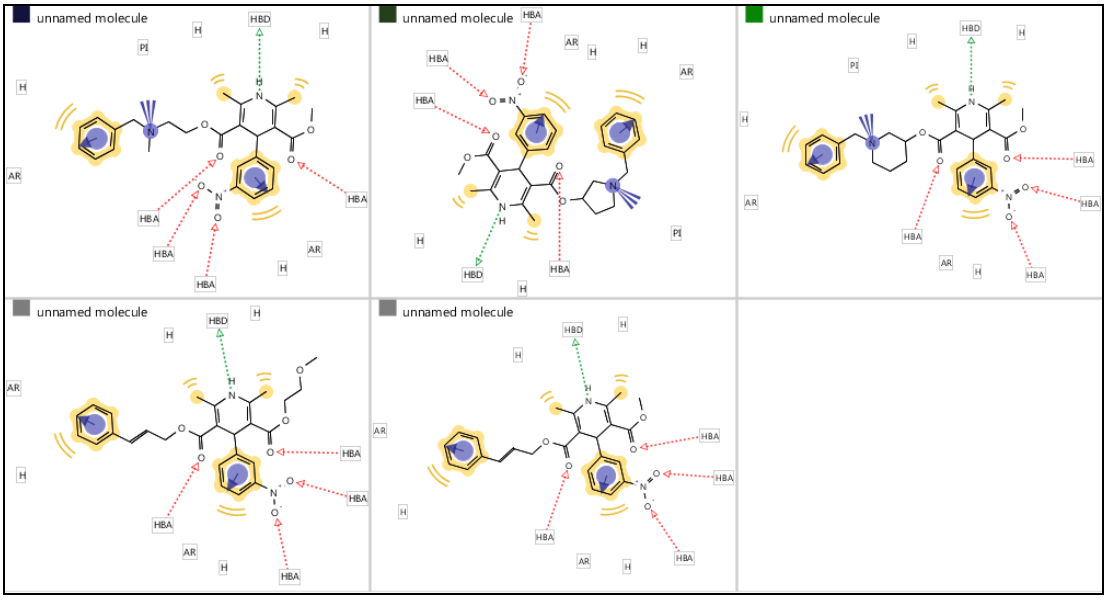
In order to consider all features and assemble them into one pharmacophore, the pharmacophore type was set to Merged Feature Pharmacophore; Pharmacophore-

Fit and Atom Overlap was set as a scoring function; and the maximum number of resulting pharmacophores was set to10.

A



B



C

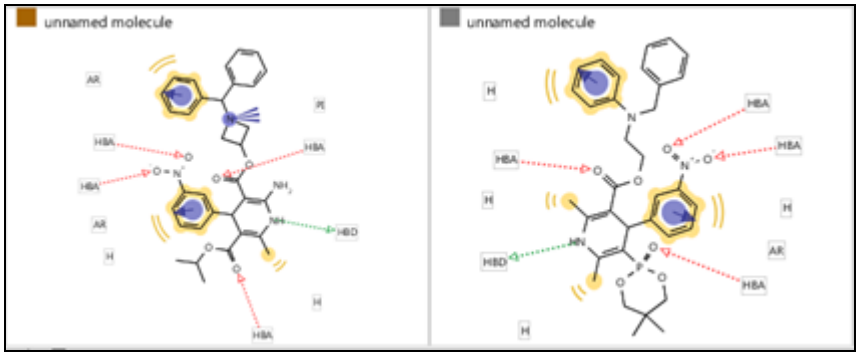


Figure 3.4. A: pharmacophore with traning set ligands mapped, B: training set ligands and C: test set ligands.

Pharmacophore validation

a) In the stage of pharmacophore creation there were two ligands (azelnidipine and efonidipine) were identified as a test set to verify the resulting pharmacophores.

b) The pharmacophore models evaluated against the Lipinski's rule to assure that the compound which fit these features is likely to be orally active drug in humans. The Lipiniski's rule of five states, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors.
- Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms).
- A molecular mass less than 500 daltons.
- A partition coefficient log P not greater than 5.

Since we are validating a pharmacophore model, not a drug molecule, the only first two criteria can only be applied for our validation.

c) Since all the training set ligands have 3-nitrophenyl group, all the pharmacophore models have showed two hydrogen bond donor features of the two oxygen atoms of the nitro group as a common features. However, according to the SAR studies and by taking in consideration those other ligands such as amlodipine, clivedipine and felodipine, nifedipine and aranidipine have different substituents or different position of substitution on 4-phenyl ring, so we concluded that the two features of 3-nitro group should not be considered as a common feature and were marked *as "Optional" features*.

d) The developed ten pharmacophore models were overlaid, the main difference between the models was the shift of the hydrophobe (yellow sphere) by 3.59 A. and the positive ionizable area (blue star) by 0.67 A.

To choose the best model, they all have been screened using one compound of our new synthesized compounds and the model which had the best Fit Score was chosen.

The chosen pharmacophore model was used for screening our newly synthesized compounds after being optimized and their energy were minimized on MMFF94.

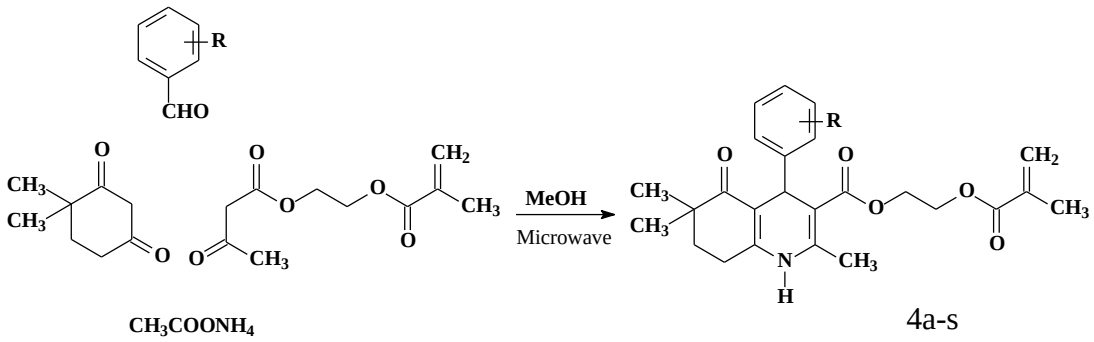
3.2. Chemistry

3.2.1. Materials and equipment

4,4-Dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, substituted benzaldehydes, ammonium acetate and solvents were purchased from Aldrich (Germany). The compounds were synthesized under a CEM Cooperation Discover SP microwave synthesis system.

3.2.2. Method of synthesis

4,4-dimethylcyclohexane-1,3-dione (0.001 mole), 2-(methacryloyloxy)ethyl acetoacetate (0.001 mole), substituted benzaldehyde (0.001 mole) and ammonium acetate (0.004 mole) were dissolved in methanol and subjected to microwave irradiation at 150 °C for 15 min. The reaction progress was monitored by TLC. After completion of the reaction, the mixture was dried under reduced pressure and the resulting precipitate was recrystallized from dichloromethane /ether mixture.



R: 2-Cl, 2-F, 2-NO_2, 3-Cl, 3-F, 3-NO_2, 3-CN, 2,3-diCl, 2,4-diCl, 2,5-diCl, 2,4-diF, 2,5-diF, 4-Cl, 4-F, 4-NO_2, 4-CN, 2-Cl 3-CF_3, 2-Cl 5-CF_3, 2-F 3-CF_3.

Scheme.1. General synthesis of the compounds

3.2.3. Analytical Methods

Melting Points

Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and were uncorrected.

Thin Layer Chromatography

TLC aluminum plates: Silica gel 60 F_{254} (Merck) were used in TLC analysis solvent system: N-hexane: ethyl acetate (50 : 50).

Method

The solvent system were poured to the jar and kept to adequate saturation. The reaction mixtures, starting materials and synthesized compounds were applied by microcapillary tubes and visualized under UV lamp (254/366 nm).

Spectrometric Analysis

IR Spectra

The IR spectra of the synthesized compounds were recorded on a Perkin Elmer Spectrum BX (ν, cm^{-1}), at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University.The synthesized compounds were applied in their powder form.

^{1}H-NMR and ^{13}C-NMR Spectra

^{1}H-NMR and ^{13}C-NMR Spectra of synthesized compounds were obtained from Varian Mercury 400 MHz Ultra Shield Spectrophotometer (DMSO-d_6, Merck; tetramethylsilaneas internal standard) at the Central Laboratory of Faculty of Pharmacy, Ankara University. The chemical shifts were expressed in δ parts per million (ppm).

Mass Spectra

Mass spectra were obtained on Micromass ZQ LC-MS Spectrophotometer with ESI+ method, at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University.

Elemental analysis

Microanalysis (C, H and N) was obtained on a Leco CHNS-932 Elemental Analyzer with accuracy 0.4%, at the Central Laboratory of Faculty of Pharmacy, Ankara University.

3.3. Myorelaxant biological activity

The myorelaxant bioilogical activity of the synthesized compounds was determined at the Department of Clinical Pharmacology, Faculty of Medicine, Gazi University.

3.3.1. Drugs

Nω-nitro-L-arginine-methylester (L-NAME) hydrochloride, indomethacin, tetraethylammonium chloride, guanethidine and nifedipine were supplied by Sigma.

L-NAME, tetraethylammonium chloride, guanethidine and nifedipine were dissolved in distilled water. Compounds and indomethacin were dissolved in dimethyl sulfoxide.

3.3.2. Method and equipment

New Zealand white rabbits, weighing 2.5-3 kg.were used in this study. Rabbits were sacrificed with i.v. injection of sodium pentobarbital (30-40 mg/kg), followed by removal of the stomach through abdominal incision.

The fundal part of the stomach was then dissected parallel to the longitudinal muscle wall. One muscle strip with approximately 15-20 mm. long and 2 mm. wide was obtained and allowed to equilibrate for a period of 60 min in 20 mL organ baths filled with Krebs'-Henseleit solution (KHS).

The composition of the Ca^{2+} free KHS solution was as follows (in mmol/l): sodium chloride 118; potassium chloride 4.7; sodium bicarbonate 25; magnesium chloride 0.54; sodium hydrogen phosphate 0.9; glucose 10.04. The solution was gassed with % 95 O_2 and % 5 CO_2 during the study and temperature was maintained at 37 ° C by a thermo-regulated water circuit. The pH of the saturated solution was 7.4.

Each strip was connected to a force transducer (FDT 10-A, May IOBS 99, COMMAT Iletişim Co., Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA) using (BSL PRO v 3.6.7, BIOPAC Systems Inc.) which also had the capacity to analyze the data.

After mounting, each strip was allowed to equilibrate with a basal tension of 1 g for 60 min. KHS was replaced with fresh solution every 15 min during this time period.

To eliminate the probability of any relaxation that can be induced by the test compounds due to an interaction with cyclooxygenase, potassium channels, adrenergic or nitric oxide pathways, all experiments were done in the presence of indomethacin (COX inhibitor, 10^{-5}M), tetraethylammonium chloride (nonspecific K^+ channel blocker, 10^{-4}M), guanethidine (adrenergic nerve blocker, 10^{-6}M) and Nω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M).

When Ca^{2+} (2.5 mM) was added to the organ bath, a contraction developed. At the plateau level of contraction, compounds (10^{-8}- 10^{-4} M) and nifedipine (10^{-10}-10^{-7} M) were applied. Concentration-relaxation for compounds and nifedipine were obtained by adding into the bath in a cumulative manner.

Responses of test compounds and nifedipine were expressed as the percentage of the precontraction using Ca^{2+} (2.5 mM). DMSO was also tested in Ca^{2+} (2.5 mM) precontracted strips.

The relaxant effects of the compounds on the tissues, precontracted with Ca^{2+} (2.5 mM), were expressed as percentage of the precontraction using Ca^{2+} (2.5 mM).

To evaluate the effects of an antagonist, the maximum response (Emax), the concentration for a half-maximal response (EC_{50}) values were calculated from the concentration-response curve (CRC) obtained in each experiment, as predicted from the Scatchard equation for drug-receptor interaction, where

Response/concentration= - 1/EC_{50} x response + maximum response/EC_{50}

The pD2 value was expressed as the negative logarithm of the EC_{50}. All data are expressed as mean ± standard error.

Statistical comparison between the groups was performed by Mann-Whitney U-test and p values less than 0.05 were considered to be statistically significant.

All procedures involving animals and their care were conducted in conformity with international laws and policies.

3.4. Antioxidant activity

The antioxidant activity of the synthesized compounds was determined at the Department of Chemistry, Faculty of Science, Hacettepe University.

3.4.1. Method and equipment

An electrochemical cell connected to voltammetric analyzer that records voltammogram of oxygen reduction using differential pulse voltammograms with

potential range E = 0 to – 2.0 V. The cell consists of pencil graphite electrode (PGE) that was first immersed into tetrabutylammonium perchlorate (TBAP)-dichloromethane solution and the oxygen reduction voltammogram in the electrolyte was scanned to obtain the oxygen reduction current value (I_{or}).

Nitrogen gas was passed from solution to extract oxygen and the oxygen reduction voltammogram in the electrolyte was scanned to obtain the residual current value (I_{res}).

0.2, 0.4 and 0.6 mg/ml of our synthesized 1,4-DHP derivatives were added to solution one by one and each time voltammogram was scanned to observe the proportional decrease of the oxygen current; and voltammograms of the electrochemical reduction current (I_{red}) were taken corresponding to the concentration of the added antioxidant at constant potential.

A value of relative change of the oxygen reduction current density ($j/(j_{or} - j_{res})$) was assigned for each concentration of each tested compound. Curves of these values for the concentrations of the tested compounds were plotted.

All the curves represent straightforward lines in the range of tested compounds concentrations and the slope angel tangent of these lines is suggested to be a coefficient of antioxidant activity (K) of the tested compounds that was calculated according to the equation:

$$K = \frac{\Delta j}{(j_{or} - j_{res})\Delta c}$$

Δj: Change of the oxygen reduction current density with the additions of tested compounds,

Δc: The change of the antioxidant concentration,

j_{or}: The oxygen reduction current density without antioxidants,

j_{res}: The residual current density after removal of oxygen by nitrogen gas.

4. RESULTS

4.1. Pharmacophore modeling

Our generated model in (Figure 4.1) showed typical features described by SAR studies which are: a HB-acceptor (green sphere) represents dihydropyridine NH, two typical hydrophobes of the 2- and 6-methyl groups (yellow sphere on the both sides of the green sphere), aromatic hydrophobe of phenyl ring (yellow sphere above the green sphere), two HB-donors of 3,5-carbonyl oxygens (red spheres on the both sides of the green sphere), and the other two HB-donors near the aromatic hydrophobe represent nitro group which were set to "optional" during pharmacophore validation.

Furthermore, the interesting finding was the appearance of hydrophobe (yellow sphere on left) and a positive ionizable feature (blue star on left), which are additional common features , related to the ester side chains, were not reported by classical SAR studies.

Figure 4.1 shows the pharmacophore model features and determines the separating distance between the hydrophobe (yellow sphere) and HB-acceptor feature (green sphere) and between the positive ionizable feature (blue star) and the same HB-acceptor feature which were 8.82 and 7.38 A respectively with a 24.97 dihedral angle.

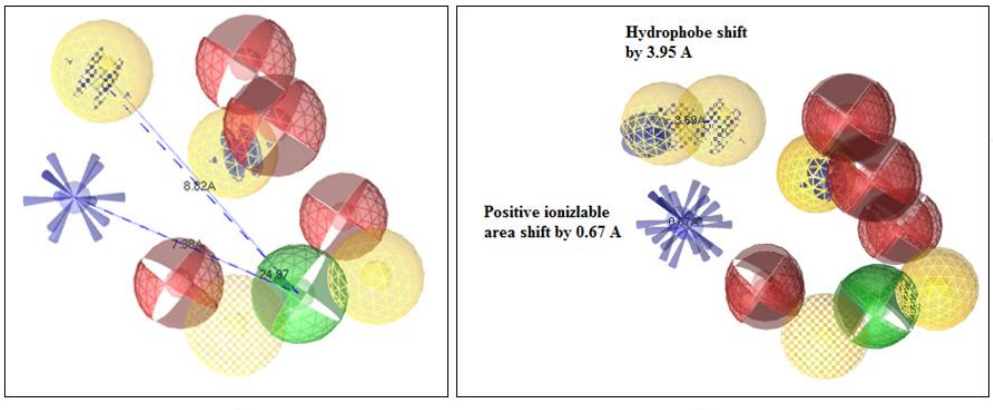


Figure 4.1. A- The pharmacophore model features. **B-** The ten pharmacophore models, during the validation process.

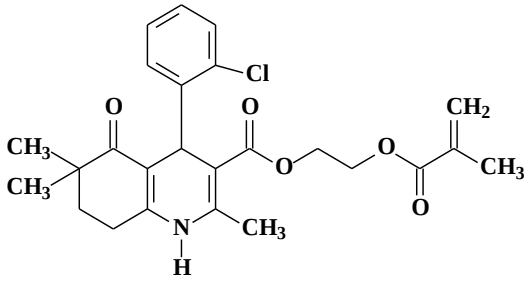
The validated paharmacophore model was used for screening the optimized compounds and the pharmacophore fit score was tabulated.

Table 4.1. Pharmacophore fit score values for the synthesized compounds.

Compound	R	Pharmacophore fit score
4a	2-Cl	0.76
4b	2-F	0.76
4c	2-NO_2	0.76
4d	3-Cl	0.75
4e	3-F	0.86
4f	3-NO_2	0.87
4g	3-CN	0.94
4h	2,3-diCl	0.69
4i	2,4-diCl	0.69
4j	2,5-diCl	0.69
4k	2,4-diF	0.69
4l	2,5-diF	0.69
4m	4-Cl	0.76
4n	4-F	0.69
4o	4-NO_2	0.75
4p	4-CN	0.76
4q	2-Cl,3-CF_3	0.58
4r	2-Cl,5-CF_3	0.73
4s	2-F,5-CF_3	0.73

4.2. Chemistry

2-(Methacryloyloxy)ethyl 4-(2-chlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4a)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2-chlorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 60%.

Melting point: 139-141 °C.

IR (cm^{-1}) 3328 (N-H stretching), 3066 (C-H stretching, aromatic) 2950 (C-H stretching, aliphatic), 1694 (C=O stretching, ester), 1635 (C=O stretching, ketone), 1434 (C-H, bending), 1207 (C-O stretching) and 765 (C-H, bending, o-disubstituted benzene).

^{1}H NMR δ 0.87 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.61-1.73 (4H; m; quinoline $H^{7,8}$), 1.83 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.31 (3H; s; CH_3), 4.13-4.31 (4H; m; COOC$\underline{H}_2$C$\underline{H}_2$OCO), 4,89 (1H; s; quinoline H^4), 5.62-5.95 (2H; s; methacryloyloxy C=C$\underline{H}_2$), 7.32-7.58 (4H; m; aromatic), 9.2 (1H; s; NH).

^{13}C NMR δ 18.36, 18.80, 23.43, 24.68, 25.26, 34.47, 35.66, 61.23, 63.14, 102.67, 109.60, 126.41, 127.14, 127.62, 129.42, 131.80, 132.46, 136.04, 145.67, 146.15, 150.09, 166.81, 166.98, 199.49.

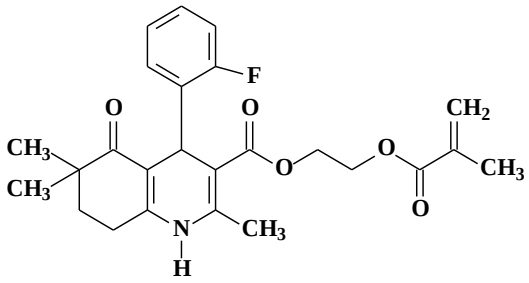
MS (m/z), 480.14 $[M+Na]^+$ (100%), 458.15, 328.08, 112.95, 59.85.

Elemental analysis for $C_{25}H_{28}ClNO_5$, MW 457.95

Calculated: C, 65.57; H, 6.16; N, 3.06.

Found: C, 65.27; H, 6.10; N, 3.07.

2-(Methacryloyloxy)ethyl 4-(2-fluorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4b)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2-fluorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 65%.

Melting point: 151-153°C.

IR (cm^{-1}) 3310 (N-H stretching), 3064 (C-H stretching, aromatic) 2950 (C-H stretching, aliphatic), 1704 (C=O stretching, ester), 1649 (C=O stretching, ketone), 1485 (C-H, bending), 1174 (C-O stretching) and 776 (C-H, bending, o-disubstituted benzene).

^{1}H NMR δ 0.79 (6H; s; 6-diCH_3), 0.93 (6H; s; 6-diCH_3), 1.63-1.75 (4H; m; quinoline $H^{7,8}$), 1.87 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.23 (3H; s; CH_3), 4.15-4.33 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.01 (1H; s; quinoline H^4), 5.62-5.94 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 6.81-7.16 (4H; m; aromatic), 9.12 (1H; s; NH).

^{13}C NMR δ 18.32, 18.71, 23.36, 24.64, 25.30, 31.73, 34.52, 61.35, 63.11, 102.13, 109.03, 115.38, 124.11, 126.47, 127.90, 131.01, 136.00, 146.30, 147.12, 150.13, 158.44, 166.82, 167.09, 199.59.

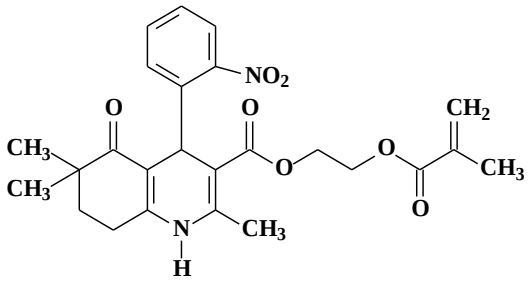
MS (m/z), 464.19 $[M+Na]^+$ (100%), 442.21, 312.13, 112.94, 59.85.

Elemental analysis for $C_{25}H_{28}FNO_5$, MW 441.49

Calculated: C, 68.01; H, 6.39; N, 3.17.

Found: C, 68.09; H, 6.46; N, 3.33.

2-(Methacryloyloxy)ethyl 4-(2-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4c)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2-nitrobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 55%.

Melting point: 125-127°C.

IR (cm^{-1}) 3297 (N-H stretching), 3053 (C-H stretching, aromatic) 2948(C-H stretching, aliphatic), 1698 (C=O stretching, ester), 1650 (C=O stretching, ketone), 1475 (C-H, bending), 1185 (C-O stretching) and 769 (C-H, bending, o-disubstituted benzene).

^{1}H NMR δ 0.79 (6H; s; 6-diCH_3), 0.92 (6H; s; 6-diCH_3), 1.62-1.75 (4H; m; quinoline $H^{7,8}$), 1.78 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.24 (3H; s; CH_3), 4.16-4.32 (4H; m; COOC$\underline{H}_2$C$\underline{H}_2$OCO), 5.20 (1H; s; quinoline H^4), 5.59-5.87 (2H; s; methacryloyloxy C=C$\underline{H}_2$), 7.81-8.22 (4H; m; aromatic), 9.3 (1H; s; NH).

^{13}C NMR δ 18.24, 18.91, 23.33, 24.66, 25.09, 34.34, 37.11, 61.38, 62.90, 101.32, 108.65, 122.48, 126.23, 126.66, 131.03, 135.92, 139.51, 146.57, 147.09, 147.64, 150.91, 166.60, 166.63, 199.78.

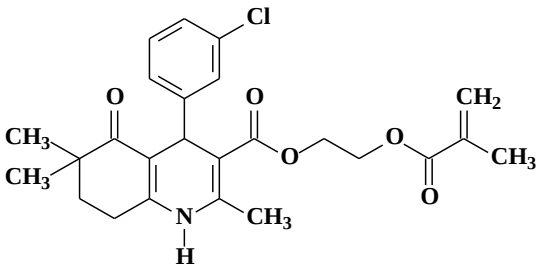
MS (m/z), 491.17 $[M+Na]^+$ (100%), 469.22, 339.1, 112.97, 59.85

Elemental analysis for $C_{25}H_{28}N_2O_7$, MW 468.50

Calculated: C, 64.09; H, 6.02; N, 5.98.

Found: C, 64.13; H, 5.90; N, 5.08.

2-(Methacryloyloxy)ethyl 4-(3-chlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4d)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 3-chlorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 70%.

Melting point: 153-155°C.

IR (cm^{-1}) 3314 (N-H stretching), 3080 (C-H stretching, aromatic) 2928 (C-H stretching, aliphatic), 1708 (C=O stretching, ester), 1642 (C=O stretching, ketone), 1469 (C-H, bending), 1190 (C-O stretching) and 865, 773 (C-H, bending, m-disubstituted benzene).

^{1}H NMR δ 0.86 (6H; s; 6-diCH_3), 0.95 (6H; s; 6-diCH_3), 1.73 (4H; m; quinoline $H^{7,8}$), 1.82 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.23 (3H; s; CH_3), 4.15-4.36 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.82 (1H; s; quinoline H^4), 5.66-5.94 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.04-7.28 (4H; m; aromatic), 9.2 (1H; s; NH).

^{13}C NMR δ 18.54, 19.09, 23.52, 24.7, 25.67, 34.69, 36.66, 61.73, 63.37, 102.50, 109.54, 126.31, 126.58, 126.69, 127.73, 130.40, 133.07, 136.22, 146.94, 150.58, 150.64, 166.91, 167.13, 200.10.

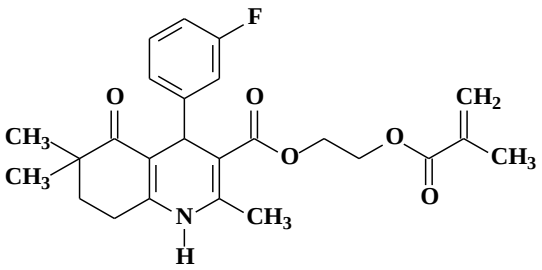
MS (m/z) 480.14 $[M+Na]^+$ (100%), 458.15, 328.08, 112.95, 59.77.

Elemental analysis for $C_{25}H_{28}ClNO_5$, MW 457.95

Calculated: C, 65.57; H, 6.16; N, 3.06.

Found: C, 65.40; H, 6.35; N, 3.19

2-(Methacryloyloxy)ethyl 4-(3-fluorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4e)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 3-fluorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 75%.

Melting point: 145-147°C.

IR (cm^{-1}) 3300 (N-H stretching), 3056 (C-H stretching, aromatic) 2929 (C-H stretching, aliphatic), 1705 (C=O stretching, ester), 1650 (C=O stretching, ketone), 1485 (C-H, bending), 1175 (C-O stretching) and 871, 769 (C-H, bending, m-disubstituted benzene).

^{1}H NMR δ 0.84 (6H; s; 6-diCH_3), 0.96 (6H; s; 6-diCH_3), 1.61-1.73 (4H; m; quinoline $H^{7,8}$), 1.83 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.30 (3H; s; CH_3), 4.12-4.34 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.81 (1H; s; quinoline H^4), 5.61-5.93 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 6.81-7.23 (4H; m; aromatic), 9.20 (1H; s; NH).

^{13}C NMR δ 18.30, 18.81, 23.33, 24.54, 25.53, 34.51, 36.28, 61.56, 63.18, 102.45, 109.30, 113.09, 114.31, 123.63, 126.40, 130.12, 136.08, 146.75, 150.32, 150.90, 161.24, 166.87, 167.08, 199.95.

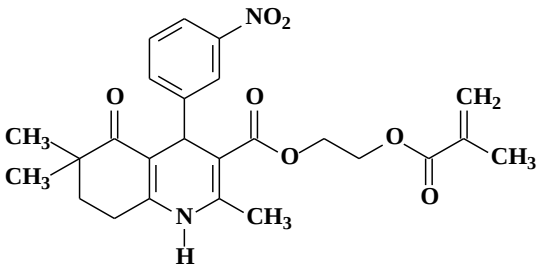
MS (m/z), 464.19 $[M+Na]^+$ (100%), 442.20, 312.13, 112.95, 59.79.

Elemental analysis for $C_{25}H_{28}FNO_5$, MW 441.49

Calculated: C, 68.01; H, 6.39; N, 3.17.

Found: C, 67.92; H, 6.57; N, 3.37

2-(Methacryloyloxy)ethyl 4-(3-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4f)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 3-nitrobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 70%.

Melting point: 132-134.

IR (cm^{-1}) 3303 (N-H stretching), 3082 (C-H stretching, aromatic) 2930 (C-H stretching, aliphatic), 1709 (C=O stretching, ester), 1643 (C=O stretching, ketone), 1464 (C-H, bending), 1186 (C-O stretching) and 872, 771 (C-H, bending, m-disubstituted benzene).

^{1}H NMR δ 0. 0.83 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.62-1.73 (4H; m; quinoline $H^{7,8}$), 1.83 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.30 (3H; s; CH_3), 4.15-4.38 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.91 (1H; s; quinoline H^4), 5.66-5.84 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.42-7.97 (4H; m; aromatic), 9.32 (1H; s; NH).

^{13}C NMR δ 18.30, 18.83, 23.35, 24.54, 25.37, 34.43, 36.87, 61.50, 63.09, 102.07, 109.21, 121.36, 122.20, 126.22, 129.84, 134.60, 135.91, 147.37, 147.83, 150.21, 150.60, 166.63, 166.76, 200.01.

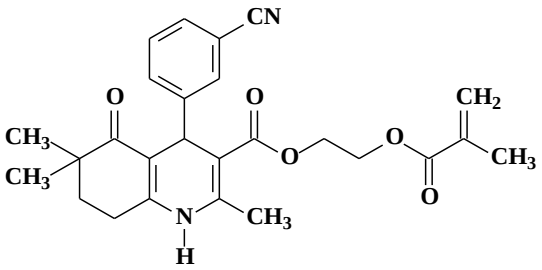
MS (m/z), 491.17 $[M+Na]^+$(100%), 469.31, 339.10, 112.97, 59.85.

Elemental analysis for $C_{25}H_{28}N_2O_7$, MW 468.50

Calculated: C, 64.09; H, 6.02; N, 5.98.

Found: C, 64.13; H, 5.72; N, 6.08.

2-(Methacryloyloxy)ethyl 4-(3-cyanophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4g)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 3-cyanobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture.Yield 60%.

Melting point: 147-149°C.

IR (cm^{-1}) 3293 (N-H stretching), 3074 (C-H stretching, aromatic) 2961 (C-H stretching, aliphatic), 2229 (CN stretching), 1699 (C=O stretching, ester), 1648 (C=O stretching, ketone), 1484 (C-H, bending), 1189 (C-O stretching) and 886, 777 (C-H, bending, m-disubstituted benzene).

^{1}H NMR δ 0.85 (6H; s; 6-diCH_3), 0.95 (6H; s; 6-diCH_3), 1.63-1.74 (4H; m; quinoline $H^{7,8}$), 1.83 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.31 (3H; s; CH_3), 4.10-4.32 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.83 (1H; s; quinoline H^4), 5.61-5.90 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.37-7.66 (4H; m; aromatic), 9.2 (1H; s; NH).

^{13}C NMR δ 18.37, 18.88, 23.33, 24.56, 25.39, 34.45, 36.71, 61.54, 63.09, 101.98, 109.18, 111.18, 119.54, 126.47, 129.62, 130.13, 131.14, 132.87, 136.00, 147.23, 149.57, 150.54, 166.72, 166.78, 199.99.

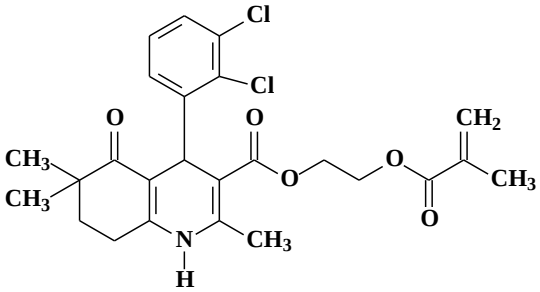
MS (m/z), 471.19 $[M+Na]^+$ (100%), 449.21, 319.13, 112.97, 59.83.

Elemental analysis for $C_{26}H_{28}N_2O_5$, MW 448.20

Calculated: C, 69.63; H, 6.29; N, 6.25.

Found: C, 69.78; H, 6.29; N, 6.42

2-(Methacryloyloxy)ethyl 4-(2,3-dichlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4h)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2,3-dichlorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 80%.

Melting point: 140-142°C.

IR (cm^{-1}) 3329 (N-H stretching), 3068 (C-H stretching, aromatic) 2930 (C-H stretching, aliphatic), 1702 (C=O stretching, ester), 1643 (C=O stretching, ketone), 1469 (C-H, bending) and 1191 (C-O stretching) and 840, 721 (C-H, bending, 1,2,3-trisubstituted benzene).

^{1}H NMR δ 0.83 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.62-1.73 (4H; m; quinoline $H^{7,8}$), 1.85 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.29 (3H; s; CH_3), 4.18-4.32 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.21 (1H; s; quinoline H^4), 5.60-5.83 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.19-7.33 (4H; m; aromatic), 9.21 (1H; s; NH).

^{13}C NMR δ 18.33, 18.86, 23.48, 24.62, 25.11, 34.47, 36.76, 61.39, 63.09, 102.51, 109.60, 126.34, 127.88, 128.21, 130.20, 130.54, 131.81, 135.90, 146.53, 148.57, 150.32, 166.71, 166.90, 199.68.

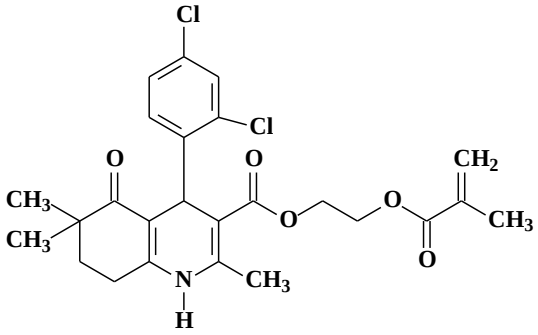
MS (m/z) 514.12 $[M+Na]^+$ (100%), 492.12, 362.03,112.95, 59.86.

Elemental analysis for $C_{25}H_{27}Cl_2NO_5$, MW 492.39

Calculated: C, 60.98; H, 5.53; N, 2.84.

Found: C, 60.98; H, 5.15; N, 2.96.

2-(Methacryloyloxy)ethyl 4-(2,4-dichlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4i)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2,4-dichlorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 75%.

Melting point: 189-191°C.

IR (cm^{-1}) 3373 (N-H stretching), 3064 (C-H stretching, aromatic) 2956 (C-H stretching, aliphatic), 1714 (C=O stretching, ester), 1693 (C=O stretching, ketone), 1469 (C-H, bending), 1189 (C-O stretching) and 885, 825 (C-H, bending, 1,2,4-trisubstituted benzene).

^{1}H NMR δ 0.82 (6H; s; 6-diCH_3), 0.92 (6H; s; 6-diCH_3), 1.61-1.73 (4H; m; quinoline $H^{7,8}$), 1.82 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.28 (3H; s; CH_3), 4.12-4.33 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.19 (1H; s; quinoline H^4), 5.65-5.90 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.18-7.31 (3H; m; aromatic), 9.20 (1H; s; NH).

^{13}C NMR δ 18.31, 18.85, 23.41, 24.65, 25.20, 34.42, 35.46, 61.31, 63.10, 102.22, 109.37, 126.30, 127.33, 128.63, 131.11, 133.03, 133.26, 135.98, 144.87, 146.66, 150.25, 166.73, 166.94, 199.54.

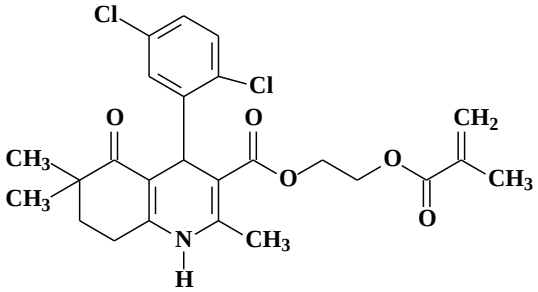
MS (m/z) 514.12 $[M+Na]^+$ (100%), 492.12, 362.03,112.94, 59.76.

Elemental analysis for $C_{25}H_{27}Cl_2NO_5$, MW 492.39

Calculated: C, 60.98; H, 5.53; N, 2.84.

Found: C, 60.97; H, 5.20; N, 3.08.

2-(Methacryloyloxy)ethyl 4-(2,5-dichlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4j)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2,5-dichlorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 76%.

Melting point: 160-162°C.

IR (cm^{-1}) 3328 (N-H stretching), 3066 (C-H stretching, aromatic) 2958 (C-H stretching, aliphatic), 1702 (C=O stretching, ester), 1637 (C=O stretching, ketone), 1438 (C-H, bending), 1185 (C-O stretching) and 881,712 (C-H, bending, 1,2,5-trisubstituted benzene).

^{1}H NMR δ 0.83 (6H; s; 6-diCH_3), 0.96 (6H; s; 6-diCH_3), 1.63-1.72 (4H; m; quinoline $H^{7,8}$), 1.86 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.30 (3H; s; CH_3), 4.12-4.33 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.09 (1H; s; quinoline H^4), 5.63-5.96 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.11-7.36 (3H; m; aromatic), 9.20 (1H; s; NH).

^{13}C NMR δ 18.31, 18.85, 23.36, 24.63, 25.18, 34.37, 36.76, 61.29, 63.08, 101.71, 108.88, 126.32, 127.65, 131.17, 131.43, 131.44, 131.59, 136.03, 146.98, 147.20, 150.54, 166.78, 166.89, 199.68.

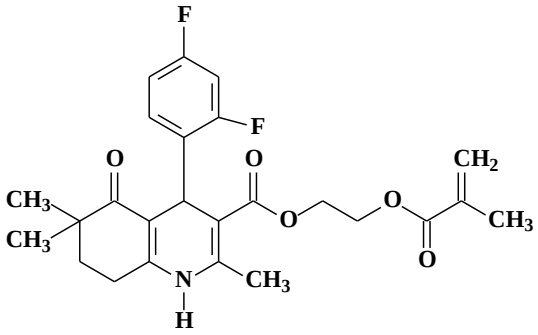
MS (m/z) 514.11 $[M+Na]^+$ (100%), 492.14, 362.04, 112.95, 59.74.

Elemental analysis for $C_{25}H_{27}Cl_2NO_5$, MW 492.39

Calculated: C, 60.98; H, 5.53; N, 2.84.

Found: C, 60.80; H, 5.64; N, 3.05.

2-(Methacryloyloxy)ethyl 4-(2,4-difluorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4k)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2,4-difluorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 80%.

Melting point: 172-174°C.

IR (cm^{-1}) 3298 (N-H stretching), 3088 (C-H stretching, aromatic) 2962 (C-H stretching, aliphatic), 1706 (C=O stretching, ester), 1648 (C=O stretching, ketone), 1469 (C-H, bending), 1181 (C-O stretching) and 879, 830 (C-H, bending, 1,2,4-trisubstituted benzene).

^{1}H NMR δ 0.84 (6H; s; 6-diCH_3), 0.93 (6H; s; 6-diCH_3), 1.64-1.76 (4H; m; quinoline $H^{7,8}$), 1.83 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.29 (3H; s; CH_3), 4.13-4.36 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.18 (1H; s; quinoline H^4), 5.63-5.95 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.26-7.41 (3H; m; aromatic), 9.21 (1H; s; NH).

^{13}C NMR δ 18.33, 18.85, 23.53, 24.80, 25.26, 34.45, 36.09, 62.81, 63.16, 99.37, 108.11, 127.30, 126.34, 127.66, 128.28, 136.08, 137.64, 139.73, 147.58, 151.03, 151.28, 166.73, 167.08, 199.59.

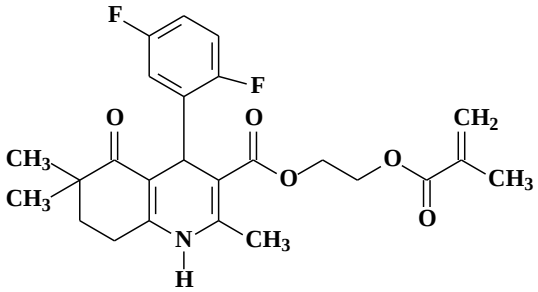
MS (m/z), 482.16 $[M+Na]^+$(100%), 460.17, 330.1, 112.95, 59.78.

Elemental analysis for $C_{25}H_{27}F_2NO_5$, M W 459.48

Calculated: C, 65.35; H, 5.92; N, 3.05.

Found: C, 65.23; H, 5.84; N, 3.16.

2-(Methacryloyloxy)ethyl 4-(2,5-difluorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4l)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2,5-difluorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 78%.

Melting point: 177-179°C.

IR (cm^{-1}) 3304 (N-H stretching), 3078 (C-H stretching, aromatic) 2959 (C-H stretching, aliphatic), 1704 (C=O stretching, ester), 1649 (C=O stretching, ketone), 1454 (C-H, bending), 1175 (C-O stretching) and 874,707 (C-H, bending, 1,2,5-trisubstituted benzene).

^{1}H NMR δ 0.82 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.63-1.74 (4H; m; quinoline $H^{7,8}$), 1.86 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.29 (3H; s; CH_3), 4.12-4.35 (4H; m; COOC$\underline{H}_2$C$\underline{H}_2$OCO), 4.99 (1H; s; quinoline H^4), 5.61-5.93 (2H; s; methacryloyloxy C=C$\underline{H}_2$), 6.84-7.11 (3H; m; aromatic), 9.23 (1H; s; NH).

^{13}C NMR δ 18.30, 188.80, 23.33, 24.60, 25.24, 32.42, 34.47, 61.36, 63.10, 101.53, 108.56, 114.58, 116.75, 117.04, 126.33, 136.01, 146.21, 150.43, 154.82, 157.2, 159.52, 166.78, 166.83, 199.61.

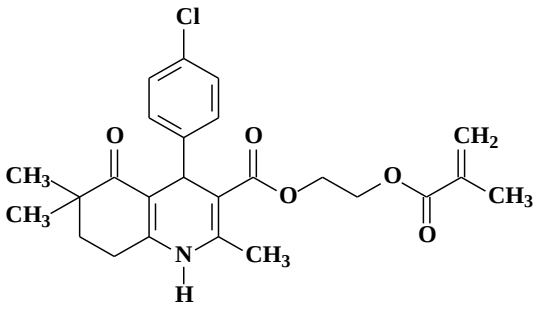
MS (m/z), 482.16 $[M+Na]^+$ (100%), 460.15, 330.11, 112.95, 59.78.

Elemental analysis for $C_{25}H_{27}F_2NO_5$, MW 459.48

Calculated: C, 65.35; H, 5.92; N, 3.05.

Found: C, 65.42; H, 5.94; N, 3.2.

2-(Methacryloyloxy)ethyl 4-(4-chlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4m)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 4-chlorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 80%.

Melting point: 207-209°C.

IR (cm^{-1}) 3376 (N-H stretching), 3068 (C-H stretching, aromatic) 2958 (C-H stretching, aliphatic), 1716 (C=O stretching, ester), 1693 (C=O stretching, ketone), 1470 (C-H, bending), 1189 (C-O stretching) and 845 (C-H, bending, p-disubstituted benzene).

^{1}H NMR δ 0.83 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.63-1.75 (4H; m; quinoline $H^{7,8}$), 1.86 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.28 (3H; s; CH_3), 4.11-4.32 (4H; m; COOC$\underline{H}_2$C$\underline{H}_2$OCO), 5.21 (1H; s; quinoline H^4), 5.61-5.87 (2H; s; methacryloyloxy C=C$\underline{H}_2$), 7.27-7.65 (4H; m; aromatic), 9.22 (1H; s; NH).

^{13}C NMR δ 18.34, 18.83, 23.32, 24.54, 25.48, 34.54, 36.04, 61.52, 63.13, 102.62, 109.59, 126.43, 128.16, 129.55, 130.62, 136.04, 146.56, 147.13, 150.14, 166.79, 166.99, 199.92.

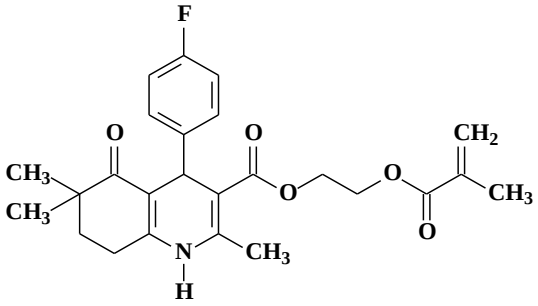
MS (m/z) 480.14 $[M+Na]^+$ (100%), 458.12, 328.08, 112.95, 59.87.

Elemental analysis for $C_{25}H_{28}ClNO_5$, MW 457.95

Calculated: C, 65.57; H, 6.16; N, 3.06.

Found: C, 65.46; H, 5.94; N, 3.21.

2-(Methacryloyloxy)ethyl 4-(4-fluorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4n)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 4-fluorobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 63%.

Melting point: 166-168°C.

IR (cm^{-1}) 3312 (N-H stretching), 3088 (C-H stretching, aromatic) 2950 (C-H stretching, aliphatic), 1704 (C=O stretching, ester), 1673 (C=O stretching, ketone), 1470 (C-H, bending), 1189 (C-O stretching) and 857 (C-H, bending, p-disubstituted benzene).

^{1}H NMR δ 0.8-1 (6H; s; 6,6-diCH_3), 1.6-1.7 (4H; m; quinoline $H^{7,8}$), 1.8 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.2 (3H; s; CH_3), 4.1-4.3 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.8 (1H; s; quinoline H^4), 5.6-5.9 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 6.9-7.1 (4H; m; aromatic), 9.1 (1H; s; NH).

^{13}C NMR δ 18.33, 18.86, 23.35, 24.57, 25.54, 34.54, 35.72, 61.43, 63.18, 102.90, 109.82, 114.93, 126.47, 129.45, 136.04, 144.31, 146.36, 150.08, 159.67, 166.89, 167.09, 199.96.

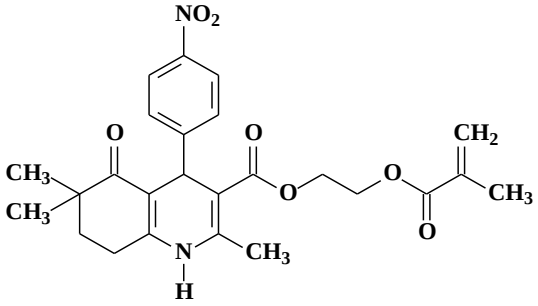
MS (m/z), 464.19 $[M+Na]^+$ (100%), 442.19, 312.12, 112.89, 59.77.

Elemental analysis for $C_{25}H_{28}FNO_5$, MW 441.49

Calculated: C, 68.01; H, 6.39; N, 3.17.

Found: C, 68.22; H, 6.58; N, 3.33.

2-(Methacryloyloxy)ethyl 4-(4-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4o)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 4-nitrobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 65%.

Melting point: 157-159°C.

IR (cm^{-1}) 3290 (N-H stretching), 3077 (C-H stretching, aromatic) 2949 (C-H stretching, aliphatic), 1705 (C=O stretching, ester), 1646 (C=O stretching, ketone), 1471 (C-H, bending), 1189 (C-O stretching) and 834 (C-H, bending, p-disubstituted benzene).

^{1}H NMR δ 0.86 (6H; s; 6-diCH_3), 0.98 (6H; s; 6-diCH_3), 1.67-1.73 (4H; m; quinoline $H^{7,8}$), 1.79 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.30 (3H; s; CH_3), 4.13-4.27 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.96 (1H; s; quinoline H^4), 5.63-5.90 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.37-8.01 (4H; m; aromatic), 9.31 (1H; s; NH).

^{13}C NMR δ 18.23, 18.80, 23.33, 24.50, 25.41, 34.40, 37.19, 61.58, 63.12, 101.83, 109.04, 123.67, 126.39, 129.04, 136.06, 146.05, 147.33, 150.62, 155.68, 166.73, 166.95, 199.96.

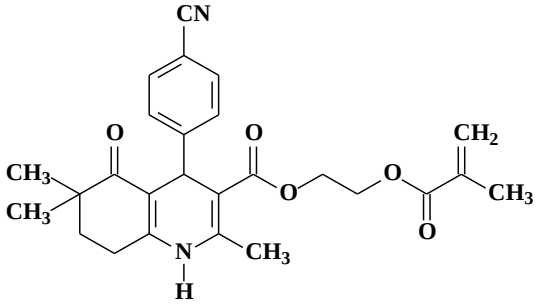
MS (m/z), 491.17 $[M+Na]^+$ (100%), 469.18, 339.13, 112.94, 59.86.

Elemental analysis for $C_{25}H_{28}N_2O_7$, MW 468.50

Calculated: C, 64.09; H, 6.02; N, 5.98.

Found: C, 63.95; H, 5.94; N, 6.13.

2-(Methacryloyloxy)ethyl 4-(4-cyanophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4p)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 4-cyanobenzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 65%.

Melting point: 172-174°C.

IR (cm^{-1}) 3297 (N-H stretching), 3076 (C-H stretching, aromatic) 2971 (C-H stretching, aliphatic), 2231 (CN stretching), 1708 (C=O stretching, ester), 1628 (C=O stretching, ketone), 1449 (C-H, bending), 1170 (C-O stretching) and 840 (C-H, bending, p-disubstituted benzene).

^{1}H NMR δ 0.88 (6H; s; 6-diCH_3), 0.97 (6H; s; 6-diCH_3), 1.75 (4H; m; quinoline $H^{7,8}$), 1.83 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.30 (3H; s; CH_3), 4.13-4.36 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 4.98 (1H; s; quinoline H^4), 5.62-5.93 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.25-7.67 (4H; m; aromatic), 9.20 (1H; s; NH).

^{13}C NMR δ 18.33, 18.85, 23.34, 24.51, 25.41, 34.45, 37.14, 61.54, 63.08, 101.93, 108.95, 109.11, 119.48, 126.43, 128.80, 132.30, 136.00, 147.20, 150.52, 153.53, 166.73, 166.77, 199.91.

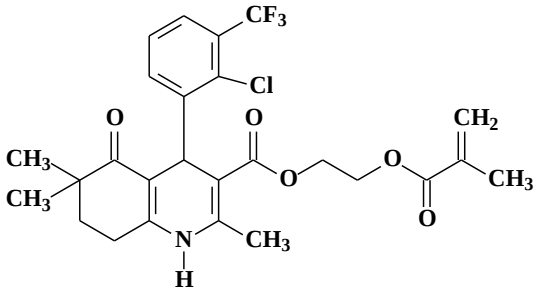
MS (m/z), 471.19 $[M+Na]^+$ (100%),449.21, 319.13, 112.97, 59.70.

Elemental analysis for $C_{26}H_{28}N_2O_5$, MW 448.20

Calculated.: C, 69.63; H, 6.29; N, 6.25.

Found: C, 69.63; H, 6.29; N, 6.35.

2-(Methacryloyloxy)ethyl 4-(2-chloro-3-(trifluoromethyl)phenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4q)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2-chloro-3-(trifluoromethyl)-benzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 55%.

Melting point: 223-225°C.

IR (cm^{-1}) 3312 (N-H stretching), 3093 (C-H stretching, aromatic) 2970 (C-H stretching, aliphatic), 1707 (C=O stretching, ester), 1666 (C=O stretching, ketone), 1476 (C-H, bending), 1187 (C-O stretching) and 865, 725 (C-H, bending, 1,2,3-trisubstituted benzene).

^{1}H NMR δ 0.80 (6H; s; 6-diCH_3), 0.95 (6H; s; 6-diCH_3), 1.61-1.77 (4H; m; quinoline $H^{7,8}$), 1.91 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.25 (3H; s; CH_3), 4.12-4.23 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.28 (1H; s; quinoline H^4), 5.60-5.86 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.31-7.57 (4H; m; aromatic), 9.25 (1H; s; NH).

^{13}C NMR δ 18.23, 18.86, 23.44, 24.6, 25.05, 34.37, 36.07, 61.11, 62.90, 102.26, 109.36, 119.68, 122.4,9 125.14, 126.32, 126.99, 127.84, 129.93, 135.91, 146.82, 148.37, 150.55, 166.62, 166.83, 199.65.

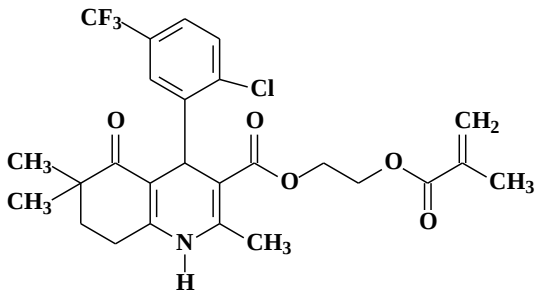
MS (m/z), 548.12 $[M+Na]^+$ (100%), 526.17, 396.05, 112.95, 59.87.

Elemental analysis for $C_{26}H_{27}ClF_3NO_5$, MW 525.15

Calculated: C, 59.37; H, 5.17; N, 2.66.

Found: C, 59.14; H, 5.14; N, 2.92.

2-(Methacryloyloxy)ethyl 4-(2-chloro-5-(trifluoromethyl)phenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4r)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2-chloro-5-(trifluoromethyl)-benzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 60 %.

Melting point: 205-207°C.

IR (cm^{-1}) 3307 (N-H stretching), 3088 (C-H stretching, aromatic) 2963 (C-H stretching, aliphatic), 1705 (C=O stretching, ester), 1637 (C=O stretching, ketone), 1470 (C-H, bending), 1190 (C-O stretching) and 871, 710 (C-H, bending, 1,2,5-trisubstituted benzene).

^{1}H NMR δ 0.81 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.61-1.77 (4H; m; quinoline $H^{7,8}$), 1.79 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.32 (3H; s; CH_3), 4.1-4.3 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.18 (1H; s; quinoline H^4), 5.26-5.84 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.45-7.51 (3H; m; aromatic), 9.26 (1H; s; NH).

^{13}C NMR δ 18.22, 18.84, 23.54, 24.66, 25.18, 34.38, 36.08, 61.11, 62.97, 102.22, 109.36, 119.6, 122.40, 125.28, 126.45, 126.90, 127.84, 129.89, 135.91, 146.93, 148.55, 150.58, 166.64, 166.89, 199.78.

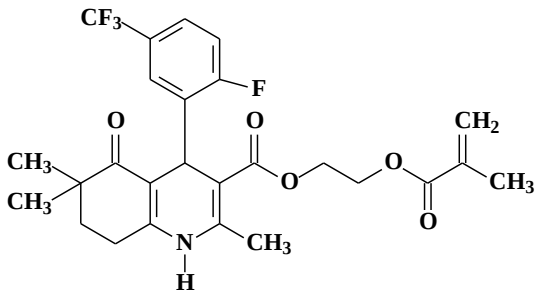
MS (m/z), 548.12 $[M+Na]^+$ (100%),526.19, 396.05, 112.95, 59.87.

Elemental analysis for $C_{26}H_{27}ClF_3NO_5$, MW 525.15

Calculated: C, 59.37; H, 5.17; N, 2.66.

Found: C, 59.48; H, 5.24; N, 2.92.

2-(methacryloyloxy)ethyl 4-(2-fluoro-5-(trifluoromethyl)phenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate(4s)



The compound was obtained by the reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, 2-fluoro-5-(trifluoromethyl)-benzaldehyde and ammonium acetate in methanol under microwave irradiation for 15 min, then was recrystallized from dichloromethane /ether mixture. Yield 50%.

Melting point: 192-194°C.

IR (cm^{-1}) 3321 (N-H stretching), 3054 (C-H stretching, aromatic) 2942 (C-H stretching, aliphatic), 1712 (C=O stretching, ester), 1644 (C=O stretching, ketone), 1485 (C-H, bending), 1167 (C-O stretching) and 869, 715 (C-H, bending, 1,2,5-trisubstituted benzene).

^{1}H NMR δ 0.81 (6H; s; 6-diCH_3), 0.94 (6H; s; 6-diCH_3), 1.63-1.75 (4H; m; quinoline $H^{7,8}$), 1.82 (3H; s; methacryloyloxy C$C\underline{H}_3$), 2.30 (3H; s; CH_3), 4.15-4.37 (4H; m; COO$C\underline{H}_2C\underline{H}_2$OCO), 5.03 (1H; s; quinoline H^4), 5.66-5.86 (2H; s; methacryloyloxy C=$C\underline{H}_2$), 7.14-7.48 (4H; m; aromatic), 9.26 (1H; s; NH).

^{13}C NMR δ 18.33, 18.85, 23.35, 24.6, 25.09, 31.83, 34.46, 62.90, 63.12, 101.54, 108.63, 116.44, 122.13, 124.58, 125.07, 126.21, 135.92, 137.04, 147.19, 157.78, 150.50, 155.24, 166.65, 166.79, 199.08.

MS (m/z), 532.18 $[M+Na]^+$(100%), 510.12, 319.13, 112.97, 59.70.

Elemental analysis for $C_{26}H_{27}F_4NO_5$, MW 509.49

Calculated: C, 61.29; H, 5.34; N, 2.75.

Found: C, 61.04; H, 5.25; N, 2.91.

4.3. Myorelaxant biological activity

Table 4.2. Maximum relaxant responses (E_{max}) and pD_2 values of the synthesized compounds on isolated strips of rabbit gastric fundus smooth muscle.

Compound	E_{max}	pD_2
4a	55,18±8,35*	4,30± 0,62*
4b	75,09±5,95*	5,19±0,47*
4c	63,22±6,49*	4,78± 0,51*
4d	66,83±4,53*	5,29±0,36*
4e	67,98±6,58*	5,22±0,52*
4f	79,01±4,03*	5,28± 0,30*
4g	73,60±6,99*	5,16± 0,54*
4h	68,48±1,88*	5,46± 0,03*
4i	33,22±5,55*	5,35± 0,44*
4j	40,71±4,01*	5,36±0,30*
4k	43,37±8,59*	4,82±0,63*
4l	62,76±6,56*	5,47± 0,52*
4m	51,63±3,72*	4,85± 0,27*
4n	81,65±7,06*	4,77± 0,55*
4o	70,07±8,16*	4,80± 0,61*
4p	57,12±5,76*	4,66± 0,46*
4q	64,52±9,63*	5,33± 0,68*
4r	56,16±4,53*	5,05± 0,36*
4s	53,49±6,76*	4,92± 0,53*
Nifedipine	98,92±0,83	7,68± 0,08

* $p < 0.05$, compared with nifedipine (n = 6)

Relaxation is expressed as a percentage of the precontraction induced by Ca^{+2} (2.5 mM). The negative logarithm of the concentration for the half-maximal response (pD_2) and E_{max} values represent mean value ± S.E.

4.4. Antioxidant activity

Table 4.3. Relative change of the oxygen reduction current density values of the compounds

	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	**4m**	**4n**	**4o**	**4p**	**4q**	**4r**	**4s**	**Nifedip.**
j_{or}	9.40	8.20	7.80	8.80	8.00	10.70	10.00	6.90	7.20	7.00	10.60	8.80	9.50	9.40	9.00	8.80	7.70	8.20	8.20	5.90
j_{res}	3.80	4.30	2.60	3.00	3.50	7.30	4.80	2.60	2.60	4.20	7.60	3.20	2.80	3.40	5.40	3.30	5.00	3.80	3.20	3.20
$j_{0.2}$	6.90	6.60	5.80	6.00	7.10	7.20	8.80	4.00	4.40	5.70	9.20	6.40	5.60	6.30	7.80	7.00	6.90	6.80	6.00	4.30
$j_{0.4}$	6.30	7.00	5.40	6.20	6.80	6.50	8.60	4.10	5.00	5.60	8.00	6.50	5.80	7.00	7.75	6.80	6.85	6.90	5.60	4.40
$j_{0.6}$	6.00	7.10	5.30	6.60	6.20	6.00	7.90	4.80	5.20	5.40	7.00	6.60	6.00	7.20	7.70	6.20	6.80	7.00	4.80	4.50
j_{or}-j_{res}	5.60	3.90	5.20	5.80	4.50	3.40	5.20	4.30	4.60	2.80	3.00	5.60	6.70	6.00	3.60	5.50	2.70	4.40	5.00	2.70
$J_{0.2}/(j_{or}-j_{res})$	1.23	1.69	1.12	1.03	1.58	2.12	1.69	0.93	0.96	2.04	3.07	1.14	0.84	1.05	2.17	1.27	2.56	1.55	1.20	1.59
$J_{0.4}/(j_{or}-j_{res})$	1.13	1.79	1.04	1.07	1.51	1.91	1.65	0.95	1.09	2.00	2.67	1.16	0.87	1.17	2.15	1.24	2.54	1.57	1.12	1.63
$J_{0.6}/(j_{or}-j_{res})$	1.07	1.82	1.02	1.14	1.38	1.76	1.52	1.12	1.13	1.93	2.33	1.18	0.90	1.20	2.14	1.13	2.52	1.59	0.96	1.67

j_{or}: the oxygen reduction current density without antioxidants.

j_{res}: the residual current density after removal of oxygen by N_2 gas.

$J_{0.2}/(j_{or}-j_{res})$, $J_{0.4}/(j_{or}-j_{res})$ and $J_{0.6}/(j_{or}-j_{res})$: values of relative change of the oxygen reduction current density for conc. 0.2, 0.4 and 0.6, respectively.

Table 4.4. Coefficients of antioxidant activity (K) of the compounds

Compound	K_1 *	K_2 **	K_{av} ***	Equation of straight line
4a	-0.54	-0.27	**-0.40**	y = -0.004x + 1.3 R^2 = 0.9552
4b	0.51	0.13	**0.32**	y = 0.0032x + 1.6367 R^2 = 0.9119
4c	-0.38	-0.10	**-0.24**	y = -0.0025x + 1.16 R^2 = 0.8929
4d	0.17	0.34	**0.26**	y = 0.0028x + 1.03 R^2 = 0.9758
4e	-0.33	-0.67	**-0.50**	y = -0.005x + 1.69 R^2 = 0.9709
4f	-1.03	-0.74	**0.88**	y = -0.009x + 2.29 R^2 = 0.9908
4g	-0.19	-0.67	**-0.43**	y = -0.0042x + 1,79 R^2 = 0.9146
4h	0.12	0.81	**0.47**	y = 0.0048x + 0.81 R^2 = 0.828
4i	0.65	0.22	**0.43**	y = 0.0043x + 0.89 R^2 = 0.9146
4j	-0.18	-0.36	**-0.27**	y = -0.0028x + 2.1 R^2 = 0.9758
4k	-2.00	-1.67	**-1.83**	y = -0.0185x + 3.43 R^2 = 0.9978
4l	0.09	0.09	**0.09**	y = 0.001x + 1.12 R^2 = 1
4m	0.15	0.15	**0.15**	y = 0.0015x + 0.8067 R^2 = 0.9643
4n	0.58	0.17	**0.38**	y = 0.0038x + 0.99 R^2 = 0.8929
4o	-0.07	-0.07	**-0.07**	y = -0.0007x + 2.1833 R^2 = 0.9643
4p	-0.18	-0.55	**-0.36**	y = -0.0035x + 1.3533 R^2 = 0.9018
4q	-0.09	-0.09	**-0.09**	y = -0.001x + 2.58 R^2 = 1
4r	0.11	0.11	**0.11**	y = 0.0013x + 1.5167 R^2 = 0.9868
4s	-0.40	-0.80	**-0.60**	y = -0.006x + 1.3333 R^2 = 0.9643
Nifedipine	0.19	0.19	**0.19**	y = 0.002x + 1.55 R^2 = 1

*: between concentrations (0.4 & 0.2), **: between concentrations (0.6 & 0.4), ***: average coefficient.

5. DISCUSSION

5.1. Pharmacophore modeling

The 3D structure of many target proteins has not been known yet, especially those transmembrane proteins as well as Ca^{2+} channels. Since the 3D structure that show the transmembrane α1 subunit, where the DHP's binding site is located, is unavailable, so we were not able to use docking or structure-based pharmacophore modeling techniques.

Since we have only information about the ligands that act as Ca^{2+} channels blockers and their interaction with Ca^{2+} channels were proved, so the pharmacophore identification were acheived by using a direct method that only rely on the ligands i.e. ligand-based pharmacophore modeling.

We suggested that the pharmacophore that can be generated if the first group ligands were used, may not provide any additional information other than that have been already reported by the classical SAR studies. So, a training set of five ligands of the second group DHP calcium channel blockers (Figure 3.2) were used for generation of a ligand-based pharmacophore model by LigandScout, then the generated pharmacophore model was validated.

The pharmacophore model showed typical features described by SAR studies which are: a HB-acceptor of the dihydropyridine NH, two typical hydrophobes of the 2,6-dimethyl groups, aromatic hydrophobe of phenyl ring, two HB-donors of 3,5-dicarbonyl oxygens, and the other two "optional" HB-donors near the aromatic hydrophobe represent nitro group.

The new finding was the appearance of hydrophobe (yellow sphere on left) and a positive ionizable feature (blue star on left), which are additional common features related to the ester side chains, were not reported by classical SAR studies.

Classical SAR studies only described the importance of the carbonyl oxygens which can be involved in hydrogen bonding with binding site residues, we suggest that those two newly described features also may participate in binding to the active site.

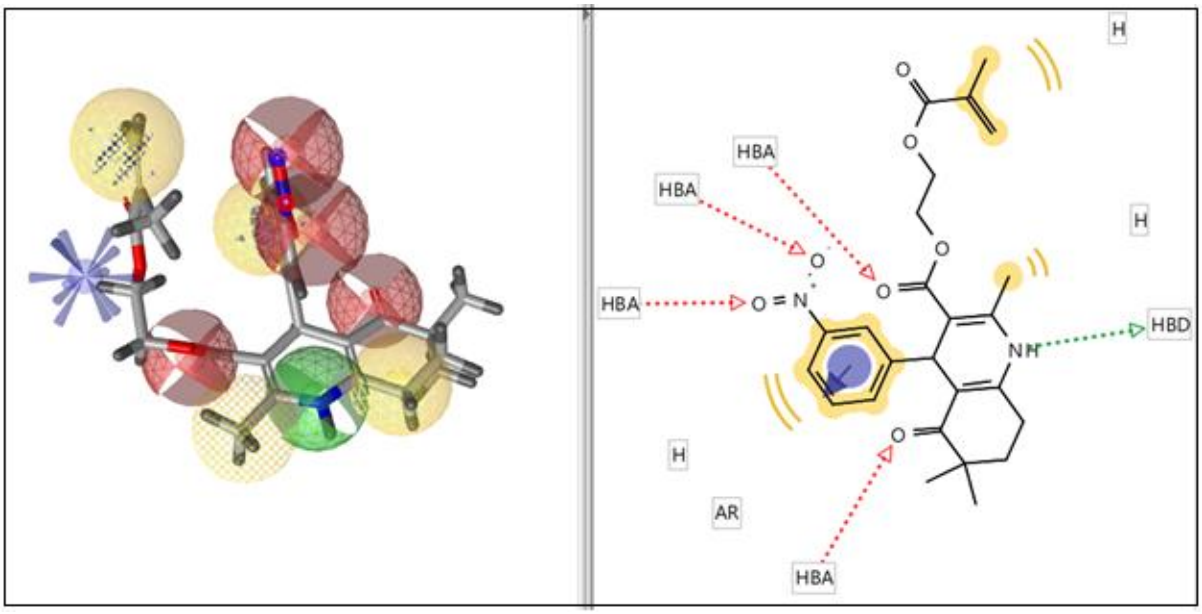


Figure 5.1. Compound **4f** mapped to pharmacophore model.

The synthesized compounds were screened out using the validated pharmacophore model, it was noticed that the hydrophobic vinyl group of the methacryloyloxy ester fits the additional hydrophobe while the positive ionizable feature was missed because methacryloloxy ester lakes nitrogens. (Figure 5.1) describes the mapping of compound **4f** to the generated pharmacophore and shows that the compound fits most of the features.

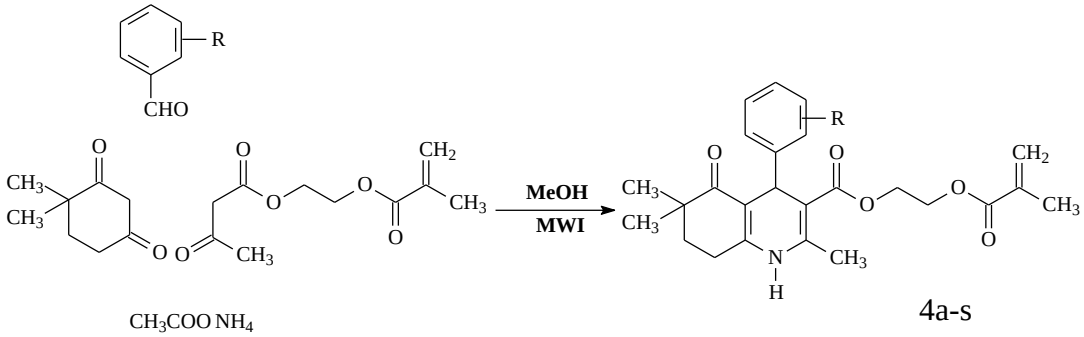
5.2. Chemistry

Microwave irradiation (MWI) as an energy source for the activation of chemical reactions has been recently introduced and gained great popularity compared to conventional reactions because of its ability to reduce reaction times, to improve yields and to simplify the work-up processes.

The success of their application extended to synthesis DHP and hexahydroquinoline (HHQ) derivatives via Hantzsch reaction (188-190, 248, 249). Thus, promoted us to use apply MWI technique to achieve our compounds.

In this study, nineteen novel 2-(methacryloyloxy)ethyl 4-(aryl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives were achieved via a four-component

reaction of 4,4-dimethyl-1,3-cyclohexanedione, substituted benzaldehydes, 2-(methacryloyloxy)ethyl acetoacetate and ammonium acetate in methanol and the mixture was irradiated by microwave for 15 minutes, thus develops an easier and faster method than the conventional method.



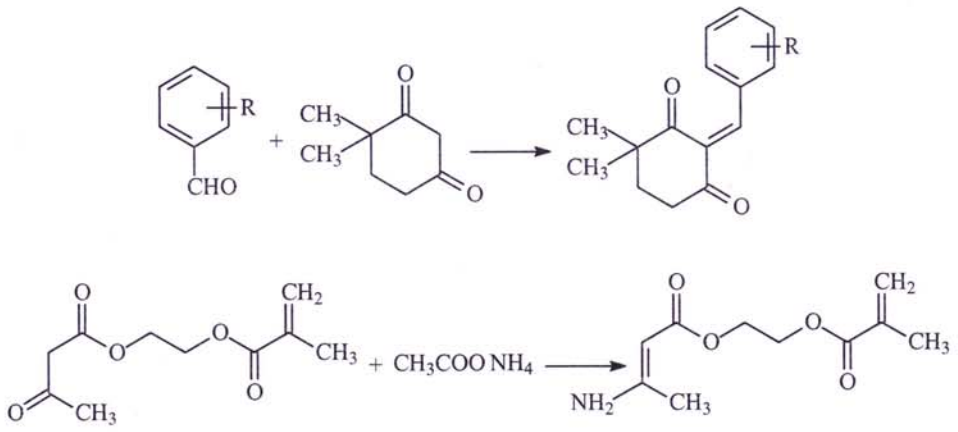
Methanol was used as a solvent because of its high dielectric loss tangent (tan δ) value that equals 0.941; this makes it a very good solvent for microwave-promoted synthetic reactions.

Furthermore, studies showed that alcohols, as a solvent for Hantzsch reaction, is much better in terms of yield than the other tested solvents such as tetrahydrofuran, acetonitrile and water (165). The time of reaction was determined after monitoring the reaction progress by TLC.

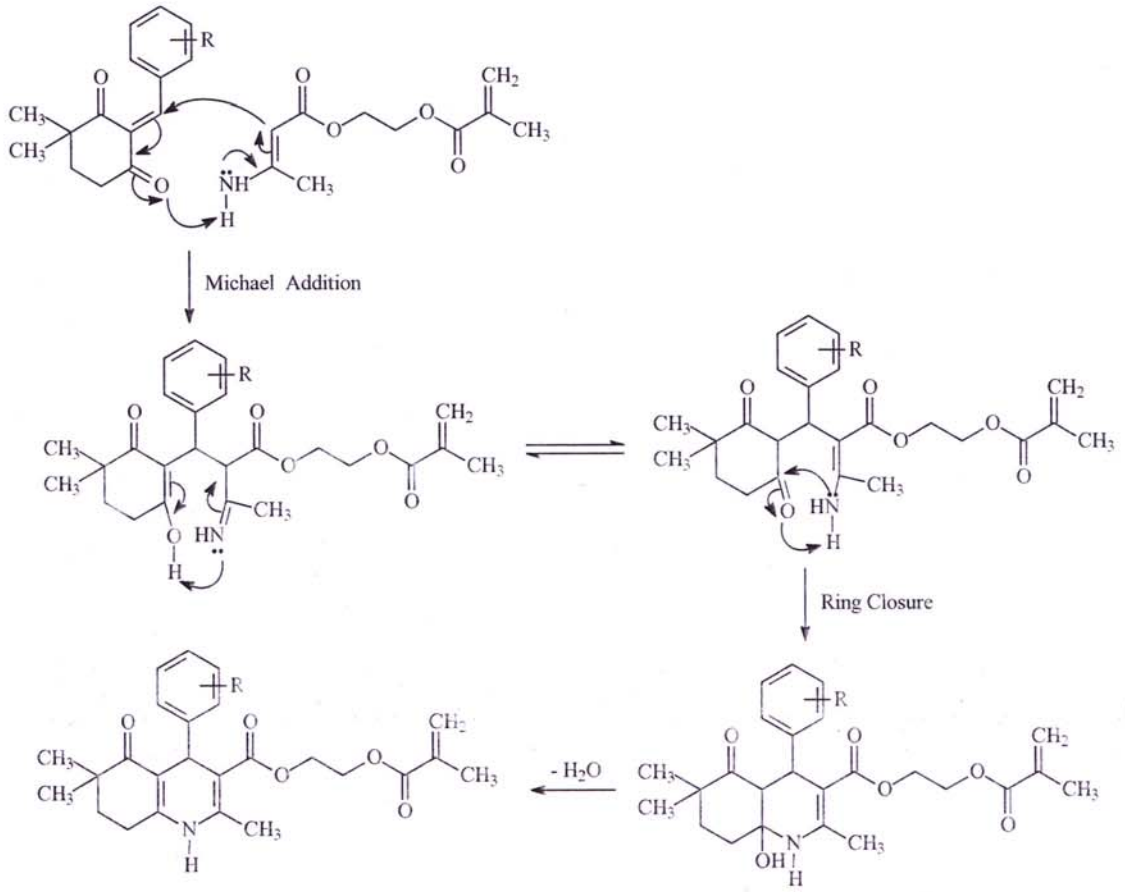
The compounds were obtained by evaporating the solvent till dryness and were crystallized from dichloromethane/ether mixture. Generally, the yield of reaction varied according to the nature of aldehyde incorporated in the reaction.

Classical Hantzsch reaction is a four-component one pot synthesis reaction of 1,4-DHPs, so there are many pathways can be involved during the synthesis of DHPs. The four components can join up in 2 + 2 manner or 3 + 1 manner. However, studies that were done by monitoring the reaction progress by NMR ensured that 2 + 2 manner pathway is involved (250).

The pathway is shown to involve the reaction of aldehyde with one of the β-dicarbonyl compound to form benzylidine or chalcone derivative, and of the ammonium acetate with the other β-dicarbonyl compound to give an enamine or aminocrotonate.



Then, a Michael addition of chalcone derivative to enamine occurs, which is shown to be rate determining step of Hantzsch reaction. The hexahydroquinoline ring is formed by 1.4-DHP ring closure and loss of water.



The structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis.

In the IR spectra, characteristic N-H stretching band appeared at about 3200-3300 cm^{-1}. Ester and ketone C=O stretching bands were seen at 1700 and 1650, respectively. The stretching bands of both aromatic and aliphatic C-H appeared at expected values.

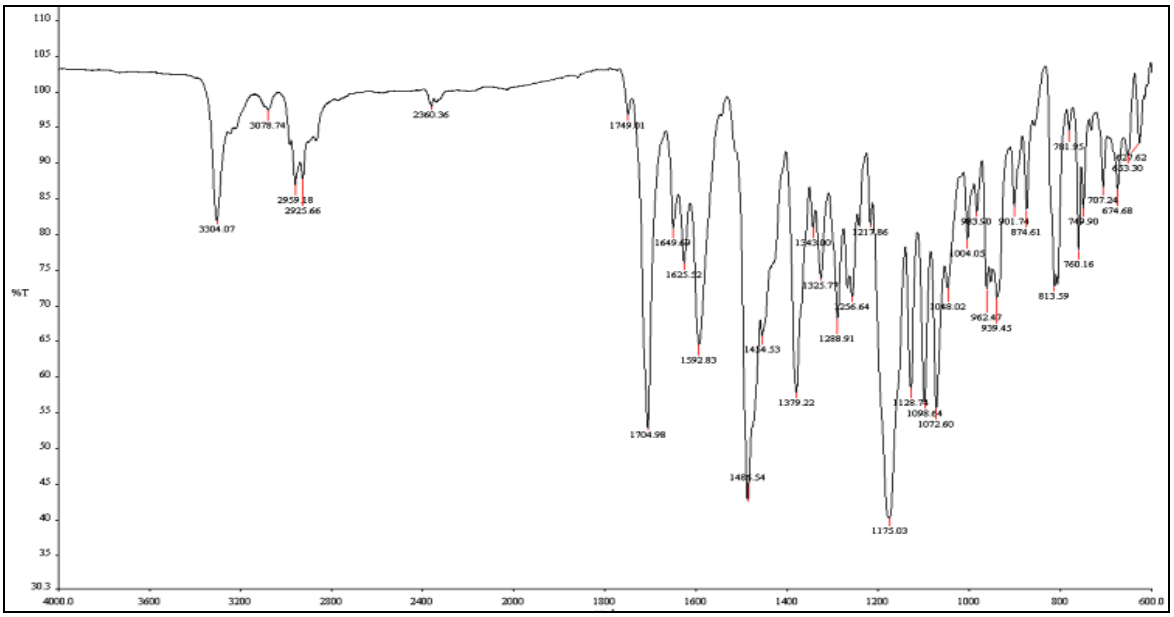


Figure 5.2. IR spectrum of the compound **4l**.

Also, the characteristic stretching band of cyano group, in the IR spectra of compounds **4g** and **4p** were seen at about 2229 cm^{-1}.

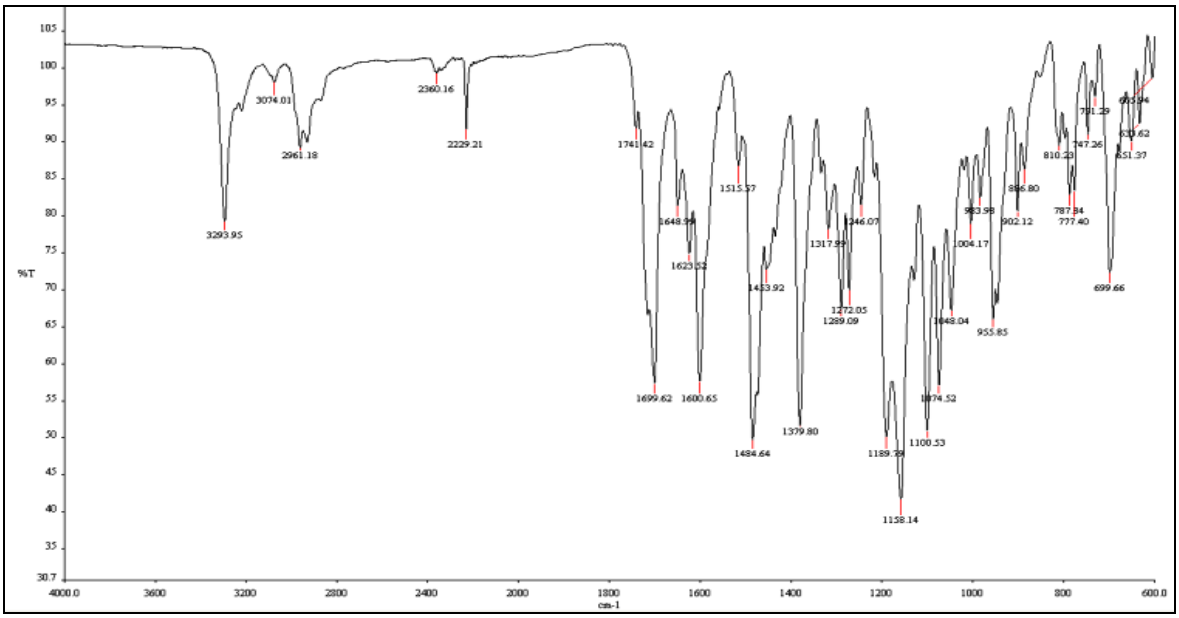
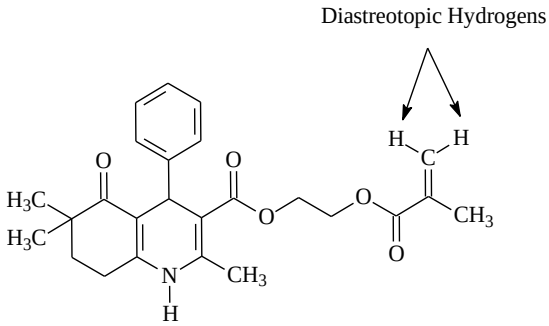


Figure 5.3. IR spectrum of the compound **4g**.

In the ^{1}H NMR spectra, HHQ 6,6-dimethyl protons were seen at 0.7- 0.9 ppm as separate singlet. The hydrogens of two methylene groups of the ester were seen as multiplet signals at about 4.00 ppm.

A singlet signal appeared at about 5.00 ppm, this signal is very characteristic for the hydrogen at C-4 of the HHQ ring.

Characteristic two signals of the diastereotopic methylidene protons of methacryloyloxy ester were seen at 5.6-5.9 ppm. They are not chemical shift equivalent because if each of them was replaced by a group, this gives compounds that are diastereomers.



The N-H proton signal was seen at 9-10 ppm. The chemical shifts of the aromatic and aliphatic protons had expected values.

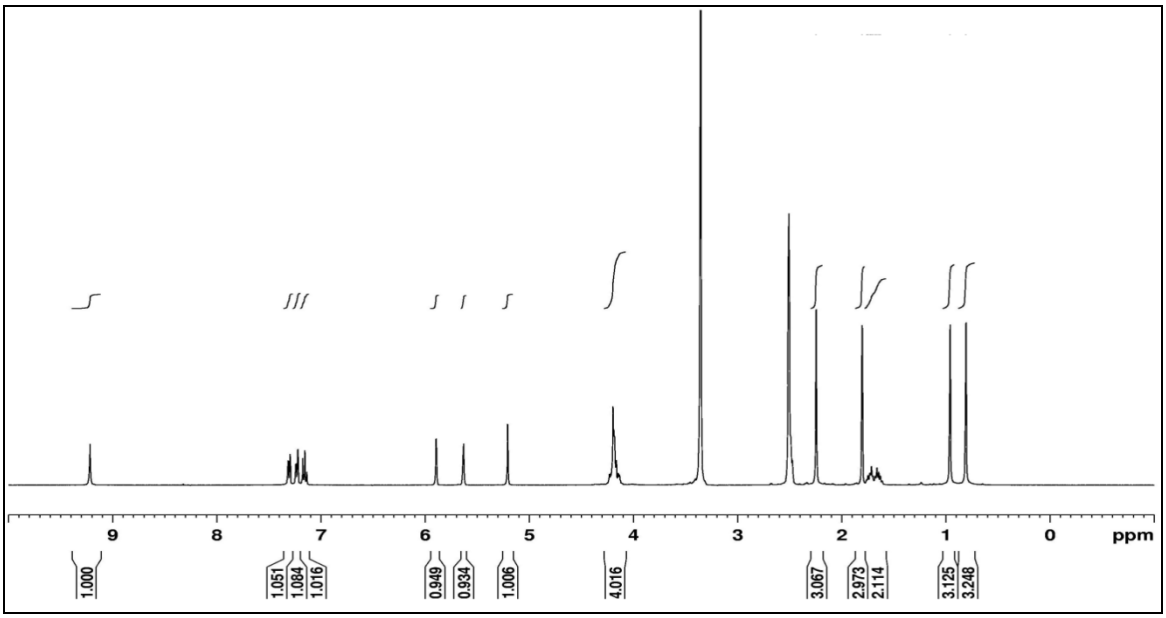


Figure 5.4. ^{1}H NMR spectrum of the compound **4h**.

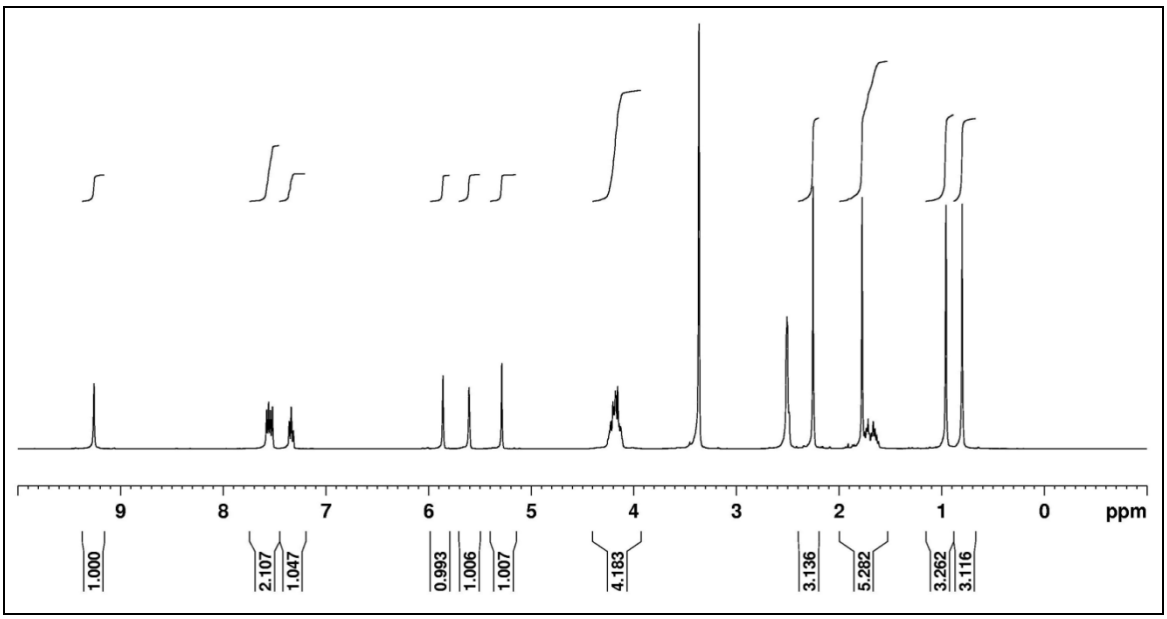
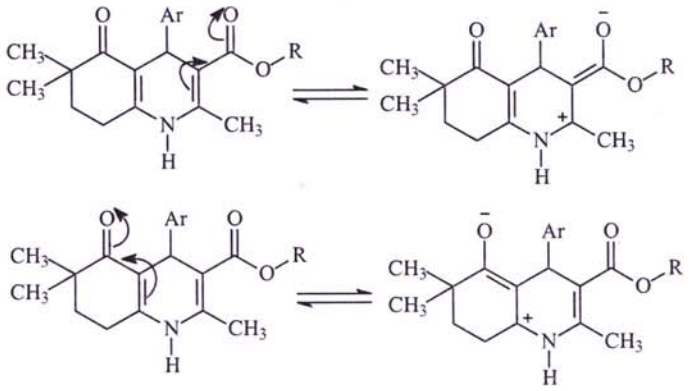


Figure 5.5. ^{1}H NMR spectrum of the compound **4q**.

The ^{13}C NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of non-equivalent carbon atoms.

HHQ carbonyl group appeared at about 199 ppm while ester carbonyl groups were seen close to166 ppm.

The effect of resonance and inductive effect of nitrogen atom cause a great difference in the chemical shift between sp^2 hybridized carbons of DHP ring, as the C-2 and C-6 were observed more deshielded at about 146 and 150 ppm, while C-3 and C-5 appeared at 102 and 109. The vinyl carbons of ester were seen at 126 and 136 ppm.



Two characteristic signals of aliphatic carbons attached to oxygens in methacryloyloxy ester (ester adjacent aliphatics) appeared at 63 and 61 ppm.

Characteristic signal of carbon of cyano group in compounds **4g** and **4p** appeared at about 119 ppm, while that of trifluoromethyl group in compounds **4q**, **4r** and **4s** was seen at about 122 ppm.

The other aromatic, aliphatic and π system carbons were seen at expected values.

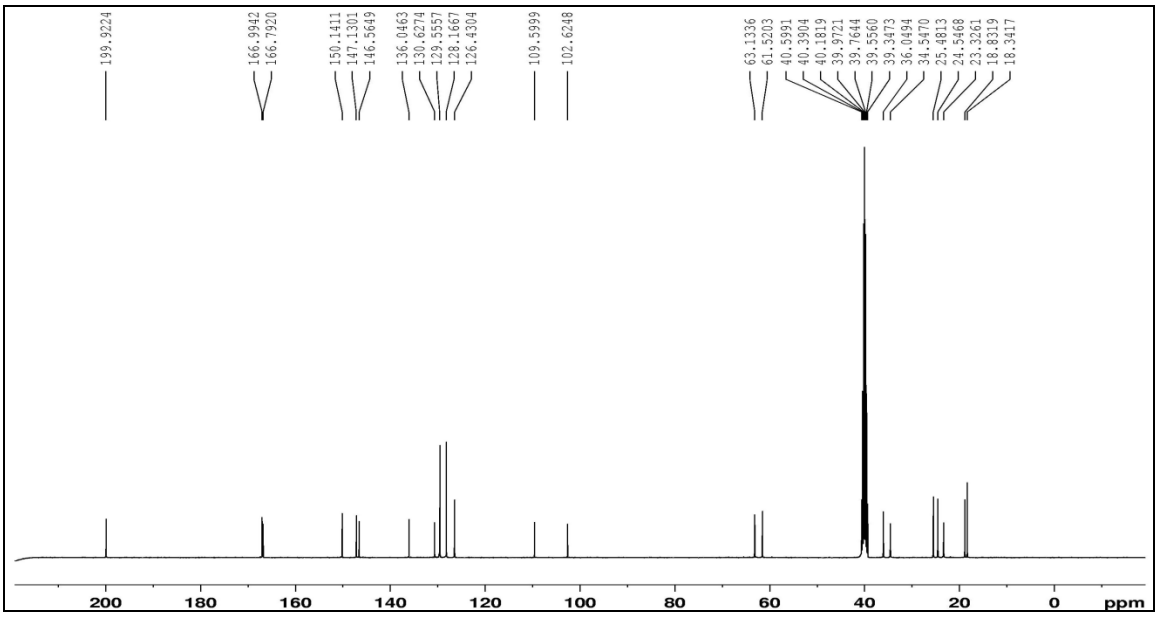


Figure 5.6. ^{13}C NMR spectra of the compound **4m**.

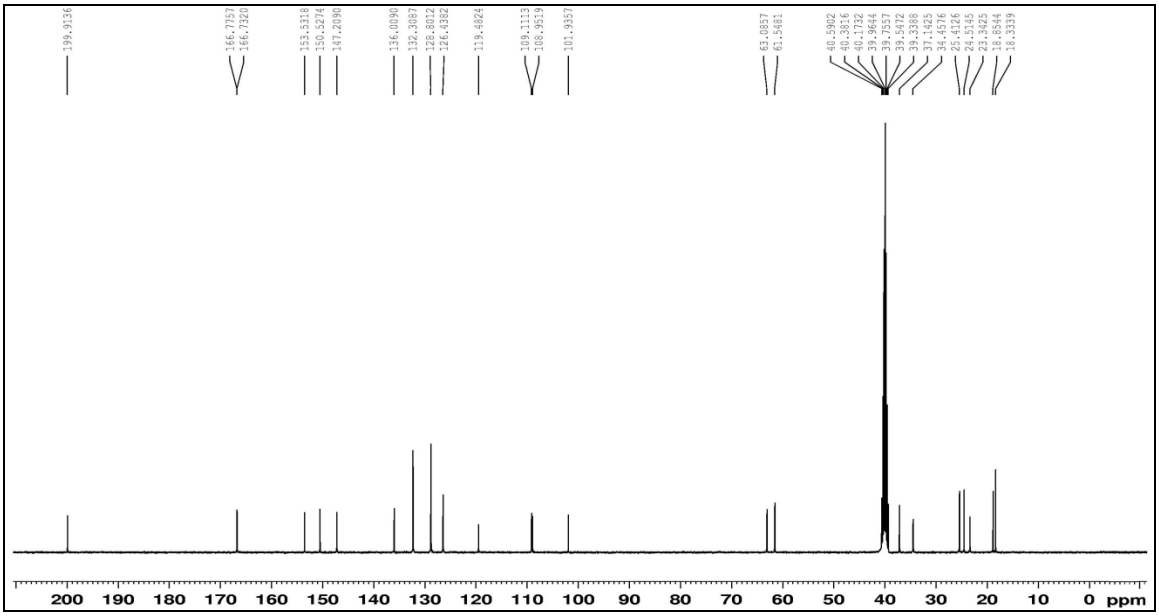
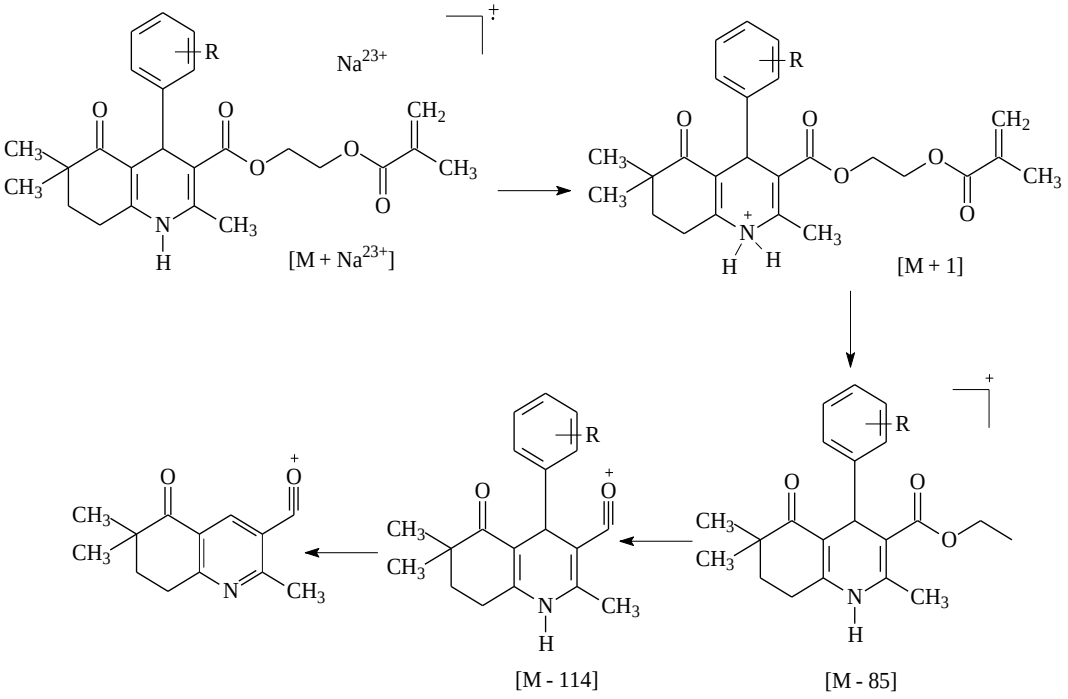


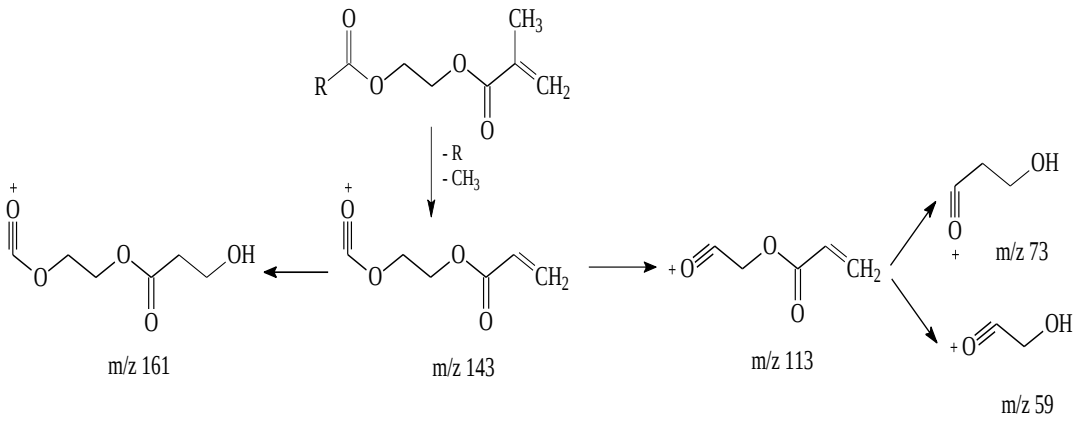
Figure 5.7. ^{13}C NMR spectra of the compound **4p**.

The mass spectra of the compounds were recorded using the electrospray ionisation technique. Molecular ion peaks $[M+Na]^+$ were seen in the spectra of all compounds due to sodium ion that was captured. Also, $[M+1]^+$peak was appeared in the mass spectra.

Ion is formed by the cleavage of the ester group that is followed by cleavage of the aryl ring from the parent molecule. Aromatization of the DHP ring to the pyridine analogue was also realized.



In further fragmentations, metacryloloxy ethyl ester was shown to be fragmented into ions that were recorded in mass spectra of the compounds.



The isotopic effect of chlorine atom appeared obviously in compounds **4a**, **4d**, **4m**, **4q** and **4r** that contain one chlorine atom as a $[M+Na+2]^+$ with relative ratios 66%, while in compounds **4h**, **4i** and **4j** that contain two chlorine atoms appeared as two peaks $[M+Na+2]^+$ and $[M+Na+4]^+$ with relative ratios 66% and 11%, respectively.

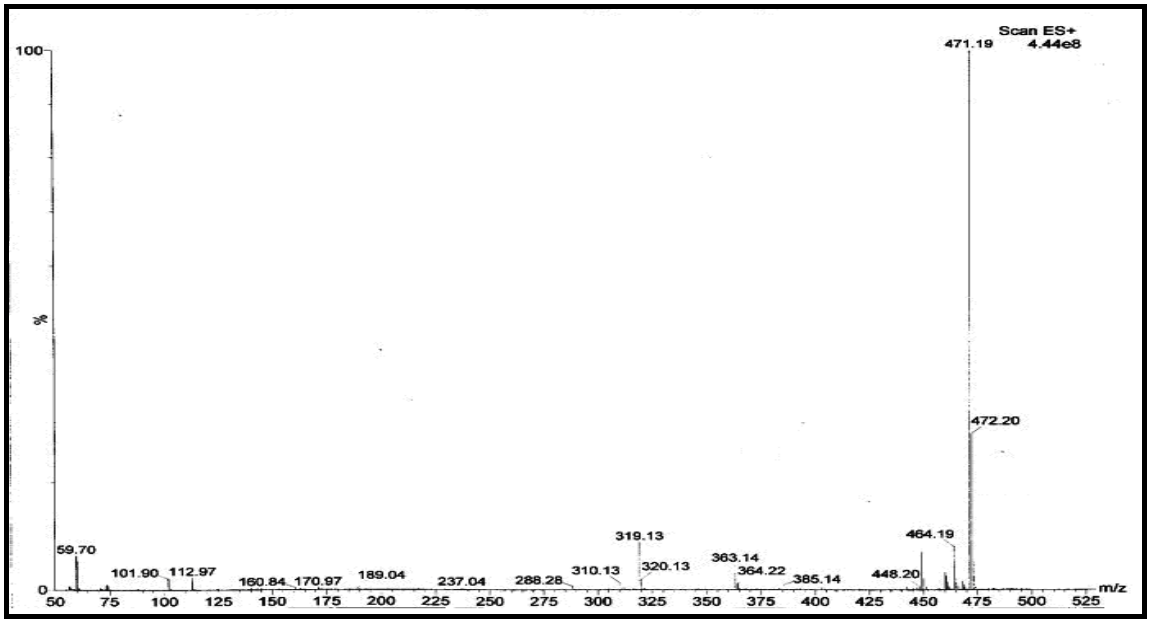


Figure 5.8. Mass spectra of the compound **4g**.

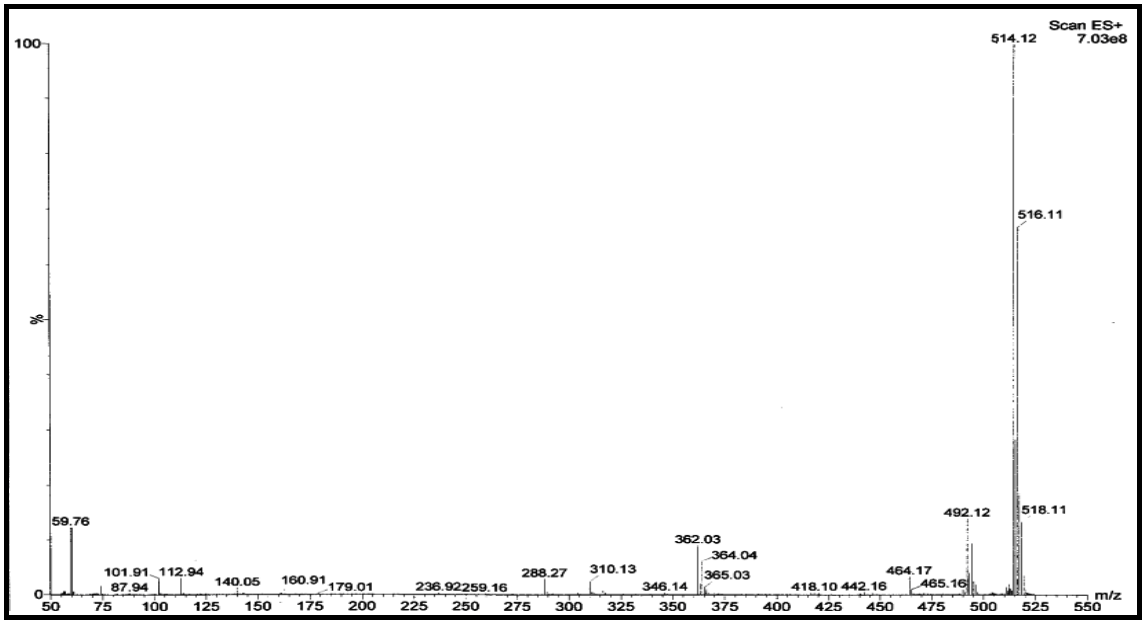


Figure 5.9. Mass spectra of the compound **4i**.

5.3. Myorelaxant activity

The maximum relaxant effects (E_{max}) and the negative logarithm of the concentration for the half-maximal response (pD_2) values of the compounds; and those of nifedipine on isolated strips of rabbit gastric fundus smooth muscle was determined.

The results indicate that the synthesized compounds and nifedipine exerted concentration-dependent relaxation responses on gastric fundus smooth muscle strips pre-treated with Ca^{2+} (2.5 mM) with the efficacy order: nifedipine>**4n**≥ **4f**≥ **4b** ≥ **4g** ≥ **4o** ≥ **4h** = **4e** = **4d**≥ **4q**≥ **4c** = **4l** ≥ **4p** ≥ **4r** = **4a** ≥ **4s** ≥ **4m**>**4k** ≥ **4j**>**4i**. Potency of compounds has been found less than nifedipine.

To eliminate the interference of other mechanisms that may induce relaxation by the interaction of test compounds with cyclooxygenase (COX) or K^+ channels; or via adrenergic or nitric oxide pathways, all experiments were done in the presence of indomethacin (COX inhibitor, 10^{-5}M), tetraethylammonium chloride (nonspecific K^+ channel blocker, 10^{-4}M), guanethidine (adrenergic nerve blocker, 10^{-6}M) and Nω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M). DMSO had no significant relaxant effect.

Thus, our results showed that these compounds had potency for relaxing isolated rabbit gastric fundus smooth muscle, possibly due to blockade of Ca^{2+} channels**.**To find the ability of the compounds to block Ca^{2+} channels, further investigation is needed.

5.4. Antioxidant activity

Coefficient of antioxidant activity (*K*) values of our compounds and that of nifedipine were calculated by using concentrations 0.2, 0.4 and 0.6 mg/ml of the synthesized compounds and the voltammogram of the supporting electrolyte was scanned each time to observe the proportional decrease of the oxygen current.

The results indicate that some of our tested compounds and nifedipine exhibited proportional decrease of the oxygen current while other showed an opposite effect.

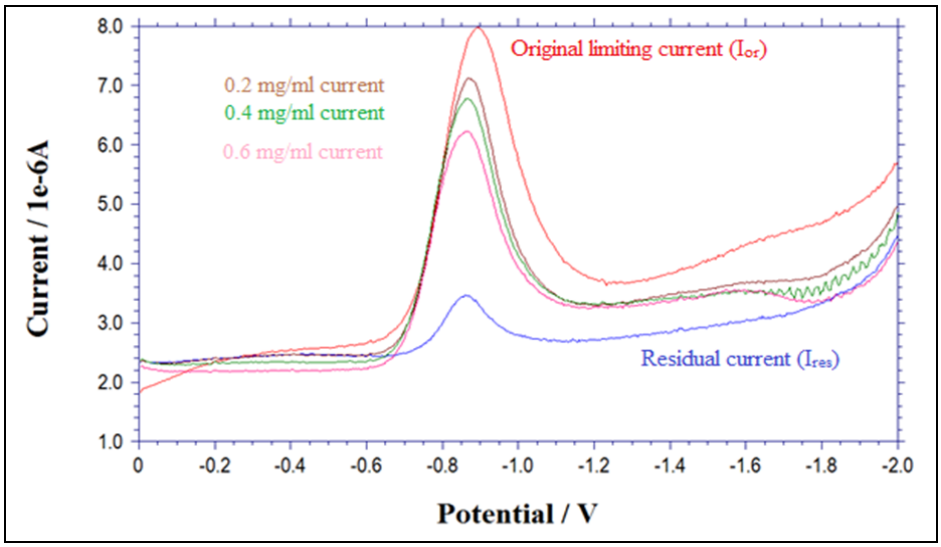


Figure 5.10. Voltammogram of the oxygen current for compound **4b**.

The order of the coefficient of antioxidant activity (*K*) values: **4f > 4h > 4i > 4n > 4b > 4d > Nifedipine > 4m > 4r > 4l > 4o > 4q > 4c > 4j > 4p > 4a > 4g >4e > 4s > 4k.**

When the relative change of the oxygen reduction current density ($j/(j_{or} - j_{res})$) values of the three concentrations of the tested compounds were plotted, it was found that the slope values of resulted straight lines were very near to the average coefficient of antioxidant activity (K_{Av}) value, that is indicate the accuracy of our results.

By comparing the average coefficient of antioxidant activity (K_{Av}) values of our tested compounds with that of other antioxidant agents that is already used in food and pharmaceutical industry or found naturally in vegetables and fruits such as: ascorbic acid (K value: 0.5), glucose (K value: 0.32), citric acid (K value: 0.18), and green tee extract (K value: 0.45); we will find that compound **4f** is a more potent antioxidant than ascorbic acid as it has a higher K value and compound **4h** is more efficient than green tea, while compounds such as **4i**, **4n** and **4b** are better than glucose in term of antioxidant activity. Also, compound **4d** shows higher K value than that of citric acid. All these compounds were efficient antioxidant agents than nifedipine.

6. CONCLUSION AND RECOMMENDATIONS

In this study, Ca^{2+} channel blocker ligands-based pharmacophore model was generated by LigandScout. Our generated model showed that used ligands have two common features can be added to the classical DHP calcium channel SAR requirements and can be considered during the design of new DHP calcium channel blockers, the two common features are hydrophobe and a positive ionizable feature.

Nineteen novel 2-(methacryloyloxy)ethyl-4-aryl-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives have been synthesized via multicomponent one pot Hantzsch reaction of 4,4-dimethylcyclohexane-1,3-dione, 2-(methacryloyloxy)ethyl acetoacetate, substituted benzaldehydes and ammonium acetate under microwave irradiation. Thus, provides an easier and faster method than classical methods. The structures of the synthesized compounds were proved by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy; and were verified by elemental analysis.

Two different biological activities were tested for our synthesized compounds: myorelaxant effect on isolated strips of rabbit gastric fundus smooth muscle by using nifedipine as standard; and antioxidant activity by differential pulse voltammetry technique.

The biological results showed that these compounds had potency for relaxing isolated rabbit gastric fundus smooth muscle, possibly due to blockade of calcium channels. To find the ability of Ca^{2+} channels blockage of these compounds further investigation is needed.

Also, some of our compounds exhibited good antioxidant activity; these compounds had coefficient of antioxidant activity value greater than that of some antioxidants prototypes such as ascorbic acid and green tea extract.

According to the results of our pharmacophore studies we suggest that condensed 1,4-DHPs system with long chain ester that carry hydrophobic group

provides a promoising scaffold for the Ca^{2+} channel modulators, and it is very advisable if we used an ester group that contain a positive ionizable atom like nitrogen and the hydrophobic group was phenyl.

Also, the use of microwave irradiation assisted synthesis with the aid of catalyst may result in improvement of reaction yield.

Furthermore, we suggest that it is advisable to find more accurate and selective biological method to determine the effect of the compounds on Ca^{2+} channels than classical methods that is conducted on tissues such as gastric fundus smooth muscles, cell-based electrophysiology may provide an excellent alternative method with high level of accuracy and selectivity.

The compounds that were shown to have higher antioxidant activity can be subjected to further investigations such *in vivo* experiments to evaluate their effects on the biological redox systems.

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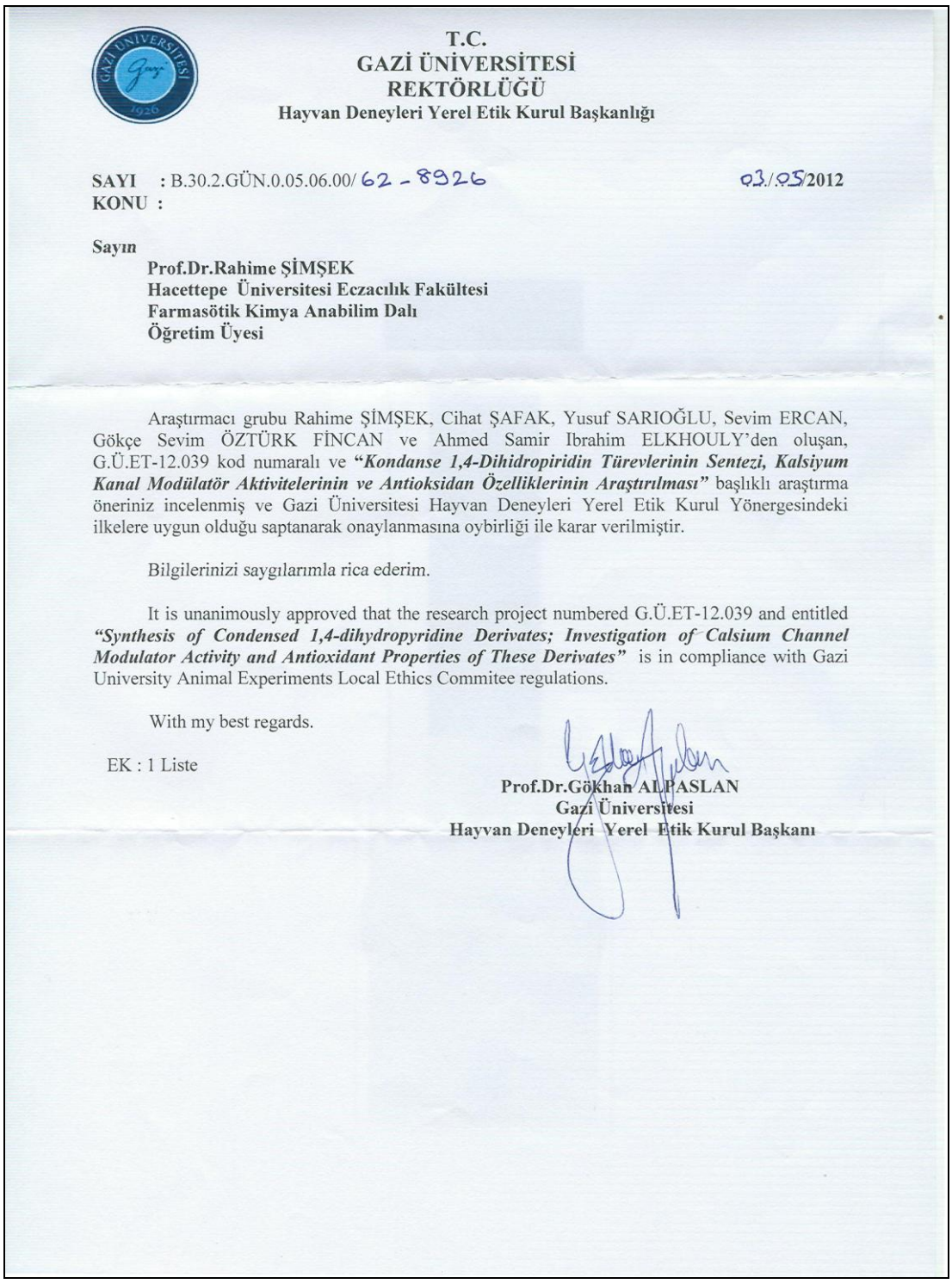
APPENDIXES

Appendix 1: Animal Experimentation Ethics Committee



T.C.
GAZİ ÜNİVERSİTESİ
REKTÖRLÜĞÜ
Hayvan Deneyleri Yerel Etik Kurul Başkanlığı

SAYI : B.30.2.GÜN.0.05.06.00/ 62 - 8926 03/05/2012
KONU :

Sayın
Prof.Dr.Rahime ŞİMŞEK
Hacettepe Üniversitesi Eczacılık Fakültesi
Farmasötik Kimya Anabilim Dalı
Öğretim Üyesi

Araştırmacı grubu Rahime ŞİMŞEK, Cihat ŞAFAK, Yusuf SARIOĞLU, Sevim ERCAN, Gökçe Sevim ÖZTÜRK FİNCAN ve Ahmed Samir Ibrahim ELKHOULY'den oluşan, G.Ü.ET-12.039 kod numaralı ve ***"Kondanse 1,4-Dihidropiridin Türevlerinin Sentezi, Kalsiyum Kanal Modülatör Aktivitelerinin ve Antioksidan Özelliklerinin Araştırılması"*** başlıklı araştırma öneriniz incelenmiş ve Gazi Üniversitesi Hayvan Deneyleri Yerel Etik Kurul Yönergesindeki ilkelere uygun olduğu saptanarak onaylanmasına oybirliği ile karar verilmiştir.

Bilgilerinizi saygılarımla rica ederim.

It is unanimously approved that the research project numbered G.Ü.ET-12.039 and entitled ***"Synthesis of Condensed 1,4-dihydropyridine Derivates; Investigation of Calsium Channel Modulator Activity and Antioxidant Properties of These Derivates"*** is in compliance with Gazi University Animal Experiments Local Ethics Commitee regulations.

With my best regards.

EK : 1 Liste

Prof.Dr.Gökhan ALPASLAN
Gazi Üniversitesi
Hayvan Deneyleri Yerel Etik Kurul Başkanı

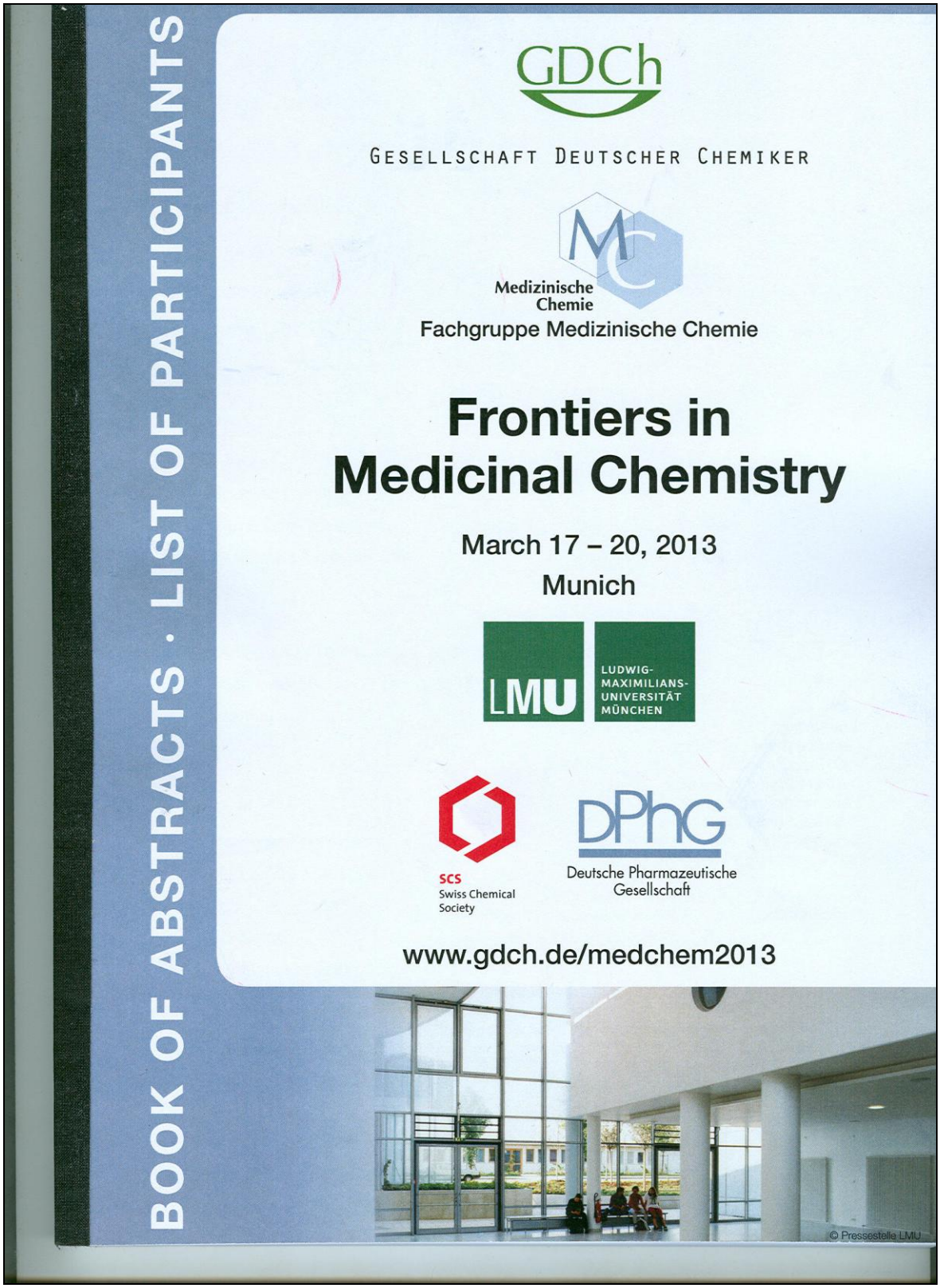
Follow **Appendix 1**

GAZİ ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU TOPLANTI KARARLARI KATILIM LİSTESİ

TOPLANTI TARİHİ : 25.04.2012	TOPLANTI SAYISI : 04
ADI-SOYADI	
Prof.Dr.Gökhan ALPASLAN	KATILDI
Prof.Dr.Aydan BABÜL	KATILAMADI
Prof.Dr.Nurten TÜRKÖZKAN	KATILAMADI
Prof.Dr.M.Tahir HATİPOĞLU	KATILDI
Prof.Dr.Mustafa ARK	KATILDI
Doç.Dr.Şule COŞKUN CEVHER	KATILDI
Uzman Dr.Şeyda DİKER	KATILDI
Arş.Gör. Esra PER	KATILDI
Dr.Kadir BAŞAR	KATILDI
İlknur ALKAN	KATILAMADI

Prof.Dr.Gökhan ALPASLAN
Gazi Üniversitesi
Hayvan Deneyleri Yerel Etik Kurul Başkanı

Appendix 2:


BOOK OF ABSTRACTS · LIST OF PARTICIPANTS
GDCh
GESELLSCHAFT DEUTSCHER CHEMIKER
Medizinische Chemie
Fachgruppe Medizinische Chemie
Frontiers in Medicinal Chemistry
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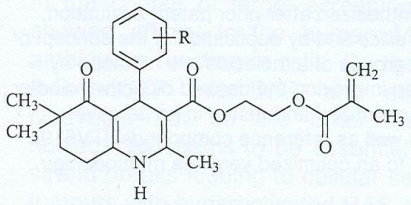
CA 03

Microwave-Assisted Synthesis and Biological Evaluation of Novel Condensed Dihydropyridine Derivatives as Calcium Channel Modulators

Elkhouly, A., Ankara/TR, Gündüz M. G., Ankara/TR, Şafak, C., Ankara/TR, Şimşek, R. Ankara/TR, Yıldırım, Ş. S., Ankara/TR, Öztürk Fincan, G. S., Ankara/TR, İşli, F., Ankara/TR, Ercan, S., Ankara/TR, Sarıoğlu, Y., Ankara/TR

Rahime Şimşek, PhD. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University 06100, Ankara, Turkey

1,4-Dihydropyridines (DHP) present a well-known class of calcium antagonists and are commercially employed for the treatment of cardiovascular diseases particularly hypertension and angina. [1] Many modifications have been carried out on the structure of nifedipine, the prototype of DHPs, in order to enhance calcium modulating effects. Among the performed modifications the introduction of bulky and lipophilic substituents as one of the esterifying groups led to new and very potent calcium antagonists. [2]



R: 2-Cl, 2-F, 2-NO_2, 3-Cl, 3-F, 3-NO_2, 3-CN, 2,3-diCl, 2,4-diCl, 2,5-diCl, 2,4-diF, 2,5-diF, 4-Cl, 4-F, 4-NO_2, 4-CN, 2-Cl 3-CF_3, 2-Cl 5-CF_3, 2-F 3-CF_3

In this study new 2-(methacryloyloxy)ethyl-4-aryl-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives were synthesized under the irradiation of microwave in order to develop a method that has the advantage of good yield and short reaction time. The structure of the synthesised compounds was proved by spectral analysis. The calcium antagonistic activities of the compounds were evaluated by determination of maximum relaxant effects (*E*max) and the negative logarithm of the concentration for the half-maximal response (pD_2) values of on isolated strips of rabbit gastric fundus smooth muscle by using nifedipine as standard.

Literature:

[1] Edraki, N., et al, *Drug Discov. Today*, **2009**, *21/22*, 1058-1066. [2] Miri, R., et al, *Bioorg. Med. Chem.*, **2006**, *14*, 4842-4849.

Appendix 3: Curicullum Vitae

Born 1981 in Monofiya, Egypt. Studied primary, secondary and high school in Monofiya. Joined the Faculty of Pharmacy at Zagazig University in 1998 and graduated in 2002. In 2003 started to work at National Organization for Drug Control and Researches (NODCAR), Cairo, Egypt. In 2009 finished my M. Sc. studies at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University.