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Scuola di Dottorato di Ricerca in
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Design and Synthesis of Heterocyclic Compounds as CB_2 Selective Agonists

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Introduction

Description

Cannabis is a genus of flowering plant that includes one or more species. The plant is believed to be indigenous to Central Asia, China, and the north-west Himalayas. The common name for *Cannabis* is hemp, although this term is sometimes used to refer only to strains cultivated for "industrial" (non-drug) use. *Cannabis* plants produce a unique family of compounds called cannabinoids, several of which produce psychical and/or physiological effects when consumed. The crude drug usually comes in the form of dried flowers and leaves, resin (hashish), or various extracts. The cultivation or possession of *Cannabis* for drug purposes is outlawed in most countries.

Figure 1

Cannabis is an annual, dioecious, flowering herb. The leaves are palmately compound, with serrate leaflets. The first pair of leaves usually have a single leaflet, the number gradually increasing up to a maximum of about thirteen (usually seven or nine), depending on variety and growing conditions. At the top of a flowering plant, this number again diminishes to a single. The lower leaf pairs usually occur in their opposite leaf arrangement

Figure 2

Taxonomy

The genus *Cannabis* was formerly placed in the Nettle (Urticaceae) or Mulberry (Moraceae) family, but is now considered along with hops (*Humulus* sp.) to belong to the Hemp family(Cannabaceae).Various types of *Cannabis* have been described, and classified as species, subspecies, or varieties:

- plants cultivated for fiber and seed production, described as low-intoxicant, non-drug, or fiber types
- plants cultivated for drug production, described as high-intoxicant or drug types
- escaped or wild forms of either of the above types.

Cannabis plants produce a unique family of terpeno-phenolic compounds called cannabinoids, which produce the "high" one experiences from smoking marijuana. The two cannabinoids usually produced in greatest abundance are cannabidiol (CBD) and/or Δ^9-tetrahydrocannabinol (THC), but only THC is psychoactive.

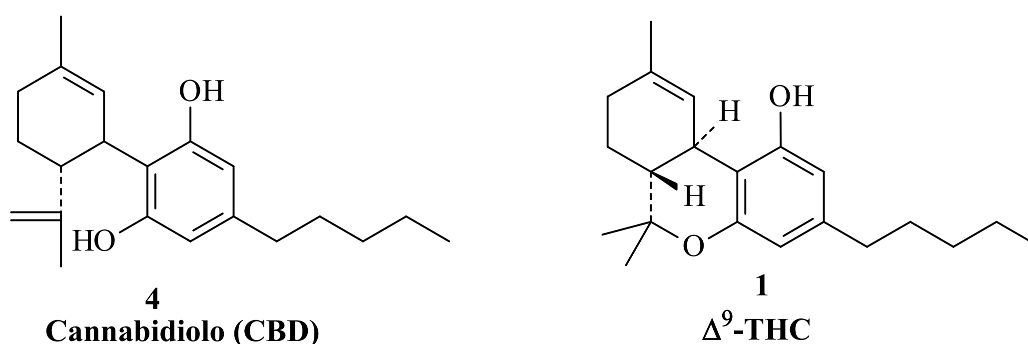
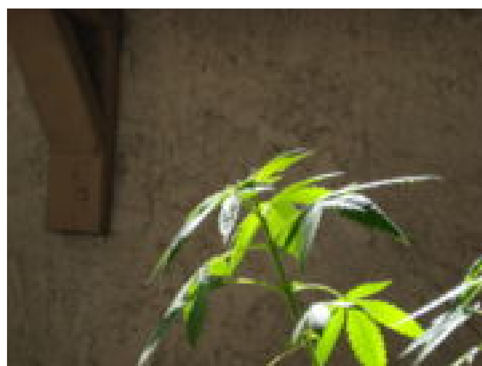


Figure 3

Since the early 1970's, *Cannabis* plants have been categorized by their chemical phenotype or "chemotype," based on the overall amount of THC produced, and on the ratio of THC to CBD. Although overall cannabinoid production is influenced by environmental factors, the THC/CBD ratio is genetically determined and remains fixed throughout the life of a plant. Non-drug plants produce relatively low levels of THC and high levels of CBD, while drug plants produce high levels of THC and low levels of CBD. When plants of these two chemotypes cross-pollinate, the plants in the first filial (F_1) generation have an intermediate

chemotype and produce similar amounts of CBD and THC. Female plants of this chemotype may produce enough THC to be utilized for drug production.



Top of *Cannabis* plant in vegetative growth stage.

Figure 4

Popular usage

The National Institute of Drug Abuse defines drug abuse as "the non-medical use of a substance for psychic effect, dependence, or suicide attempt.

Marijuana (also known as ganja) and hashish are psychoactive products of the plant *Cannabis sativa* L. subsp. *indica* (= *C. indica* Lam.). The herbal form of the drug consists of dried mature inflorescences and subtending leaves of pistillate ("female") plants. The resinous form consists primarily of glandular trichomes collected from the same plant material. It has been reported that commercial hashish is often less potent than high quality seedless marijuana.[1] However, carefully produced and screened hashish is at least three times as potent as the highest quality herb.[2] The major biologically active chemical compound in *Cannabis* is THC. It has psychoactive and physiological effects when assumed, usually by smoking or ingestion. The minimum amount of THC required to have a perceptible psychoactive effect is about 5 mg. A related compound, Δ^9-tetrahydrocannabidivarin, also know as THCV, is produced in appreciable amounts by certain drug strains. This cannabinoid has been described in the popular literature as having shorter-acting, flashier effects than THC, but recent studies suggest that it may inhibit the effects of THC. Relatively high levels of THCV are common in African dagga (marijuana), and in hashish from the northwest Himalaya

The nature and intensity of the immediate effects of cannabis consumption is related to the dose, the species or hybridization of the source plant, the modality of consumption, the user's psychical and physical characteristics (such as tolerance), and the environment of consumption. This is sometimes referred to as set and setting. Smoking the same cannabis

either in a different frame of mind (set) or in a different location (setting) can alter the effects or perception. What the user does under the influence can also affect the effects of cannabis. For example, if the user does nothing they will feel relaxed and sleepy, whereas if they engage in intense physical or psychical activity they will feel energised. Effects of cannabis consumption may be loosely classified as cognitive and physical. Anecdotal evidence suggests that drug varieties of *Cannabis sativa* subsp. *sativa* tend to produce more of the cognitive or perceptual effects, while *C. sativa* subsp. *indica* tends to produce more of the physical effects.

ECS (endocannabinoid system)

The endocannabinoid system includes cannabinoid receptors, their endogenous ligands, the anandamide transporter protein and two enzimes, fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL).[3]

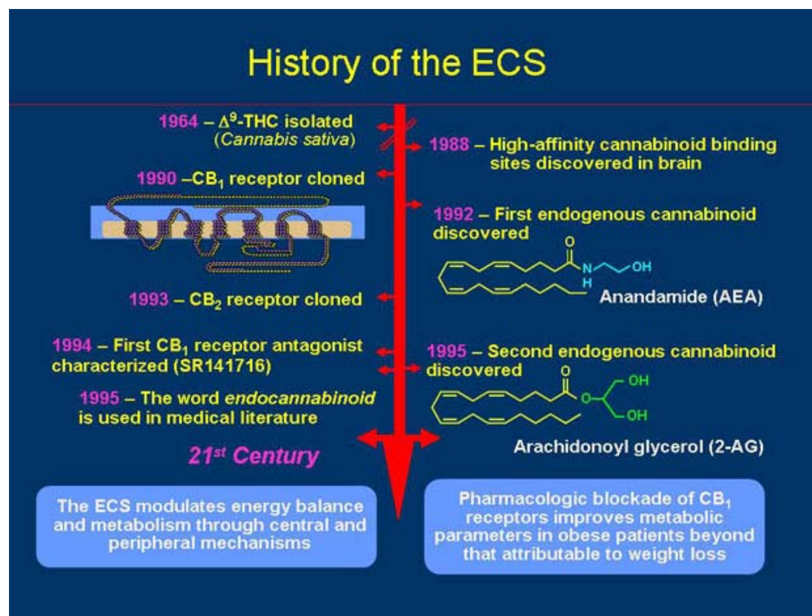


Figure 5

Cannabinoid receptors

Cannabinoid receptor types are denoted by the abbreviation CB and numbered in the order of their discovery by a subscript. At present, two cannabinoid receptor types have been unequivocally identified, named CB_1 and CB_2. The CB_1 receptor is the most abundant G protein-coupled receptor expressed in the brain. Especially in the hypothalamus, pituitary gland and in the mesolimbic dopamine circuits. It has detected not only in neurones but also in astrocytes and microglial cells. It is also found in a variety of peripheral tissues such as adipose tissue, liver, muscle, the gastrointestinal tract, pancreas, urinary bladder, lung, adrenal gland, testis, ovary, uterus and prostate and in rat adipose tissue. The CB_2 receptor is limited essentially to the cells associated with the immune system, like spleen, thymus, and tonsils.[4] CB_2 receptors were found to be expressed in brain microglial cells under inflammatory conditions,[5] and recent studies using human neutrophils indicate that the CB_2 receptor may suppress neutrophil migration during inflammation. It is also located in retina[6], skin[7] and some malignant cells.[8]

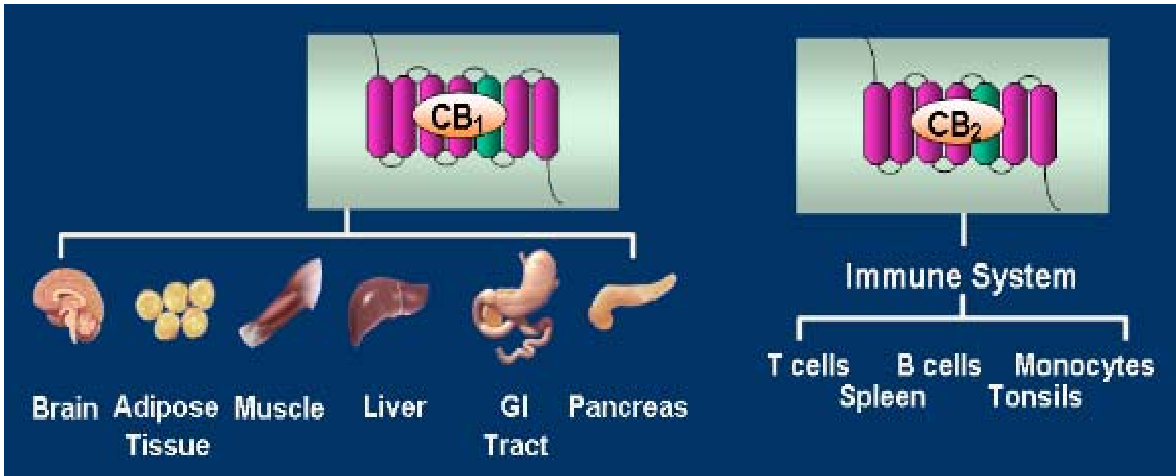


Figure 6

The human CB_1 and CB_2 receptors exhibit 68% identity within the transmembrane regions, 44% identity throughout the whole protein. A new putative cannabinoid receptor gene has been recently identified in the invertebrate *Ciona intestinalis* that share 28% sequence identity with the human CB_1 and 24% sequence identity with the human CB_2.[9]

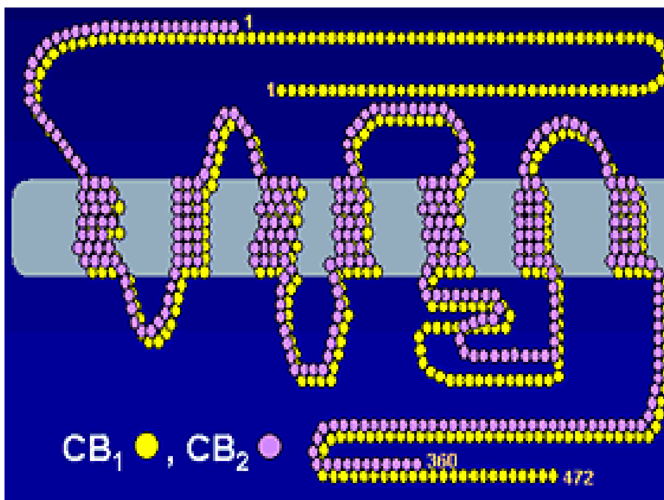


Figure 7

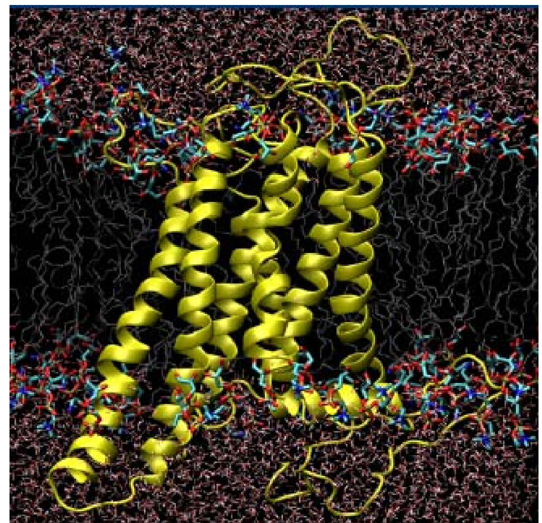


Figure 8

Endogenous ligands of cannabinoid receptors

The individuation of endogenous ligands for these receptors has been yielded after cannabinoid receptors discovery. The endogenous ligand of the CB_1 receptor is anandamide whereas the endogenous ligand of the CB_2 is 2-arachidonilglicerolo (2-AG) (Figure 9). They are synthesized from membrane-derived phospholipids whose biologic effects are mediated through coupling with the specific, widely expressed ECS receptors located presynaptically.[10] The synthesis of anandamide is Ca^{++}-dependent and is produced locally by the phospholipase D-mediated cleavage of the membrane precursor called N-arachidonoyl-phosphatidylethanolamine. While the synthesis of 2-arachidonilglicerolo is produced by the DAG lipase cleavage of the membrane precursor. Because endocannabinoids are lipophilic compounds derived from membrane phospholipids, they do not need to be stored in synaptic vesicles like other neurotransmitters.[11] In the brain, they are produced by neurons at their sites of action and act on demand, generating a transient, rapid effect before being hydrolyzed and inactivated by FAAH.[11, 10] Because of their lipophilic nature and the mechanism of their synthesis and release, endocannabinoids are considered as local neuromodulators.[12]

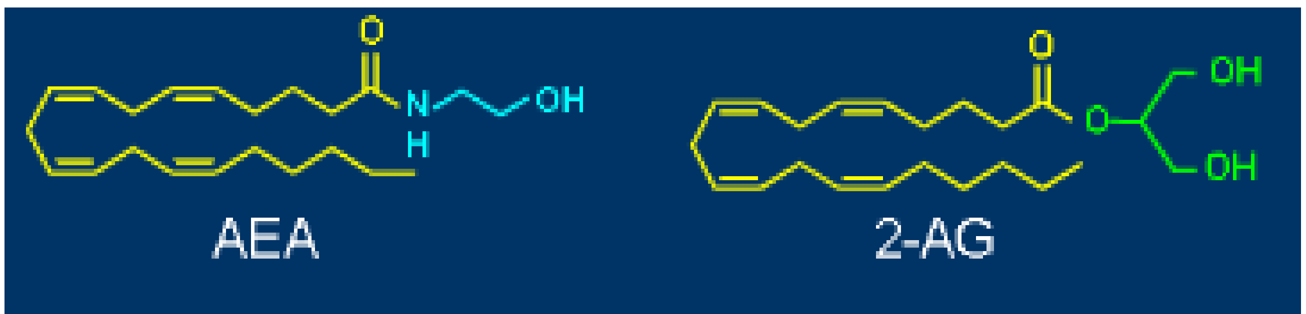


Figure 9

Signal transduction activated by cannabinoid receptors

The protein G, which is coupled to receptor, is constituted by the α and βγ subunits and the G protein (Gi/Go) associated to cannabinoid receptors is sensible to pertussis toxin. The affinity of CB_1 and CB_2 for G_i or G_o proteins may be different as revealed by several studies on cannabinoid ligand binding or regulation of [^{35} s] GTPγS binding. Whereas, activation of both of them display a high affinity for G_i, agonist stimulation of CB_1 also result in a high-affinity saturable interaction with G_0 but CB_2 receptor do not interact efficiently with G_0. It has been reported that the affinity of CB_1 receptor for G_0 is ten fold higher than that of the CB_2.[13] It has been described that the juxtamembrane C-terminal region of CB_1 receptors (amino acids 401-417) and the second and third intracellular loops are critical for $G_{i/o}$ protein coupling and that the distal C-terminal tail domain profoundly modulates both the magnitude and kinetics of signal transduction.[14] In the CB_2 receptor it has been described the existence of two cysteins, C313 and C320, that are located in this C-terminal region, that may play important roles for receptor- G protein coupling and receptor desensitization. Also, the third transmembrane domain in the CB_2, particularly the Asp-Arg-Tyr motif, may be crucial for interacting with G proteins because mutations of highly conserved aspartate residues in the second tranmembrane domain receptors have also been described to disrupts G-protein coupling. Some effects are independent by interaction with G proteins. The former are negative regulation of adenylate cyclase. An effect mediated by G protein is inhibition of calcium channels. In fact cannabinoid agonists reduce the amplitude of voltage-gated calcium currents in neuronal cells through $G_{i/o}$ proteins. Cannabinoids also modulate K^+ channels and cannabinoid as THC cause a depression of inward sodium current determining a depression of action potentials.[15,16] CB_2 receptor seem to be independent of channel activation.

GPCRs (G protein-coupled receptors) can stimulate the protein kinase (MAPK) cascade and induce cellular proliferation. The mammalian MAPK family consists of three subfamilies with multiple members: the extracellular signal-regulated kinases (ERK), the Jun amino-terminal kinases/stress-activated kinases (JNK/SAPK), and the p38 MAPKs. While ERK is involved in regulation of cell division and growth, the other two subfamilies are activated by stress signals and inflammatory cytokines and have been related with cellular death and immune disorders.

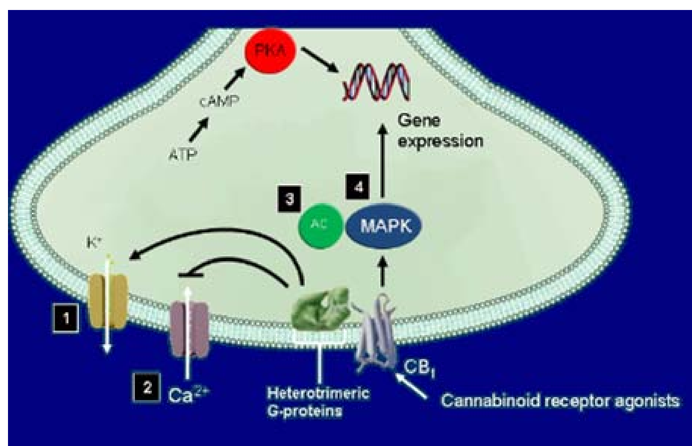


Figure 10

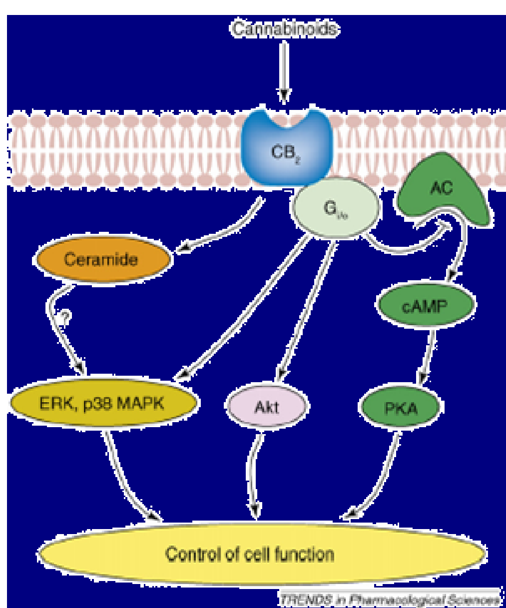


Figure 11

Biological roles of endocannabinoids system

The endo-cannabinoid system is implicated in two major biological roles: modulation of neurotransmitter release and modulation of immune functions. Cannabinoids has been demonstrated to inhibit the evoked release of Ach, GABA, NA, DA, 5-HT, Glu, Gly, d-Asp, and CCK.[17] The effect of cannabinoid on the function of immune cell is still unclear and requires elucidation but they produce a deleterious effect on the immune response causing the impairment of macrophage functions, perturbation of immunoglobulin production and down-regulation of immune cells activity. It has been evidenced that THC can suppress the human immune response in a host of leukocyte subsets and alveolar macrophages. The degranulation of mast cells induced by substance P is fully abrogated by the endogenous ligands at

cannabinoid receptors. The mechanism by which CB_2 ligands modulate mast cell activation is by generating NO and PGE_2. It is involved in the controlling vascular homeostasis[18] and synaptic transmission.[19] NO can be induced by proinflammatory factors under pathological conditions. NO production is stimulated by anandamide in human monocytes. This stimulation could explain some cannabinoids effects like vasodilatation and neurotransmission release inhibition. Cannabinoids could be used as therapeutic agents in NO-mediated inflammation leading to neurodegeneration. In addition AEA and exogenous cannabinoids induce arachidonic acid mobilization and activation of the enzymes of arachidonic acid cascade in many cells. It is important to note that AEA degradation into cells by fatty acid amide hydrolase (FAAH) yield arachidonic acid that could mediate some biological actions of endocannabinoids like vasorelaxation but, in this case, the effect is independent of cannabinoid receptors.[20] (Figure 12)

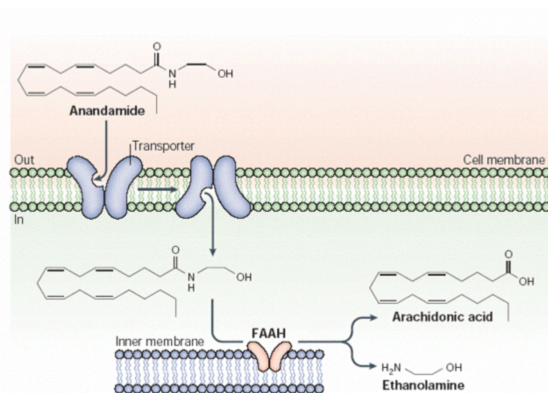


Figure 12

Three enzymes are involved in the release of arachidonic acid from membrane phospholipid domain: phospholipase D, phospholipase C and phospholipase A_2. The three enzymes have been shown to be activated by cannabinoids. PLD cause phospholipid hydrolysis yielding arachidonate containing diacylglicerols generation that, after hydrolysis by DAG lipases, may release arachidonico acid. The primary pathway is represented by activation of PLA_2 which liberate arachidonic acid from phospholipids. The main mechanism of cytosolic PLA_2 activation is phosphorylation by MAP kinase. The release of arachidonic acid induced by THC in hippocampal neurones can be blocked by the CB_1 receptor antagonist SR141716 but not by pre-treatment with pertussis toxin, suggesting an involvement of cannabinoid receptor by a mechanism independent of Gi coupling. It has been recently shown that cannabinoids may modulate arachidonate metabolic enzymes. In human platelets THC inhibit COX-2 activity blocking the synthesis of its pro-inflamatory metabolites with the redistribution of

products towards lipoxygenase pathway.[21] In human neuroglioma cells THC and methanandamide stimulated COX-2 mRNA expression and subsequent PGE2 synthesis via a non cannabinoid receptor-mediated mechanism.

Role of cannabinoids

For over 4000 years, *Cannabis sativa L* extract have been widley used as recreazional drug and as therapy for a variety of disorders. The discovery of psychoactive principle of *Cannabis sativa,* delta 9-tetrahydrocannabinol initiated research into the physiological role of cannabinoids. In the last years our understanding has been modified significantly by new discovery.

Cannabinoids are best know for their effects on CNS functions. They produce euphoria, alterations in cognition and analgesia, have anticonvulsivant properties and affect temperature regulation, sleep and appetite. However, cannabinoids also possess immunomodulatory activity and anti-infiammatory properties. Many disease of the CNS including Alzeimer disease, Parkinson's disease, AIDS dementia and mainly multiple sclerosis (MS) involve inflammation, and cause an upregulation of cytochines and other infiammatory mediators in the CNS; Therefore cannabinoids may be potential therapeutic agents in neurological diseases.

Recent studies revealed mainly role of cannabinoids in:

Cognition: the most prominent among the various consequences of CB_1 receptor activation by exogenous agonist are disrupotive effects on woking meoty, i.e. on processes necessary to learn and react to new information that differs from session to session.

Emotionally: blockade of CB_1 receptors by SR141716A caused an increase in anxiety-related behaviour. [22, 23, 24] In contrast , lower doses of SR141716A had no effects.[25] Data obtained in mice were more inconsistent. Administation of SR141716A either decreased[26] or increased anxiety-related behaviour, depending on the genetic background of animals and the test situation.

Multiple sclerosis (MS): is the most important chronic inflammatory demyelinanting disorder of the CNS. The cannabis and cannabinoid agonist may be effective in ameliorating symptomatology of MS, especially spasticity and pain. The benefical effects of cannabinoids agonist the symptomatology associated with chronic inflammatory demyelinnanting pathologies, may be exerd at multiple levels: by improving motor function, by limiting neuroinflammation, by promoting remyelination.

Immune modulation: the cannabinoid CB_2 receptor is expressed abundately in various types of inflammatory cells in particolary in B cells. The CB_2 receptors has been associated with most of immunomodulatory activity of cannabinoids, but also CB_1 may be linked to cannabinoid-mediated alterations of immune cell reactivity. Cannabinoids exibit immunosopressive properties.

Appetite and energy regulation: the ECS is postulated to connect the physical and emotional responses to stress with appetite and energy regulation (general stress-recovery system). Stimulation of the ECS may possibly occur as a consequence of obesity, leading to increased levels of endocannabinoids, which disrupt the feedback mechanism involved in energy balance. The ECS affects energy balance, glucose homeostasis, and lipogenesis because of the cannabinoids receptors are located in the adipose tissue, the liver, the pancreas and the skeletal muscle (Figure 13).

Obesity: activation of the ECS increases food intake and promotes weight gain. The blockade of the CB_1 receptor reduces body weight in animals through central and peripheral action.

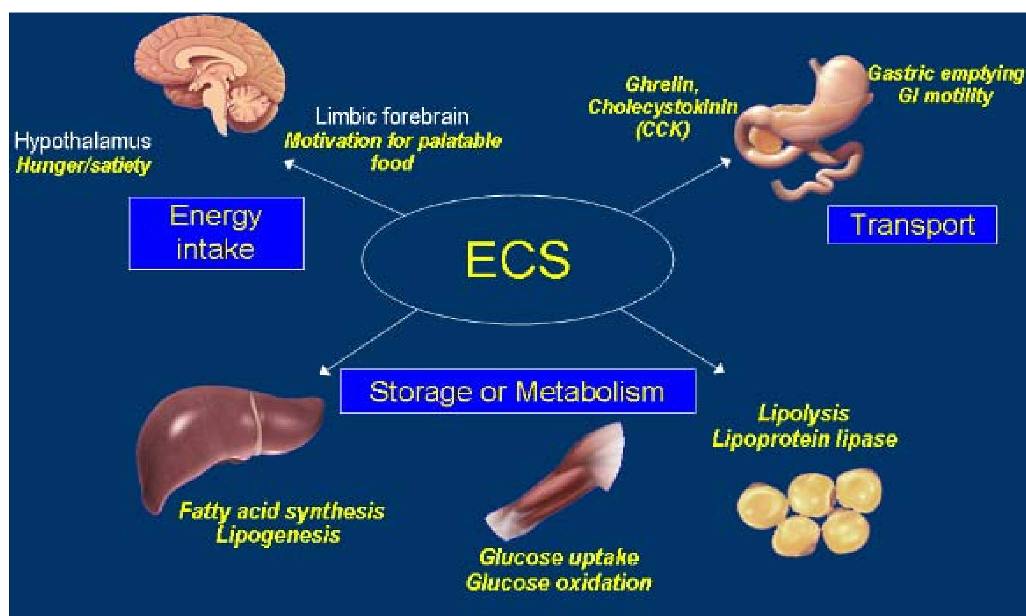


Figure 13

Other Functions of the ECS

The ECS appears to play an important role in the regulation of hormonal balance through actions on endocrine axes. In general, the ECS has been shown to inhibit hypothalamic-pituitary-adrenal functions and to modulate fertility. Recent data demonstrate that FAAH protein expression and activity varies as a function of the mouse estrus cycle[27] and that CB_1 receptor expression and function correlate with larval organogenesis of *Xenopus laevis.*[28] In rats, the CB_1 receptor has been shown to mediate cardiopressor and vasodilator effects of endogenous AEA[29] and in mice AEA was shown to modulate cough sensitivity.[30] Other functions of the ECS in normal physiology may be related to immune modulation, neuroprotection, and bone mass regulation.[31-37] Taken together, these data indicate the ECS has pleiotropic functions with important implications for human physiology.

Pharmacological role of CB_2 receptors

The physiological and putative therapeutic potential of the CB_2 receptor largely remains unexplored; however, recent data indicate that CB_2 cannabinoid receptors participate in the control of peripheral pain, inflammation, osteoporosis, growth of malignant gliomas, tumors of immune origin, and immunogical disordes such as multiple sclerosis. Recent studies have now implicated CB_2 receptors in neuroprotective activity of cannabinoids, mainly through a series of glia-dependent anti-inflammatory actions.[7]

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (asl), is a neurodegenerative disease characterized by rapid progressive degeneration of motor neurons in the brain and spinal cord, paralysis and death within 2-5 years   from the diagnosis.[38] Asl is the third most common neurodegenerative cause of adult death, after Alzheimer' s disease and Parkinson's disease.[39] Most cases of asl are sporadic and are probably acquired, while approximately 10% are familial. Despite a variety of putative underlying oxidative stress, neuroinflammation, autoimmunity, a defect in neuronal glutamate transport, glutamate toxicity and mutations of superoxide dismutase gene.[40] Recent evidence indicates that asl is a disease characterized by chronic inflammation.[41, 42] Microglia are the resident macrophages of CNS and in response to CNS injury microglia quickly convert to on "active" state during which they change to on amoeboid shape, up-regulate the cell-surface expression of a variety of surface antigens and

secrete several pro-inflammatory molecules[43] including interleukin-1β, tumour necrosis factor-α, nitric oxide,[43] oxygen radical, glutamate, proteases and contribute to the pathogenesis of neurologic disorders. The microglial activation in the CNS suggest a primary neuroinflammatory state with deleterious effects on surrounding neurons.[44] Recent study reported elevated levels of CB_2 receptors in microglia isolated from post-mortem human spinal cord of ASL patients[45] moreover recently in vitro studies demonstrate that CB_2 receptors are up-regulated in microglia in response to inflammatory stimuli[46] and that CB_2 agonist suppress microglial activation.[47] In 2005 Jared Ehrart et al showed that the CB_2 agonist JWH 015 inhibits INF-γ- induced microglial CD40 expression. They proved that when CB_2 is stimulated by the presence of JWH 015 the pro-inflammatory molecules were significantly reduced. Recent Studies demonstrate that treatment at onset with the non selective CB_1/CB_2 agonist Win 55-212 produces a significant rightward shift in the survival curve on G93A mice reflected by an increase of 8.8 days in the survival interval. Administration of the selective CB_2 agonist AM1241 results in a highly significant extension of survival with mice living 56% longer after symptom onset than controls. These results suggest that CB_2 agonist may be effective as pharmacological agents with several distinct advantages for the management of this devastating disease.[48]

Pancreatic cancer

Pancreatic cancer is one of the most malignant and aggressive forms of cancer.[49] About 95% of pancreatic cancers cases are ductal adenocarcinomas. The anatomic localization of the pancreas and the non-specific nature of the symptoms result in a complex and delayed diagnosis. The 85% of patients show metastasic infiltrations in proximal lymphatic nodes, liver, or lungs, and only 15% to 20%[49] of the tumors are typically found rescetable. <20% of the operated patients survive up to 5 years. However the median survival for the affected patients remains about 1 years. The department of biochemistry and molecular biology I of university of Madrid and institute national de la Sante et de la Recherche Medical of France investigated the effect of cannabinoids on pancreatic tumor cells. First, they determined the expression of cannabinoid receptors in four different human pancreatic tumor cell lines: Panc 1, MiaPaCa 2, Capan 2 and BxPc 3 cell lines. In these cells, mRNA for cannabinoid receptors, was expressed whereas mRNA levels for these receptors were very low in normal pancreatic tissue.[50] This difference was confirmed by immunofluorescence analysis of CB receptors both in human biopsies from pancreatic cancer and in pancreatic tumors generated in mice. Next,

they incubated these cells with THC and observed a decrease in cell viability in the four lines tested. The MiaPaCa 2 cells were the most sensitive, whereas Panc1 were the less. Incubation with the CB_2 selective antagonist SR144528 but not with the CB_1 selective antagonist SR141716, prevented THC-induced loss of cell viability in both lines. THC led to caspase 3 activation, a characteristic of apoptotic cell death.[50] The stress-regulated protein p8 is involved in THC-induced apoptosis of pancreatic tumor cells. Next, they identified genes downstream of p8 that could participate in the antitumoral effect of THC. To evaluate the antiproliferative effect of cannabinoids on pancreatic tumors in vivo, they first generated tumor by s.c. injection of MiaPaCa2 cells in immunodeficient mice. The treatment with THC or the CB_2 selective cannabinoid agonist reduced the growth of the pancreatic tumors. The synthetic cannabinoid agonist (WIN 55,212-2) induces apoptosis of pancreatic cancer cells via the same endoplasmatic reticulum stress-related proapoptotic pathway as THC. The cannabinoid treatment decreases the growth and spreading of pancreatic tumor cells not only to adjacent locations but also to distal tissues.[50]

Inflammation

Activation of cannabinoid CB_1 and CB_2 receptor subtypes suppresses pain behaviour resulting from tissue injury and inflammation. The ability of peripheral cannabinoid mechanisms to modulate the development of inflammatory nociception is well established, less is known about peripheral cannabinoid antihyperalgesic mechanisms after the establishment of chronic inflammation. Local administration of agonist suppressed tactile allodynia and mechanical hyperalgesia with the expected pharmacological specificity. However, antihyperalgesia efficacy and pharmacological specificity for the CB_2 selective agonist was less robust in testsof thermal compared to mechanical hypersensitivity. Local administration of cannabinoids suppress capsaicin-evoked calcitonin gene-related peptide release in rat spinal cord in vitro,[51] suggesting a possible neuronal mechanism of action.CB_2 receptor protein has been identified in microglial cultures of neonatal rat spinal cord,[51] suggesting the existence of additional non-neuronal substrates capable of mediating the antihyperalgesic actions. Activation of CB_2 receptor on skin keratinocytes stimulates production of β-endorphin to induce antinociception through activation of μ-opioid receptor.[52] Locally administered CB_1 and CB_2 selective agonists (respectively ACEA and AM 1241)induced qualitatively similar suppressions of allodyna and hyperalgesia. Local administration of either ACEA and AM 1241 at site of inflammation may suppress antihyperalgesic efficacy by reducing primary

afferent sensitization, effects consistent with the observation that cannabinoids suppress capsaicin-evoked calcitonin gene-relatedpeptide release.[51,53] Carrageenan also enhances C-fibre-mediated responses and windup in spinal dorsal horn neurons effects, that enhance spinal neuronal excitability. These effects are also modulated by both CB_1[54] and CB_2 specific mechanisms.[55]

Inhibition of tumor angiogenesis by cannabinoids

Active angiogenesis is involved in the progression of the majority of solid tumors. The vascular endothelial cells, like glioma cells, express functional CB_2 receptors. The treatment with CB_2 cannabinoid agonist, as JWH-133, inhibits angiogenesis not only by targeting vascular endothelial cells directly, but also by interfering with proangiogenic factor expression.[56] Cannabinoid administration reduced the expression of vascular endothelial growth factor (VEGF), the most potent and ubiquitous proangiogenic factor and of angiopoietic 2 (Ang2), which also contributes to the angiogenic process. Cannabinoid treatment decreased the activity and expression of matrix metalloproteinase-2 (MMP-2), a proteolytic enzyme that allows tissue breakdown and remodelling during invasive angiogenesis, whereas tissue inhibitor of metalloproteinase 2 (MMP-2) expression remained unaffected.[56] Therefore cannabinoids prevent blood vessel formation by inhibition vascular endothelial cell migration and survival. Cannabinoids induce apoptosis of tumor cells and suppress proangiogenic factor and MMP production, further blocking tumor growth and angiogenesis.

Bone mass

In vertebrates, bone mass and shape are determined by continuous remodelling consisting of the concerted and balanced action of osteoclasts, the bone resorbing cells, and osteoblasts, the bone forming cells. Osteoporosis results from the impairment of this balance, leading to bone loss and increased fracture risk.[57] It has been recently reported that bone remodelling is subject to central through patways that involve signalling by the hypothalamic receptors for leptin and neuropeptide Y[58,59] which are also associated with the regulation of endocannabinoid brain levels.[60] Osteoblasts, osteoclasts and their precursors express cannabinoid receptor (CB_2). The endocannabinoid system is essential for the maintenance of normal bone mass by osteoblastic and osteoclastic CB_2 signaling. A CB_2 specific agonist regulates the activity of these cells and attenuates ovariectomy (OVX)- induced bone loss. It enhances endocortical osteoblast number and activity and restrains trabecular

osteoclastogenesis. CB_2 is expressed also in macrophages and immune cells. CB_2 agonists inhibit the expression of proresorptive cytokines, such as TNF and IL-1.[61] CB_2 activation stimulates the expression of IL-1 receptor antagonist, which is normally present in bone and suppresses osteoclast formation.[57] In addition, endocannabinoids enhance NO synthase activity and its release from monocytes and mast cells.[62,63] NO inhibits bone formation and resorption[64] and therefore this endocannabinoid activity is consistent with the CB_2 mediated suppression of bone remodelling implied by the high bone turnover in the CB_2-null mice.

Prevention of Alzheimer's disease

Alzheimer's disease, the most common form of dementia, is characterized by the deposition of β-amyloid peptide. This deposition induce microglial activation.[65, 66] CB_2 receptors are localizated in microglial cells. Cannabinoid receptor activation protects hippocampal or granule cerebellar neurons from excitotoxicity. In vivo cannabinoids decrease hippocampal neuronal loss and infarct volume after cerebral ischemia, acute brain trauma and ouabain-induced excitotoxicity.[67] These effects have been ascribed to inhibition of glutamate transmission, reduction of calcium influx, and subsequent inhibition of noxious cascades, such as tumor necrosis factor-α-generation and oxidative stress.

Cannabinoids induce glioma stem-like cell differentiation and inhibition gliomagenesis

Malignant gliomas remain the most deadly human brain tumors. A characteristic of gliomas is their molecular and cellular heterogeneity. Recent findings support the existence of a stem cell-derived origin for different types of cancers.[68,69] In particular, glioma-derived stem-like cells (GSCs) have been isolated from both human brain tumors[70-74] and several glioma cell lines. GSCs are crucial for the malignancy of gliomas[76] and may represent the consequence of transformation of the normal neural stem cell compartiment.[75] The department of biochemistry and molecular biology I of university of Madrid, the department of medical chemistry and natural products of Hebrew university and the research unit of La Paz university hospital Madrid showed that GSCs express different elements of the CB system including G protein-coupled receptors (CB_1 and CB_2), the ionotropic receptor TRPV1 and eCB-degrading enzymes. Cannabinoid receptor are expressed and functionally active in neural progenitors in which they regulate cell proliferation and differentiation. In fact the cannabinoid agonista promote GSC differentiation and reduce gliomagenesis in vivo. The malignancy of human brain tumors inversely correlates with their degree of differentiation whereas their mitotic activity is inversely correlated with their increased expression of mature

glial and neuronal markers. To identify the potential action of cannabinoids on GSCs, they investigated the changes in gene expression induced by the synthetic cannabinoid agonist HU-210 in GBM-GSCs. It significantly altered the expression of 11 genes of the 266 genes analyzed.[76] Among them , seven genes involved in regulation of the cell cycle and cell proliferation (CDK4, CDKN1B, FGFR1, FGFR3, EGF receptor) were downregulated by cannabinoid stimulation. The transcript levels of neuronal MAP2 and the tumor suppress RBL1 were increased. These results suggest that CB receptor activation regulates essential GSC functions such as cell proliferation and differentiation. CB_1 receptor activation resulted in increased expression of the early neuronal marker β-tubuline III. U87-MG-GSCs previously cultured in the presence of HU-210 or JWH-133 were less efficient as tumor-initiating cells, moreover cannabinoid-treated GSCs generated tumors with a lower growth rate, resulting in smaller tumor size compared with vehicle-treated cells. Tumors generated by cannabinoid-treated GSCs showed decreased neurosphere-forming activity and reduced cell proliferation. These observations confirm that cannabinoids inhibit stem-like cell-initiated gliomagenesis.[76]

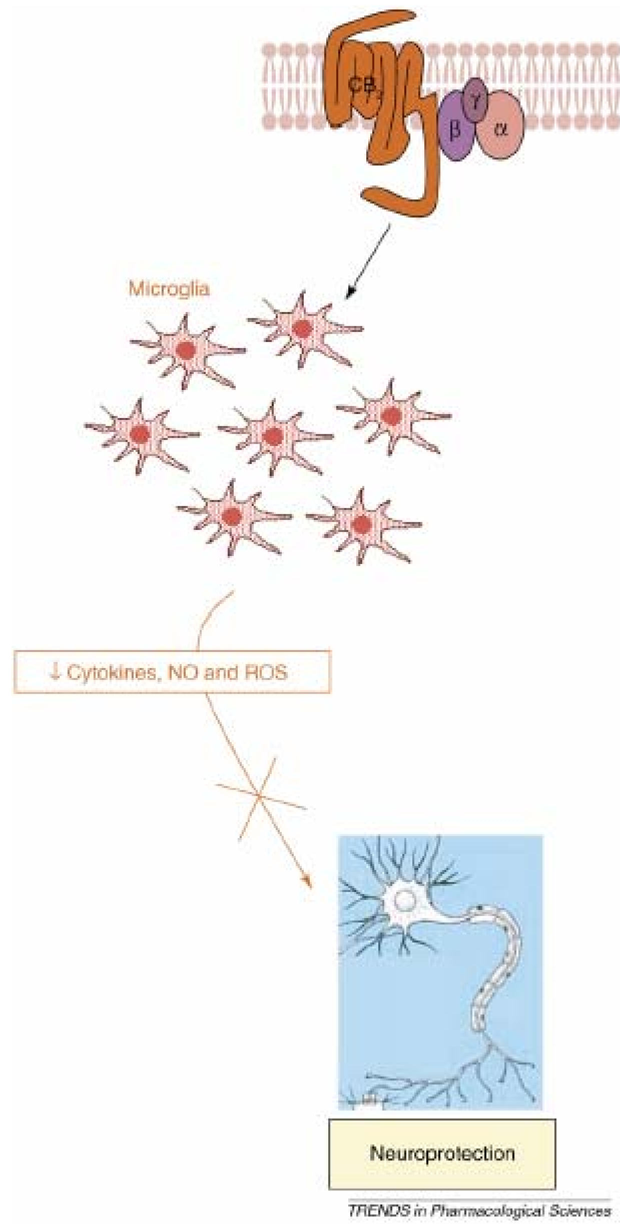


Figure 14

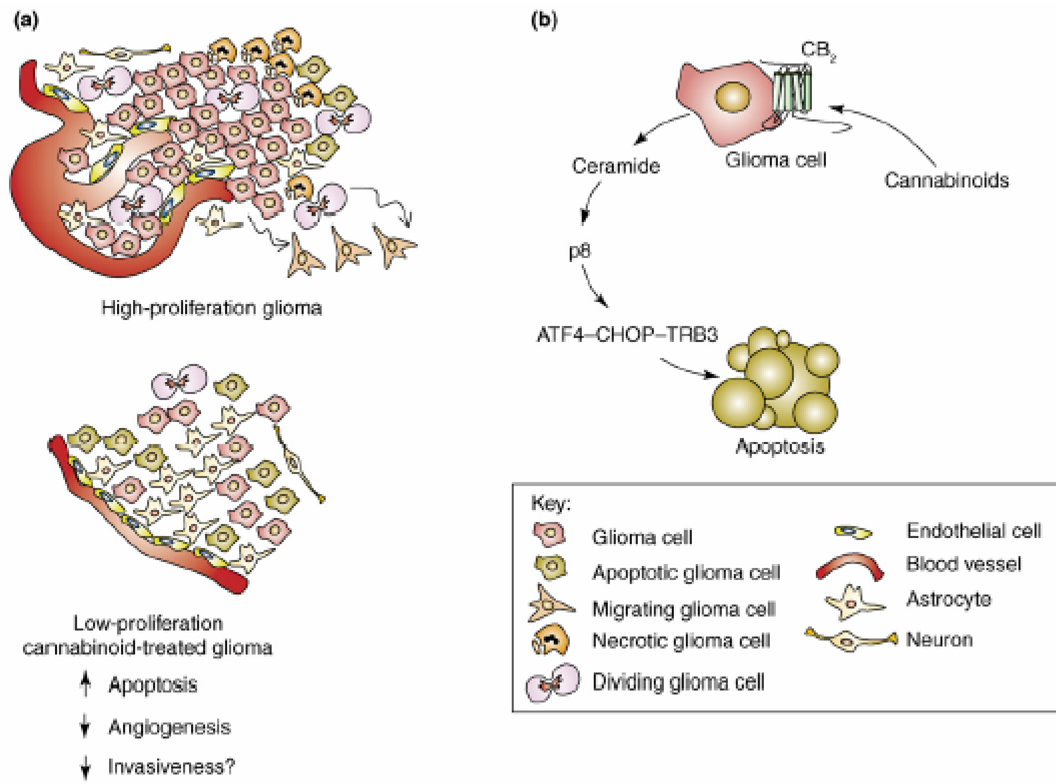


Figure 15

Agonist and antagonist CB_2

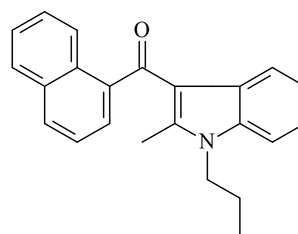
In pharmacology an agonist is a substance that binds to a specific receptor or specific receptor and triggers a response in the cell. It mimic the action of an endogenous ligand (such as hormone or neurotrasmitter) that binds to the same receptor. Although many cannabinoid receptor ligands show little, or at best modst, selectivity for either receptor, a number of synthetic compouds are known which have significant selectivity for the CB_2 receptor. These include cannabinoid indoles, traditional dibenzopyran and tricyclic pyrazoles.

Agonist:

Indoles : Following the observation that WIN-55,212-2 shows significant CB_2 selectivity (Ki= 3.3 nM CB2, Ki= 62.3 nM CB1), Showalter *et al.* Investigated the structure-activity relationship of ligands for the CB_2 receptor and found that JWH-015 has high affinity for the CB_2 receptor. Almost simultaneously the Merck Frosst group repoted that several indoles structurally related to L768242 are selective for CB_2 receptor (Ki=14 nM).

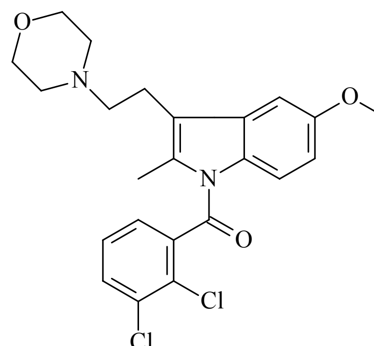
JWH-015[12] Ki CB2 13.8 nM

Ki CB1 383 nM



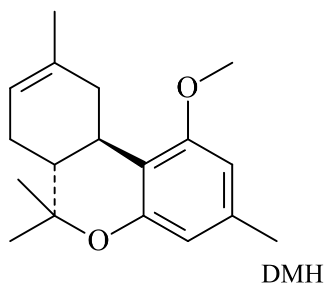
L768242[13] Ki CB2 14 nM

Ki CB1 2043 nM

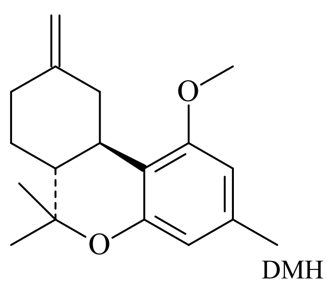


Traditional dibenzopyran: The traditional cannabinoids, such as Δ^9-THC have similar affinities for both receptors. Several years ago it was found that 1-methoxy-Δ8-THC-DMH(L759633, JWH-143) and 1-methoxy-Δ9-THC-DMH (L759656, JWH-142) both have high affinity for the CB2 receptor. But 1-deoxy-Δ8-THC-DMH (JWH-057) has considerably greater affinity for the CB2 receptor. These observations led Huffman et al. to prepare a

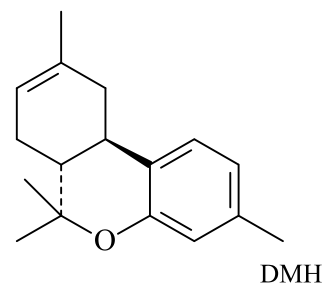
number of 1-deoxy-Δ8-THC analogues in particular JWH-056, JWH-139, JWH-133, JWH-065 have high affinity for the CB2 receptor.



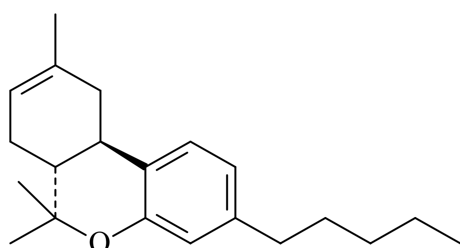
L759633



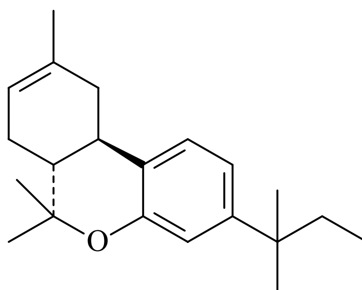
L759656



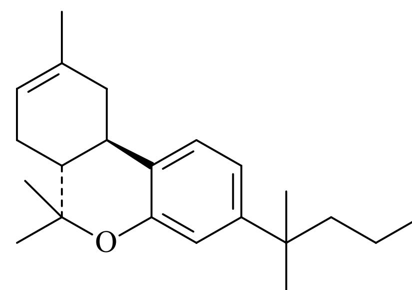
JWH-057



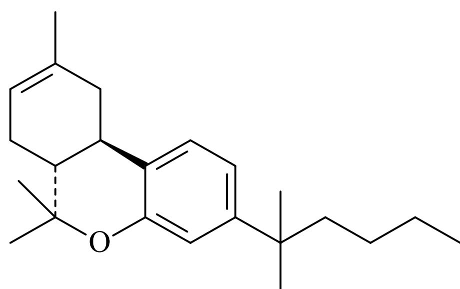
JWH-056



JWH-139



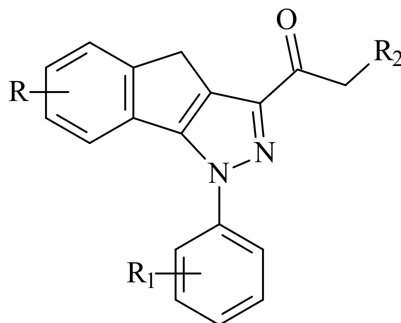
JWH-133



JWH-065

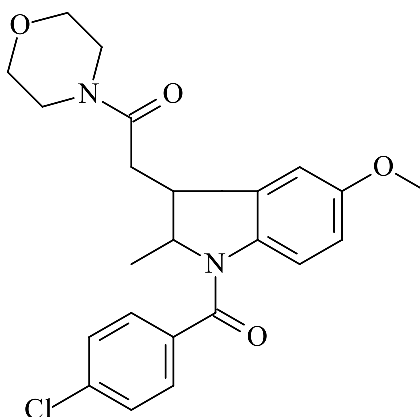
Tricyclic pyrazoles: several of which are highly selective for the CB_2 receptor [40]. These compounds were based on the Sanofi CB_1 and CB_2 inverse agonists, SR141716A and SR144528, but with the addition of a one carbon bridge from C4 of the pyrazole to the ortho-position of the C5 aromatic ring. Moreover they contain a relatively small substituent at C-6

of the aryl group at C-5 of the pyrazole ring. The highly selective compound yield chloro substituent in 5 on aryl group.



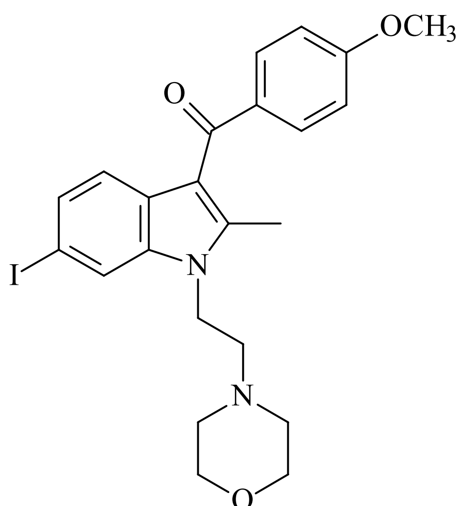
Inverse agonist : is an agent which binds to the same receptor binding-site as an agonist for that receptor but exerts the opposite pharmacological effect.

BML-190 is an inverse agonist, this compound has at best modest affinity for the CB_2 receptor (Ki 435 nM).

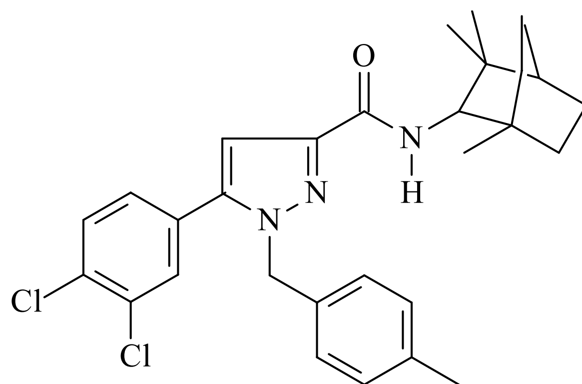


Antagonist:

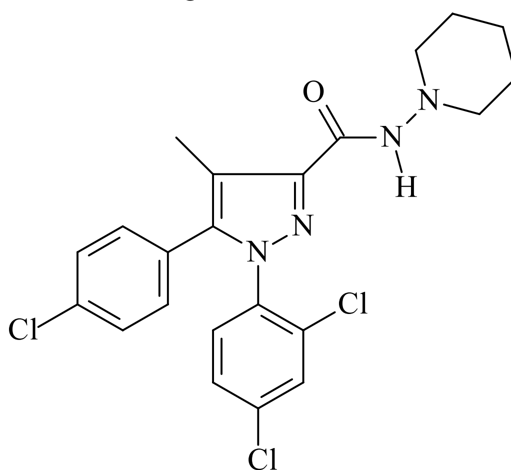
AM630 has been demonstrated to be a cannabinoid receptor antagonist in the mouse brain and vas deferens. Studies conducted in guinea pig brain reveal that AM630 antagonizes the stimulatory effect of the cannabinoid agonist WIN 55,212-2 on [35SlGTFjS binding.



SR144528 : is a CB_2 receptor-selective cannabinoid antagonist. In the GTPγS binding assay, SR144528 antagonized a number of cannabinoid receptor agonists (K_B values ranging from 26.3 to 76.6 nM) in rat cerebellar membranes and in rat whole brain membranes (K_B=50.8 nM). SR144528 also antagonized CP 55,940-stimulated GTPγS binding in a CB_2-expressing cell line (K_B=6.34 nM).



SR 141716: is a CB_1 cannabinoid antagonist.



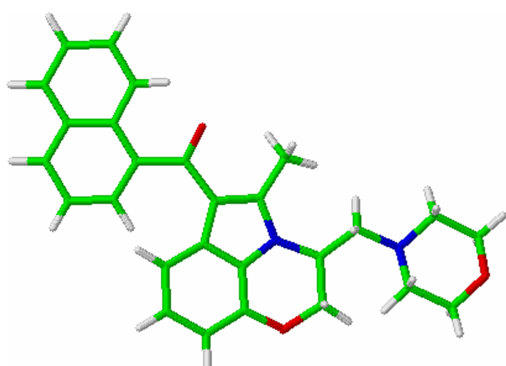
Introduction of experimental part

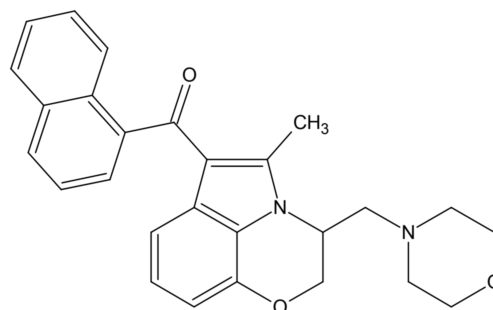
INTRODUCTION OF EXPERIMENTAL PART

PART 1

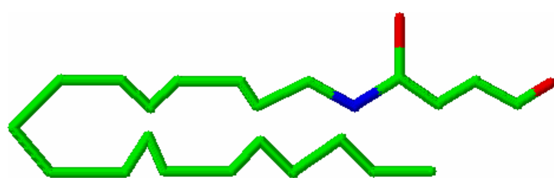
Models of the CB_1 and CB_2 receptors were generated using the bovine rhodopsin structure determined at 2,8 Å as a template using an automated docking approach (AUTODOCK 3.0). The length of the transmembrane helices of the two receptor was defined by aligning the rhodopsin primary sequence with both receptor sequences. Some aminoacids of the rhodopsin were replaced with well-known aminoacids of CB receptors to obtain the three dimensional model.[77]

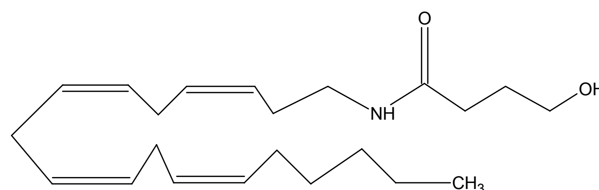
The optimization of the model had been obtained through docking of WIN55212-2, an agonist of the cannabinoids receptors which shows a high activity in both receptors in particular CB_2, and of anandamide (AEA), the most well-known endogenous ligand and which has a completely different structure with respect to WIN55212-2.





WIN55212-2





AEA

Both receptors showed two binding pocket that are delimited by the transmembrane domain 3 (TM3), TM4, TM5 and TM6.

WIN55212-2 had been inserted with the morpholinic group positioned between TM3 and TM4, while the naphthyl substituent was directed toward the central core of TM5 and TM6. In

this manner, the lipophilic core of the ligand was able to interact with W5.43 (TM5) and W6.48 (TM6), (figure 16) and in the CB_2 receptor the naphthyl ring with F5.46 (TM5) and the morpholinic group with S3.31 (TM3) (figure 16). Therefore in the CB_1 the binding site is characterized by a lipophilic pocket delimited by F3.36 (TM3), W5.43 (TM5) and W6.48 (TM6), which principally interact through aromatic stacking with the naphthyl and indole ring system, while the morpholinic group is positioned in a secondary lipophilic pocket formed by L3.26 (TM3), P4.60 (TM4) and L4.61 (TM4). The CB_2 binding site is similar to the CB_1 one but the WIN55212-2 has a slightly different orientation. In the CB_2 site the ligand interacts with F5.46 (TM5) and not with F3.36 (TM3) to stabilizing the naphthyl ring. Regarding the secondary lipophilic pocket in which the morpholinic group is positioned, the substituent interacts with L3.27 (TM3), P4.60(TM4), L4.61 (TM4) and S3.31 (TM3) forms a hydrogen bond with the oxygen atom of the morpholinic group (figure 16). Both receptors show a large lipophilic pocket occupied by the naphthyl and indole ring of WIN55212-2 and a secondary one corresponding to the morpholinic position.

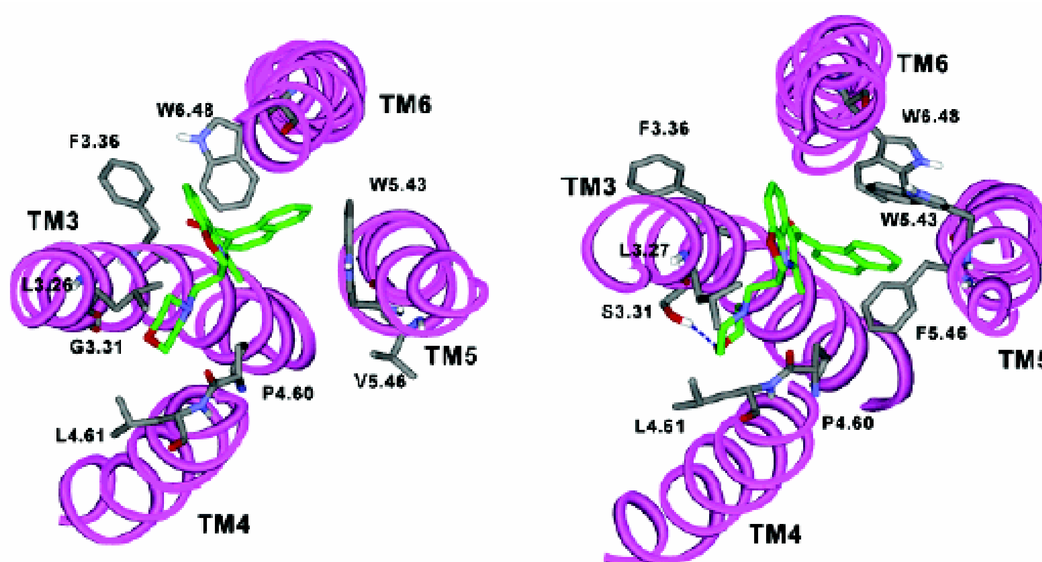
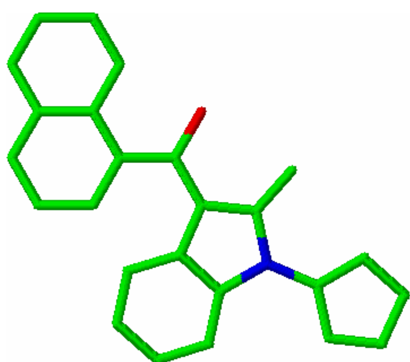
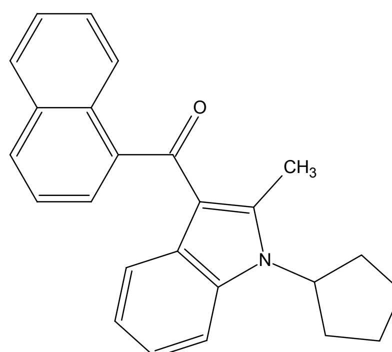


Figure 16

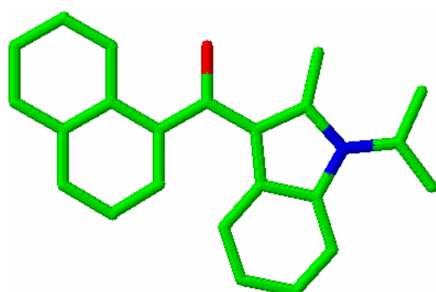
In order this investigation continued with the study of relevant literature indole ligands that problaby interact in the WIN55212-2 binding site and that were docked into the CB_2 model. The indole derivatives show a binding position very similar to the one observed for WIN55212-2 .

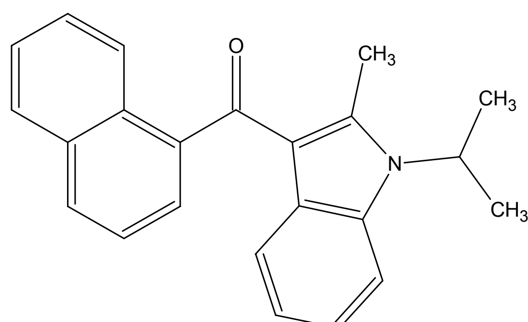
The indole JWH-007 and analog compounds as JWH-015 are less potent than WIN55212-2 .





JWH-007

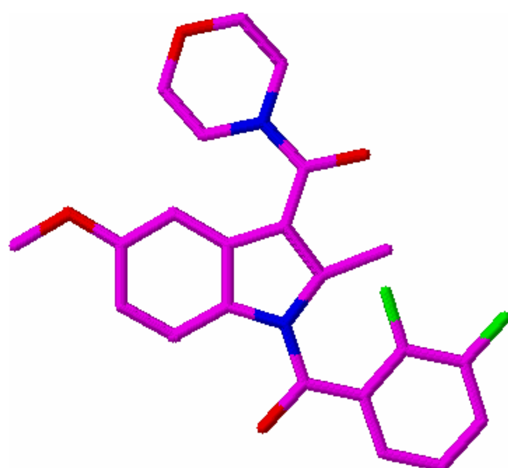


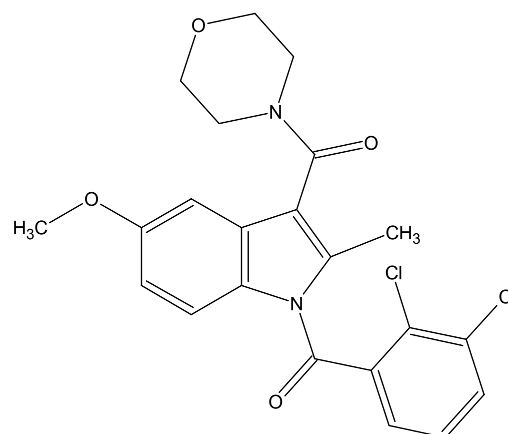


JWH-015

This because the indole ring interacts with L3.26 (TM3), I3.29 (TM3) and L6.59 (TM4). The pentyl substituent is inserted into the secondary lipophilic pocket formed by L3.27 (TM3), P4.60 (TM4) and L4.61 (TM4), but of course it is not able to form the H bond with S3.31 (TM3) and this could be one of the reasons for the lower affinity of this ligand, compared with that of the WIN55212-2 affinity.

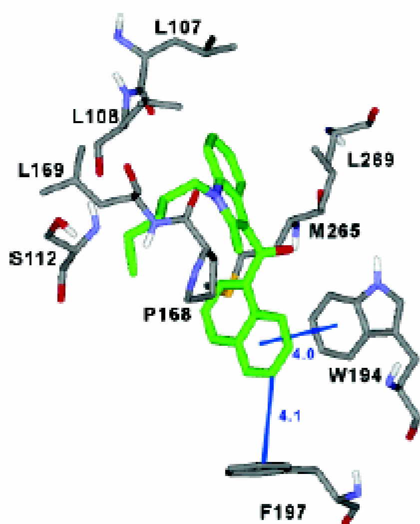
The indole derivatives bearing the morpholinic ring linked to the C3 position of the indole system and the aromatic group linked to the N1 position (in an opposite manner compared with WIN55212-2) show a different placement of the indole ring in the CB_2 binding site, compared with the indole position of the 3-(1-naphtoyl)indole analyzed. (see JWH-007)

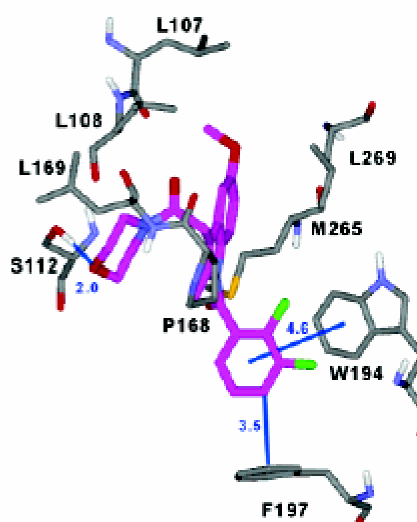




indole **J**

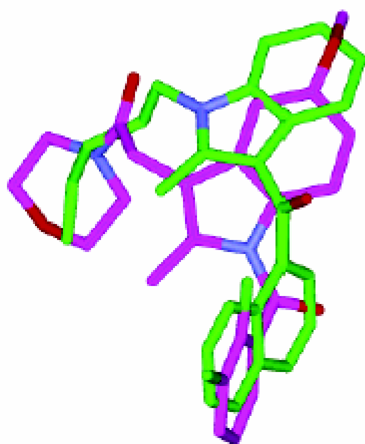
In particular for indole **J** the substituent position 3 (acetylmorpholine) is inserted into the secondary lipophilic pocket and forms the H bond with S3.31, while the benzoyl substituent interacts with W5.43, F5.46. Comparing the position JWH-007 with the compound **J** we observe that the aromatic and morpholinic group have the same disposition, but for this reason, unlike JWH-007, the indole ring of compound **J** is upset, with the nitrogen directed toward the intracellular side of the receptor. These observation might suggest that the nitrogen of the indole system should not be important for the interaction, and that the role of the whole indolic system could be only that of an aromatic core able to place the substituents in the right disposition in the CB_2 receptor binding site.





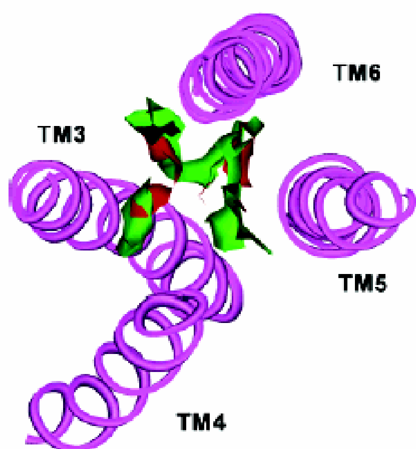
JWH-007

Indole compound **J**

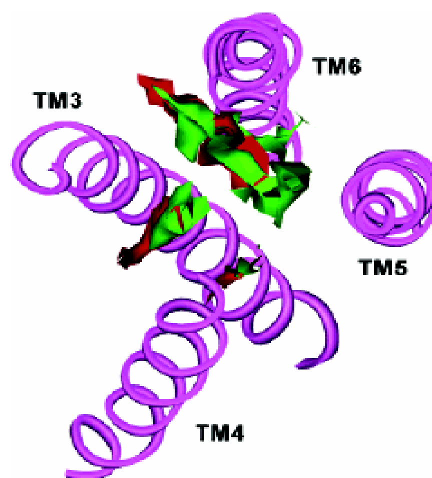


Superimposition of the two ligands: JWH-007 and compound **J**.

These analysis suggest that the CB_2 binding site is similar to the CB_1, with a primary lipophilic pocket delimited by F3.36, W5.43, W6.48 (TM5 and TM6) and the secondary lipophilic pocket delimited L3.27, P4.60, L4.61 and Ser3.31 (TM3 and TM4). An analysis of the interaction of some agonists with both receptors showed that CB_2/CB_1 selectivity is mainly determined by the presence of the nonconserved residue S3.31 (TM3) and F5.46 (TM5) in CB_2. These residues are localized in the binding pocket that is delimited by TM3, TM4, TM5 and TM6 (Figure 17).



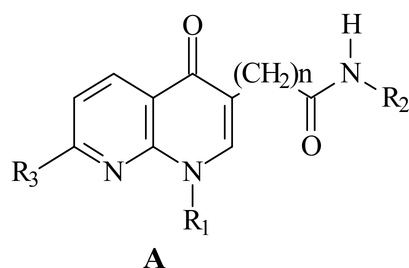
The binding pocket of the CB_1 receptor.



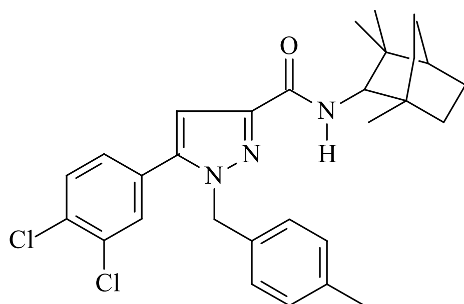
The binding pocket of the CB_2 receptor.

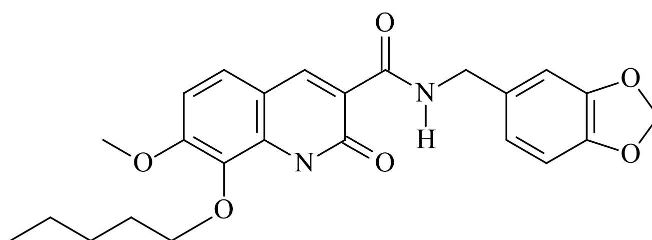
Figure 17

Previously were synthesized a serie of 1,8-naphthyridin-3-carboxamide derivatives of general structur **A.**[78] These compounds are characterized by the presence of important structural requirement shown by other classes of cannabinoid ligands, such us an aliphatic or aromatic carboxamide group in position 3, present in quinoline derivatives like JTE-907 and in arylpyrazole derivatives like SR-144528, and alkyl or arylalkyl substitutent in position 1, present in aminoalkylindole derivatives like WIN-55212-2 and SR-144528.



A



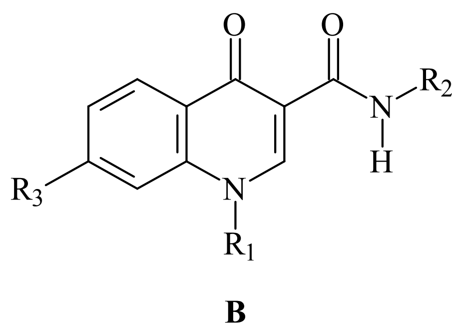


SR-144528 **JTE-907**

The binding results of these compounds for CB_1 and CB_2 receptors were assessed by competition experiments with [^{3}H]CP-55,940 in mouse cerebral membranes and mouse spleen homogenate, respectively.[78]

The results obtained show that the 1,8-naphthrydine derivatives examined generally exhibit a higher affinity for the CB_2 receptor than for the CB_1 receptor. Furthermore, some of these compounds showed a good CB_2 selectivity with the CB_1/CB_2 ratio >20. It was also possible to hypothesise that in order to obtain a good affinity on the CB_2 receptor, the presence is necessary in position 1 of a n-alkylic or benzylic substituent, which my be substituted, and in position 3, of a aliphatic carboxamide, such us cyclohexylamide directly linked to the 1,8-naphthyridine nucleus.

Bearing this in mind, I designed and synthesized new 7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives (structure **A** n=0), analogues derivatives in which the methyl group was removed or substituted with a chlorine atom, fluorine atom, metoxy group and dimethylamino group and new quinolin-4(1*H*)-on-3-carboxamide derivatives of general structure **B.**



B

The synthesis of the new 1,8-naphthyridine derivatives are described in Schemes 1-5. As reported in Scheme 1 the treatment of 2-amino-6-methylpyridine with ethoxymethylenemalonic acid diethyl ester (EMME) at 110°C for 2h gave the diethyl ester derivative **1**, which was heated in dowtherm A for 1h to give the derivative 7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxylic acid ethyl ester **2**.[79] The heating of ester **2** with the suitable amine provided the corresponding amide derivatives **3-5**. The treatment of carboxamide derivatives **3-5** in anhydrous DMF with NaH for 1 h and then with the appropriate benzyl chloride or arylalkyl chloride or 4-(2-chloroethyl)-morpholine provided the desired 1,8-naphthyridin-4-one derivatives **6-14** (Scheme 1).[80]

The treatment of 2,6-diaminopyridine with acetyl chloride in anhydrous dioxane at room temperature for 2 h gave acetyl derivative **15**, which was purified by flash-chromatography (AcOEt). The 7-acetamido-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid ethyl ester **16** was synthesized following the method reported in literature**.** [81]

Compound **16** was heated at 120 °C in sealed tube with 4-methylcyclohexylamine or cyclohexylamine for 24h (Scheme 2). Under these conditions, the hydrolysis of the acetamido group also takes place, and thus, the 7-amino-3-carboxamide derivatives **17** and **18** respectively were obtained. Diazotization of these compounds carried out in aqueous 37% hydrochloride acid at 40°C for 3h afforded the 7-chloro derivatives **19** and **20**, (Scheme 3) which, by reaction with benzyl chloride or arylalkyl chloride or 4-(2-chloroethyl)-morpholine, in the same condition above reported gave the desired compounds **21-25** (Scheme 3). The diazotization of compound **18,**[83] carried out in aqueous 50% thetrafluoroboric acid at –5°C

during the addition of the $NaNO_2$ and then at r.t. for 1h 30 min, gave the corresponding 7-fluoro derivative which was purified by flash-chromatography. The treatment of carboxamide derivative **26**, in anhydrous DMF with NaH for 1h and then with the 4-(2-chloroethyl)-morpholine for 24h provided the desired derivative **27**. The reaction of the compound **22** with the sodium metilate, prepared from sodium in MeOH, at 80°C for 5h 30 min, gave the derivative **28** (Scheme 3).

The 7-chloro derivative **20**, by reaction with dimethylamine in sealed tube a 120°C for 24h gave the N,N-dimethylamino derivative **29** (Scheme 4). The treatment of carboxamide derivative **29**, with NaH and then with the 4-(2-chloroethyl)-morpholine provided the desired compound **30**. The carboxamide **31** was obtained by dehalogenation of **20** with H_2 in the presence of Pd/C as a catalyst. The treatment of carboxamide derivative **31**, in anhydrous DMF with NaH for 1h and then with benzyl chloride or arylalkyl chloride or 4-(2-chloroethyl)-morpholine, gave the desired compounds **32-34** (Scheme 4).

As reported in Scheme 5, the diazotization of compound **18**[78] with $NaNO_2$ in aqueous 96% sulfuric acid gave the 7-hydroxy derivative **35**, which, by reaction with *o*-fluorobenzyl chloride under the same conditions described above, gave *N*-cyclohexyl-1-(*o*-fluorbenzyl)-7-(*o*-fluorobenzyloxy)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide **36**.

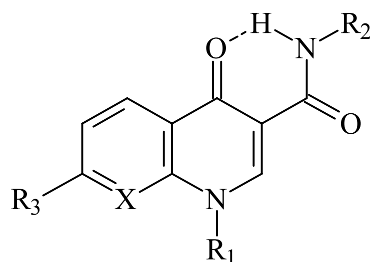
The synthesis of the new quinoline-4(1H)-on-3-carboxamide derivatives are described in Scheme 6.

The suitable aniline was heated with EMME to gave the diethyl ester derivatives **37**[83]**-39**.

Successful these compounds was heated in dowtherm A for 1 h to afforded the 7-substituited ethyl ester **40-42**.

The heating of ethyl ester **40-42** with the appropriate amine at 120°C for 24h in sealed tube provided the carboxamide derivatives **43-46** (Scheme 6). The treatment of carboxamide derivatives **43-46** in anhydrous DMF with NaH for 1 h and then with the suitable benzyl chloride or 4-(2-chloroethyl)-morpholine provided the desired compounds **47-54** (Scheme 6).

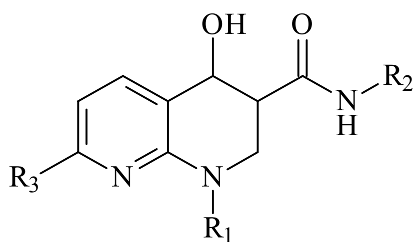
The studies of automated docking have showed that all the 1,8-naphthyridine derivatives tested could form an intramolecular H bond between the carbonylic oxygen and the amidic NH, creating a pseudocycle planar with the naphthyridine ring (structure C), and our studies suggested that this interaction was quite strong.[77]



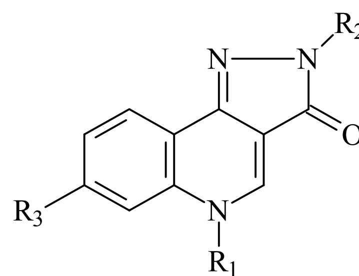
C

To verify the ability of our CB_2 model to discriminate between active and inactive ligands and also to verify whether the formation of a planar pseudocycle was important for interaction inside the CB receptor, I synthesized to tested some new compounds characterized by:

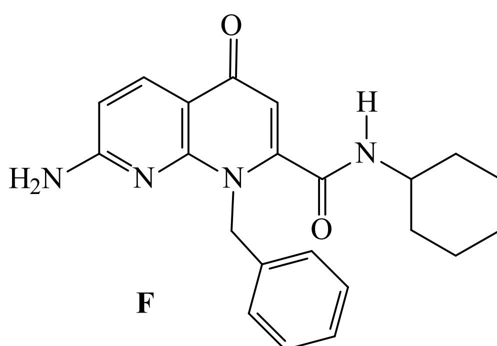
- the presence of a hydroxy group in position 4 of the naphthyridine nucleus, instead of the carbonyl oxygen atom, and by partial removal of the aromaticity of the cycle.(general structure **D**).
- closing of the O in position 4 with the amidic NH in a cycle to five atoms, to mime the pseudocycle planar of the energetic form more stable. (general structure **E**).
- shifting from position 3 to position 2 of the heterocyclic nucleus, of the carboxamide group (structure **F**).



D



E



F

The synthesis of compounds of general structur D is outlined in Scheme 7. The reaction of the 1,8-naphthyridin-4-one derivatives[78] **55-57** with sodium borohydride in anhydrous ethanol gave the N1-substituted 4-hydroxy-7-methyl-1,2,3,4-tetrahydro-1,8-naphthyridin-3-carboxamides **58-60**.

The synthesis of 3H-pyrazol-[4,3-c]-quinolines **66-70** is reported in Scheme 8. When the quinolin-4(1H)-on-3-carboxylic acid ethyl esters **39** and **40**[83] were heated with phosphoryl trichloride at 120°C for 15 min the corresponding ethyl 4-chloro-3-quinolinecarboxylate **61** and **62**[85] were formed. These intermediates were subsequently converted into compounds **63-65** by reaction with the appropriate hydrazines in xylene at 150 °C for 13 h (73–80% yields). The reaction of these with benzylchloride or 4-(2-chloroethyl)-morpholine hydrochloride under the same conditions described above gave the desired compounds **66-70** (30–60% yields).

The synthesis of compounds of structure **F** is outlined in Scheme 9.

The derivative 7-acetamido-1,8-naphthyridin-4(1H)-on-2-carboxylic acid methyl ester **72**[85] was synthesized following the method reported in the literature starting from 2-acetylamino-6-aminopyridine **15** with acethylendicarboxylic acid dimethyl ester at 180°C for 24h, and than was heated in Dowtherm A for 1h and was purified by flash-chromatography (AcOEt/MeOH 10:1).

The heating of **72** at 120 °C in sealed tube with cyclohexylamine for 24 h affording the 7-amino-2-carboxamide derivative **73**. This compound, by reaction with benzylchloride under the same conditions described above, gave the 1,8-naphthyridine derivative **74** in very low yield.

Results and discussion

CB_1 and CB_2 affinity

Affinities at CB_1 and CB_2 receptors for the 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives **6-14**, **21-25**, **32-36**, **58-60**, were determined by measuring their ability to displace [3H]CP-55,940 from its binding site in a membrane preparation from mouse brain (minus cerebellum) and mouse spleen homogenate, respectively.

For the compounds **7** and **8** and for the other compounds **27-30**, **47-54**, **66-70** and **74** the binding assay were tested using membranes from HEK-293 cells transfected with either the human CB_1 and CB_2 receptors.

The CB_1 and CB_2 receptor binding assay results for the new compounds are summarized in Table 1. The Ki values of WIN-55,212-2, HU-210, AM630, JWH-133, ACEA, SR141716A and SR144528 are also included in the table as reference compounds for the CB_1 and CB_2 cannabinoid receptors.

The results obtained for the compounds **7** and **8**, show that there are not substantially differents between the Ki values obtained utilizing membrane preparation from mouse brain (minus cerebellum) and mouse spleen homogenate, and membranes prepared from HEK-293 cells. For this reason the SAR was made considering the totality of 1,8-naphthyridin-4-one derivatives.

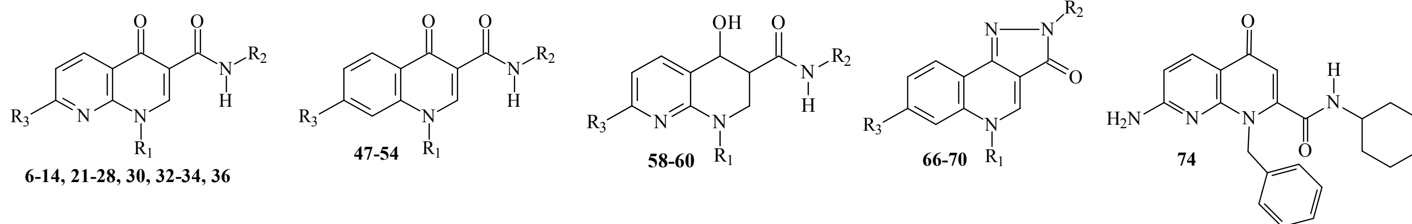


Table 1

compd	R_1	R_2	R_3	$KiCB_1$ nM mouse	$KiCB_2$ nM mouse	$KiCB_1/KiCB_2$	$KiCB_1$ nM human	$KiCB_2$ nM human	$KiCB_1/KiCB_2$
6	benzyl	4-methylcyclohexyl	methyl	NT	NT	NT			
7	p-fluorobenzyl	4-methylcyclohexyl	methyl	8.7±1.6	1.4±0.1	6	5.6	0.79	7
8	o-fluorobenzyl	4-methylcyclohexyl	methyl	37.5±5.4	8.4±0.3	4	56	7.9	7
9	benzyl	cycloheptyl	methyl	143.2±9.1	5.1±1.3	28			
10	p-fluorobenzyl	cycloheptyl	methyl	4.3±0.6	1.0±0.1	4.3			
11	o-fluorobenzyl	cycloheptyl	methyl	149.4±1.8	13.4±4.7	11			
12	1-ethyl-4-phenylpip	cyclohexyl	methyl	>1000	>1000				
13	phenethyl	cyclohexyl	methyl	>1000	16.3±1.2	>62			
14	p-methoxybenzyl	cyclohexyl	methyl	>1000	35.8±2.1	>28			
21	ethylmorph	cyclohexyl	Cl	>1000	25	>40			
22	benzyl	cyclohexyl	Cl	463.6±1.1	24.6±4.7	19			
23	p-fluorobenzyl	cyclohexyl	Cl	495.0±39.4	21.4±1.0	23			
24	o-fluorobenzyl	cyclohexyl	Cl	171.2±12.3	18.1±2.7	9.5			
25	ethylmorph	4-methylcyclohexyl	Cl	>1000	40.5±7.7	>25			
27	ethylmorph	cyclohexyl	F				>1000	13	>76.9
28	benzyl	cyclohexyl	OCH_3				560	11	51
30	ethylmorph	cyclohexyl	$N(CH_3)_2$				>5600	94	>59.4
32	p-fluorobenzyl	cyclohexyl	H	384.1±25.3	13.0±1.4	29			
33	benzyl	cyclohexyl	H	>1000	48.6±12.0	>21			
34	ethylmorph	cyclohexyl	H	>1000	67.2±11.6	>15			
36	o-fluorobenzyl	cyclohexyl	o-F-benzyl	NT	NT	NT			
47	ethylmorph	cyclohexyl	H				NT	NT	
48	benzyl	cyclohexyl	H				560	79	7
49	ethylmorph	cyclohexyl	Cl				>1000	38	>26
50	benzyl	cyclohexyl	OCH_3				170	79	2.1
51	ethylmorph	cyclohexyl	OCH_3				2920	79	37
52	benzyl	cycloheptyl	H				17	6.4	2.6
53	ethylmorph	cycloheptyl	H				290	28	10.3
54	benzyl	cyclohexyl	Cl				NT	NT	
58	o-fluorobenzyl	cyclohexyl	methyl	>1000	>1000				
59	ethylmorph	4-methylcyclohexyl	methyl	>1000	>1000				
60	benzyl	cyclohexyl	methyl	>1000	>1000				
66	benzyl	cyclohexyl	H				620	790	0.78
67	benzyl	cyclohexyl	Cl				560	1640	0.34
68	ethylmorph	cyclohexyl	H				>5600	>7900	-----
69	ethylmorph	phenyl	H				2800	2280	1.2
70	ethylmorph	cyclohexyl	Cl				NT	NT	-----
74	benzyl	cyclohexyl	NH_2				5600	7900	0.7
Win55212-2							21	2.1	10
HU-210							0.18	0.15	1.2
AM630							840	36	23
ACEA							5.3	95	0.06
JWH-130							680	3	227
SR141716A							16	1640	0.01
SR144528							>50000	5.4	-----

CB_1 receptor affinity

The results reported in Table 1 show that some of the compounds characterized by the presence in position 1 of *orto* or *para*-fluorobenzyl group possess an interesting affinity at the CB_1 receptor. In particular, the 1,8-naphthyridine derivatives **7**, **8**, and **10** exhibit a considerable affinity but are not selective for this receptor. The other compounds showed very low affinity at this receptor.

CB_2 receptor affinity

The results obtained indicate that, the *N*-benzyl-1,8-naphthyridine derivatives possess a higher affinity than the *N*-ethylmorpholino derivatives, as is clear from a comparison of compounds **7-11**, **14**, **22-24, 28, 32-33** and **36,** with **21**, **25**, **27**, **30** and **34**. For the *N*-benzyl- 1,8-naphthyridine derivatives (**7-11, 14, 22-24**, **28**, **32-33**), the presence of an atom of fluorine on the benzyl increases the affinity, above all if the substitution is in the para position. In particular, the *p*-fluorobenzyl-1,8-naphthyridine derivatives **7** (Ki =1.4 nM and Ki =0.79 nM determined from mouse and human respectively) and **10** (Ki **=**1.0 nM) proved to be the compounds with the highest affinity in this series. Furthermore, the *N*-phenethyl-1,8-naphthyridine derivative **13** and the *N*-p-methoxybenzyl-1,8-naphthyridine derivative **14** showed a good affinity, with a Ki of 16.3 nM and Ki of 35.8 nM respectively. In contrast, the compound bearing a 1-ethyl-4-phenylpiperazinyl group in position 1 of the naphthyridine nucleus (**12**) possesses a very low affinity, with a Ki value greater than 1000.

Furthermore, the substitution of the methyl group in position 7 of the 1,8-naphthyridine nucleus with an atom of chlorine or fluorine, or with methoxy group, or dimethylamino group, or the lack of any substituent in the same position (**21-25**, **27-30**, **32-34**), generally determines the maintenance or an increase in the affinity (except for compounds **23** and **33**, which showed a 4-fold and 5-fold decrease in affinity compared with the methyl analogues[78]).

Compounds **47-54** in which the naphthyridin-4-one system was substituted by the quinoline-4-one, possess a good affinity with Ki value $<$80nM. However this affinity is lower than one of the corresponding 1,8-naphthyridine derivatives, as is clear from a comparison of **48-50**, with **21**, **28** and **33.**

The 4-hydroxy-1,2,3,4-tetrahydro-1,8-naphthyridine derivatives **58-60** exhibit a poor affinity toward the CB_2 receptor, with Ki values greater than 1000.

The compounds characterized by the 2H-pyrazolo[4,3-c]quinolin-3(5H)-one central scaffold (**66**-**70**), showed a very low affinity toward both CB receptor subtypes.

The shifting of the carboxamide group from position 3 to position 2 (compound **74**) of the heterocyclic nucleus together with the substitution of the methoxy group in position 7 with the amino group causes a drastic decrease of affinity toward both cannabinoid receptors, as confirmed by a comparison of this compound (**74**) with compound **28**.

Test for CB_2 Functionality

To assess the functionality of the studied compounds at CB_2 receptors, functional studies on human basophils were performed. Activation of CB_2 receptors is known to down-regulate the immunological activation of guinea pig mast cells and human basophils.[86,87] *N*-Cyclohexyl-1-benzylquinolin-4(1*H*)-on-3-carboxamide (**48**) and *N*-cyclopentyl-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide,[78] which is structurally analogous to the 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives, were used for functional studies. Human basophils, pretreated with these compounds (1 nM to 1 μM, 30 min of preincubation, 37 °C), showed a reduced expression of CD203c in response to immunological stimulation (anti-IgE 1 íg/mL, 30 min, 37 °C). The inhibition was reversed by the selective CB_2 antagonist SR 144528 (SR, 100 nM, 30 min of preincubation, 37 °C) but not by AM251 (AM, 100 nM, 30 min of preincubation, 37 °C), a selective CB_1 antagonist.

These results show that compounds **48** and *N*-cyclopentyl-7-methyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide,[78] exert a CB_2-mediated inhibitory action on immunological human basophil activation. Furthermore, we hypothesize that the quinolin-4-(1*H*)-on-3-carboxamide **47, 49-54** and the 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives **6-14**, **21-28**, **30**, **32-34** and **36** possess the same kind of activity. This result indicated that these compounds behaved as CB_2 receptor agonist.

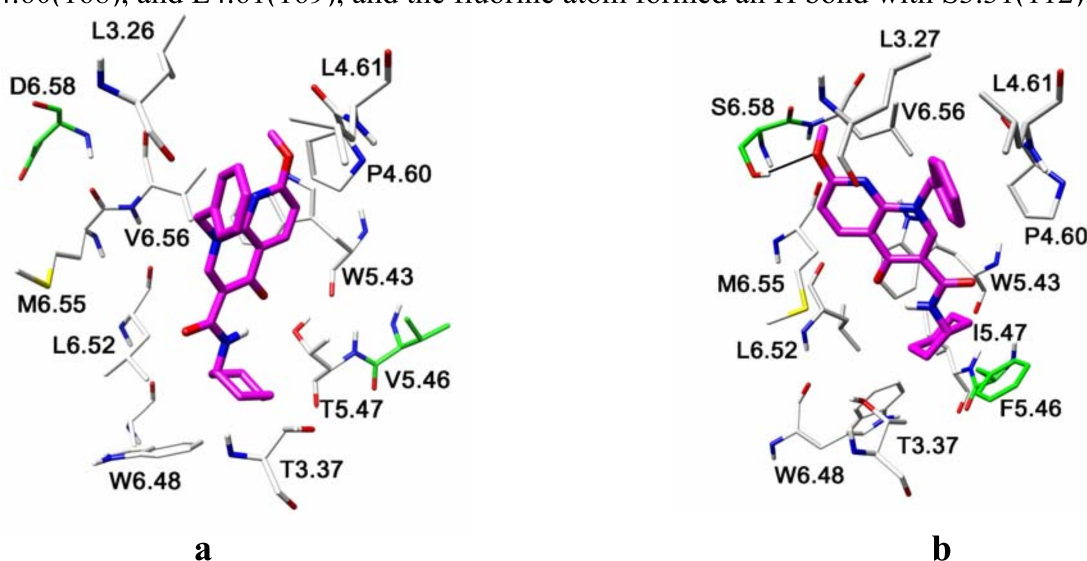
Molecular Modeling

The synthesized ligands were docked using AUTODOCK 3.0,[88] in the CB_1 and CB_2 receptor models prepared by molecular modeling.[77]

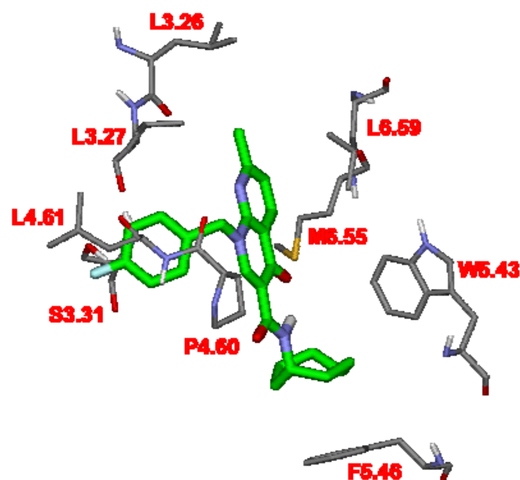
Figure 18 (**a**, **b**) shows the docking of compound **28** into both receptors; in the CB_1 receptor model, the naphthyridine and the cyclohexyl ring predominantly interact in the lipophilic pocket delimited by W5.43(279), T5.47(283), W6.48(356), L6.52(360), and V6.56(364), while the benzyl group is positioned in a secondary lipophilic pocket formed by L3.26(190), P4.60(251), and L4.61(252) (figure 18).

In the CB_2 receptor model, the benzyl ring of the ligand interacted similarly to the CB_1 receptor in the lipophilic pocket delimited by L3.27(108), P4.60(168) and L4.61(169) (figure 18) whereas the naphthyridine ring was rotated about 130° (counterclockwise sense from the extracellular point of view) and mainly interacted with W5.43(194), M6.55(265), and L6.60(269). Furthermore the methoxy substituent formed an H bond with the non-conserved S6.58(268) (substituted by D366 in the CB_1 receptor) and the cyclohexyl group, beyond the lipophilic interactions with T3.37(118), W5.43(194), and W6.48(258), feels the effect of a strong interaction with F5.46(197), which is a non-conserved residue (V282 in the CB_1) (figure 18). The interactions with S6.58(268) and F5.46(197), together with the different disposition of the naphthyridine ring, could be the reason for the CB_2 versus CB_1 selectivity of this ligand.

The docking of compound **10** showed that the cycloheptyl substituent was directed toward the intracellular side of the receptor, interacting with W5.43(194) and F5.46(197) (see Figure 18 **c**). As for the *p*-F-benzyl group, it interacted in a lipophilic pocket formed by L3.27(108), P4.60(168), and L4.61(169), and the fluorine atom formed an H bond with S3.31(112).



a **b**



c

Figure 18

The docking of the inactive compounds **58-60** revealed that the lack of planarity determines a different position of the central lipophilic core, determining weaker interactions with the receptor. As shown in Figure 19, the central core of **60**, compared with the position of the naphthyridine ring of *N*-cyclohexyl-7-methyl-1-benzyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide,[78] that was shifted further way from TM3 and directed toward TM5, determining weaker interactions with M6.55(265) and L3.27(107); furthermore, this arrangement determined a weaker interaction of the cyclohexyl ring with F5.46(197). From this consideration we can confirmed the hypothesis about the fundamental role of the presence of a planar pseudocycle with the naphthyridine nucleus obtained by an intramolecular H bond between the carbonylic oxygen and the amidic NH.

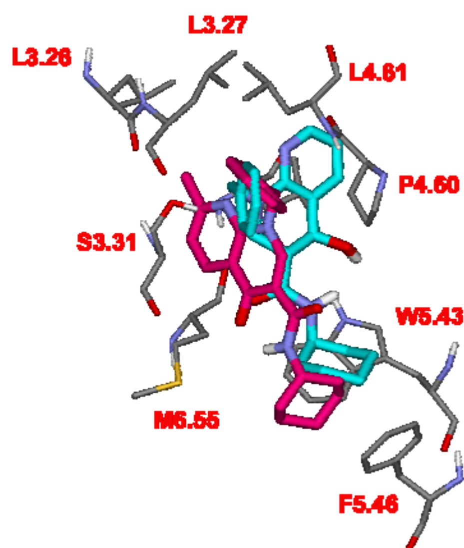


Figure 19

As shown in Figure 20, compound **67** disposition in the CB_1 binding, site very similar to the one observed for compound **28**, with the same lipophilic interactions, in agreement with the very similar CB_1 affinity value. On the other hand, in the CB_2 binding site, the benzyl group was directed toward S6.58(268) and lost lipophilic interactions inside the pocket delimited by L3.27(108), P4.60(168) and L4.61(169), which explains its low CB_2 affinity.

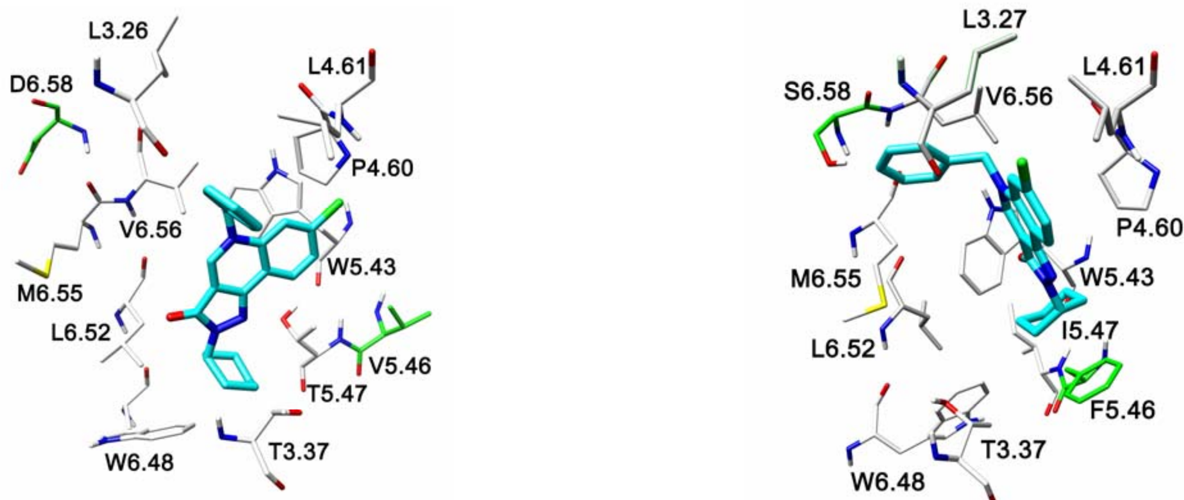


Figure 20

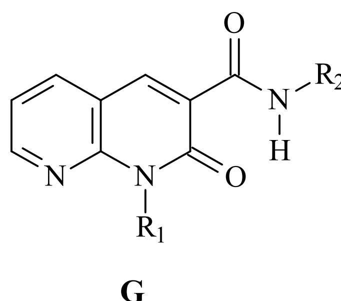
These analysis suggested that the preservation of good CB_2/CB_1 selectivity and the improvement of the CB_2 affinity seemed to require the presence of a lipophilic or aromatic R_1 substituent with an H bond acceptor atom capable of interacting in the CB_2 receptor with the nonconserved S3.31(112), belong to the second lipophilic pocket of the receptor. The substitution of the carboxycyclohexylamide group in position 3 of the 1,8-naphthyridine nuclear with a carboxycycloheptylamide group and 4-methylcarboxycyclohexylamide group determine an increase in the affinity toward the CB_2 receptor. Infact these groups interact with the residues W5,43(194) and F5,46(197), belong to the first lipophilic pocket of the receptor (TM5).

The presence in position 7 of the heterocyclic nucleus of an H bond acceptor atom or goup (chlorine, fluorine or methoxyl group) capable of interacting with the nonconserved residue S6.58(268) in the CB_2 receptor seemed to be one of the reasons for the high selectivity value.

INTRODUCTION OF EXPERIMENTAL PART

PART 2

Successful I synthesized new 1,8-naphthrydine of general structure G characterized by the presence of a carbonilic group in position 2, to verify if this shift could be create a new interaction of binding site receptor.



G

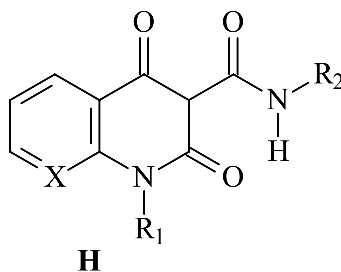
The synthesis of compounds of general structur G is outlined in Schemes 10.

The heating of 2-aminopyridin-3-carboxaldheyde with ethyl malonate and a few drops of piperidine at 90°C for 4h afforded the ethyl 2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxylate derivative **75**.[90]

The treatment of ethyl ester **75** with the appropriate amine in sealed tube for 24 h at 120°C provided the desired carboxamide derivatives **76-81**.

Successful the treatment of carboxamide derivatives **76-81** in anhydrous DMF with NaH and than with the suitable benzylchloride or aryl-alkylchloride or 4-(2-chloroethyl)-morpholine provided the desired 1,8-naphthyridin-2-one derivatives **82-93** (Scheme 10).

Moreover new 1,8-naphthrydine and quinoline derivatives characterized by the presence of a carbonilic group in positions 4 and 2, were synthesized to verify the effect of a further carbonilic group on the affinity and on the selectivity toward both CB receptor subtypes.



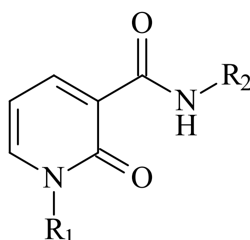
H

The synthesis of these compounds is outlined in Scheme 11. The esterification of 2-aminonicotinic acid with concentrated H_2SO_4 and CH_3OH at 80°C for 7h gave the methyl 2-aminonicotinate **94**. The heating of compound **94** with EMME at 80°C for 6h gave the derivative 1,8-naphthrydin-2,4-dione **95**. The reaction of **95** with cyclohexylamine at 120°C for 24h in sealed tube provided the carboxamide derivative **96**, which for treatment in anhydrous DMF with NaH and then with the suitable benzyl chloride or 4-(2-chloroethyl)-morpholine at 50 °C for 24h provided the desired compounds **97** and **98**.

The synthesis of quinoline compounds is reported in Scheme 12. The reaction of aniline with the triethyl methanetricarboxylate in microwave in open vessel at 210°C, P 200 W, p 200 psi for 15 min gave the carboxamide derivative **99**.

The compounds **100** and **101** were obtained from **99** by similar condition above reported for the preparation of **97** and **98**.

Finally to verify the importance of the presence of both aromatic rings in the central lipophilic bicyclic core, I have synthesized the pyridin compounds with general structure **I.**



I

The monocyclic derivatives were obtained as reported in Scheme 13, starting from 2-hydroxynicotinic acid which by reaction with concentrated H_2SO_4 and CH_3OH at 80°C in microwave, at 200 W, 100 psi and for 55 min.

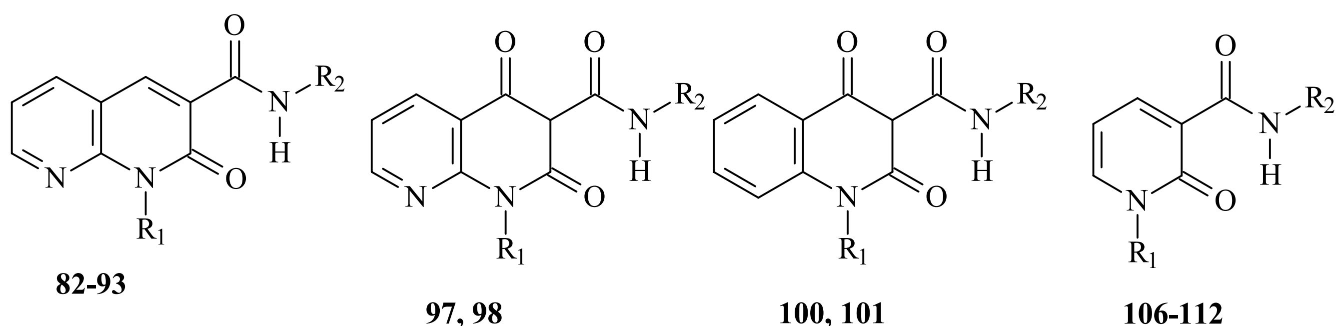
The heating of ester **102** with the appropriate amine provided the carboxamide derivatives **103-105** which were tranformed in to desired compounds **106-112** by reaction with NaH and the suitable chloro derivatives at room temperature.

Results and discussion

CB_1 and CB_2 affinity

Affinities at CB_1 and CB_2 receptors for all these compounds (**82-93, 97, 98, 100, 101** and **106-112**) were tested using membranes from HEK-293 cells transfected with either the human CB_1 and CB_2 receptors.

The CB_1 and CB_2 receptor binding assay results for these new compounds are summarized in Table 2. The Ki values of WIN-55,212-2, HU-210, AM630, JWH-133, ACEA, SR141716A and SR144528 are also included in the table as reference compounds for the CB_1 and CB_2 cannabinoid receptors.



compd	R_1	R_2	Ki CB_1 nM human	Ki CB_2 nM human	$KiCB_1/KiCB_2$
82	benzyl	cyclohexyl	560	31	18
83	p-fluorobenzyl	cyclohexyl	56	2.2	25.5
84	butyl	cyclohexyl	5600	70	80
85	benzyl	4-methylcyclohexyl	>1000	3.5	>285.7
86	ethylmorph	4-methylcyclohexyl	1000	1.9	526.3
87	ethylmorph	cyclohexyl	560	7.9	70.9
88	benzyl	β-phenylethyl	Non sol.		
89	benzyl	4-fluoro-phenylethyl	>1000	190	5.3
90	benzyl	cycloheptyl	960	22	43.6
91	p-fluorobenzyl	4-methylcyclohexyl	96	0.9	106.7
92	p-fluorobenzyl	cycloheptyl	9.6	0.7	13.7
93	ethylmorph	cycloheptyl	18	3	6
97	Ethylmorph	cyclohexyl	NT	NT	
98	benzyl	cyclohexyl	560	79	7.1
100	Ethylmorph	phenyl	NT	NT	
101	benzyl	phenyl	5600	200	2.8
106	benzyl	cyclohexyl	800	200	4
107	ethylmorph	cyclohexyl	n.d.	7900	
108	p-fluorobenzyl	cycloheptyl	40	7.9	5
109	p-fluorobenzyl	cyclohexyl	70	20	3.5
110	benzyl	cycloheptyl	56	7.9	7
111	ethylmorph	cycloheptyl	950	79	12
112	p-fluorobenzyl	4-methylcyclohexyl	NT	NT	
Win552122			21	2.1	10
HU-210			0.18	0.15	1.2
AM630			840	36	23
ACEA			5.3	95	0.06
JWH-130			680	3	227
SR141716A			16	1640	0.01
SR144528			>50000	5.4	-----

CB_1 receptor affinity

The results reported in Table 2 show that some of the compounds characterized by the presence in position 1 of *p*-fluorobenzyl or ethylmorpholin group possess an interesting affinity at the CB_1 receptor in particular compounds **83**, **91-93** and **108-110** showed a Ki value <100 nM**.** The other compounds showed very low affinity at this receptor.

CB_2 receptor affinity

The new compounds, 1,8-naphthrydin-2-one **82-93** generally showed a high affinity and selectivity for CB_2 receptor. Infact compounds **83**, **85-87**, and **91-93** showed a $KiCB_2 < 10$ nM, in particular compounds **91** and **92** showed a subnanomolar affinities. Some of these compounds possess an important selectivity on CB_2 receptor. In particular compounds **85, 86** and **91** showed a $Ki(CB_1)/Ki(CB_2)$ of >285.7, 526.3 and 106.7 respectively.

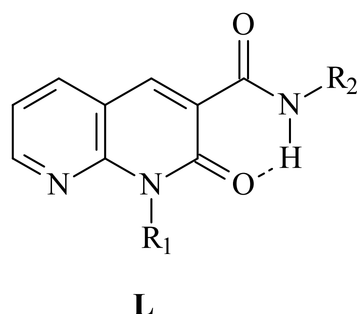
From these results it's clear that the 1,8-naphthrydin-2-one **82-93** exhibit a higher affinity and selectivity for CB_2 receptor than the corresponding 1,8-naphthrydin-4-one.

The compound **98** characterized by the presence of two carbonilic groups possess an interesting affinity for the CB_2 receptor (Ki= 79nM) with a low selectivity.

Regards the new monocyclic derivatives the compounds **108** and **110** showed a remarkable affinity for CB_2 receptor. (Ki= 7.9 nM) The compounds **109** and **111** showed a relatively good affinity (Ki CB_2 20 and 79 nM respectively.) In the light of these results it is clear that the pyridine compounds (**106-112**) represent a new classes of heterocyclic derivatives as ligands of CB_2 cannabinoid receptor.

Molecular Modeling

Studies of automated docking have showed that the compounds 1,8-naphthyridin-2-one analogously to compounds 1,8-naphthyridin-4-one form an intramolecular hydrogen bond between the carbonilic oxygen and the amidic's carbonolic form a pseudocycle planar to heterocycle ring. (structure L); moreover the amidic's carbonilic form a hydrogen bond with residual of Thr 114 (T3.37) that is not conservated in CB_1 receptor (figure 21).



L

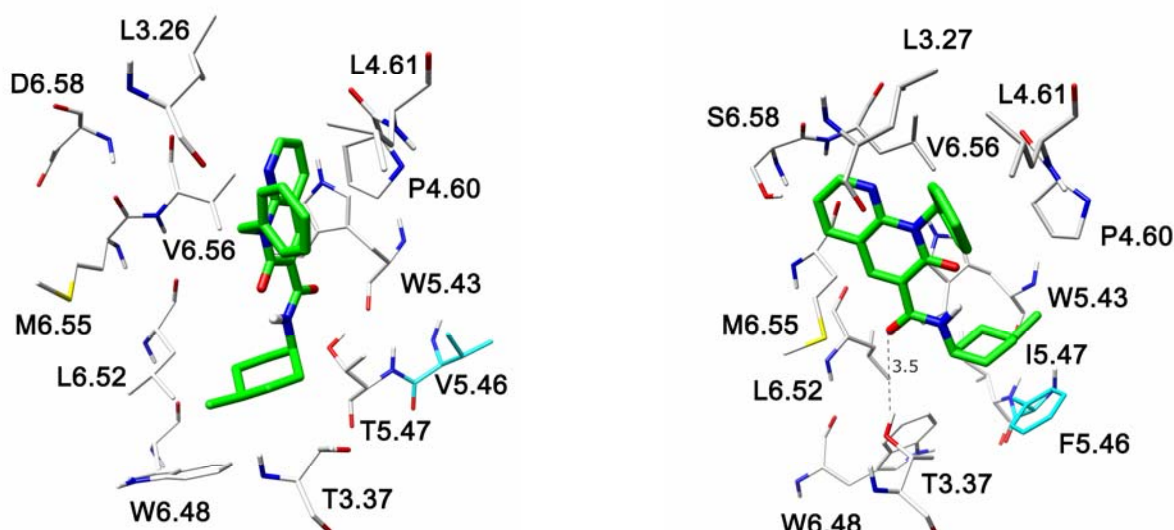


Figure 21

The results obtained for these compounds suggested that the good CB_2/CB_1selectivity and the improvement of the CB_2 affinity respect of the analogues compounds 1,8-naphthyridin-4(1H)-one seemed to be produced by the presence of a carbonilic group in position 2 that determine a different rearrangement of all molecule into the receptor, giving an interaction between the amidic's carbonilic with the residue of T3.37 (T114) in the CB_2 receptor.(see figure 22)

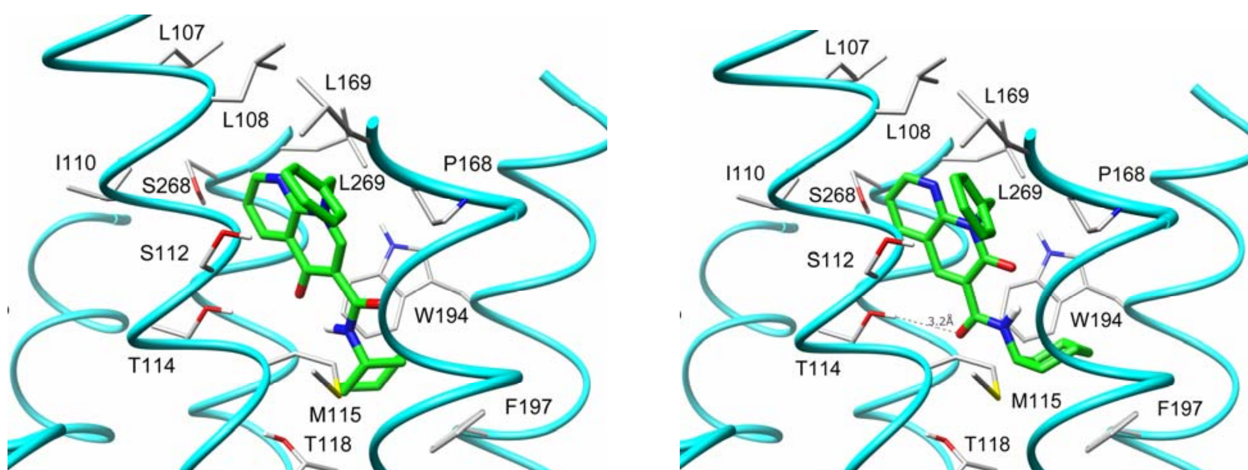
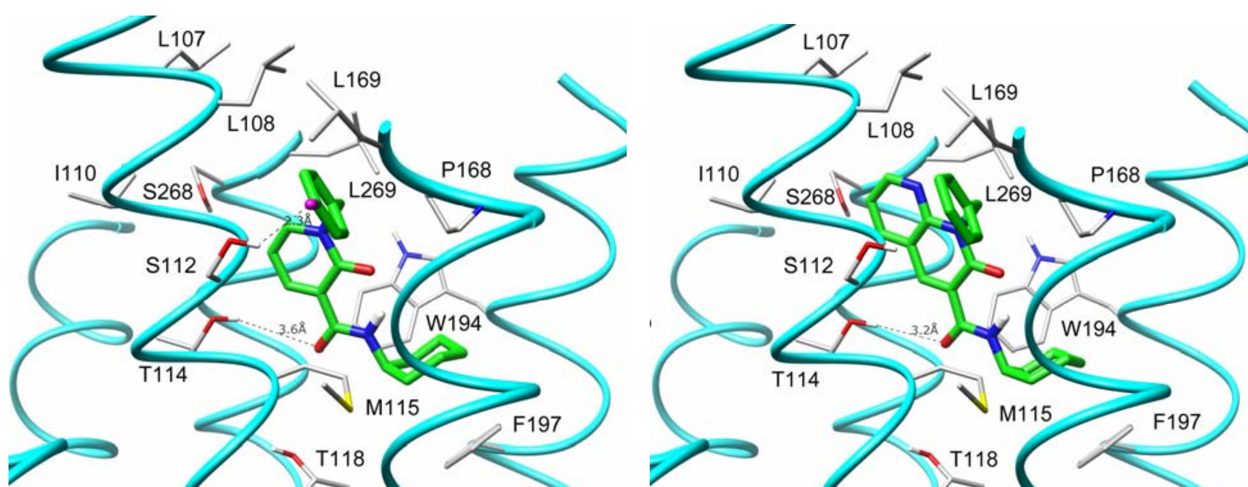


Figure 22

Finally, the virtual screening in the CB_2 receptor of monocyclic compounds (**106-112**) revealed that these ligands seem interacting like the 1,8-naphthyridin-2-one in the binding pocket of the receptor conserving the same interactions. (see figure 23)



Docking of monocyclic derivatives. Docking of naphthyridine derivatives.

Figure 23

Antinociceptive effects

The *N*-Cyclohexyl-7-methoxy1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide **(28)** which showed a high CB_2 affinity and selectivity (Ki = 11 nM, $Ki(CB_1)/Ki(CB_2) = 51$) was tested to determine antinociceptive effects in the mouse hot-plate test.[91,92] Furthermore, mouse motor coordination was evaluated in the rotarod test.[93,94] Compound **28** exhibited an antinociceptive effect at the dose of 50 mg kg^{-1} p.o. in the mouse hot-plate test 15 and 30 min after administration.

Table3. Effect of AM630 on antinociception induced by derivative **28** in the mouse hot-plate test

Treatment 1 p.o.	Treatment 2 p.o.	N° of mice	Licking latency (s)			
			Before pre treatment	After treatment		
				15 min	30 min	45 min
CMC	CMC	12	14.8±0.6	15.5±0.7	16.8±0.6	14.7±0.5
CMC	AM630 0.6 mg kg^{-1}	14	15.2±0.4	16.6±0.6	18.0±0.4	16.3±0.4
CMC	**28** 10 mg kg^{-1}	10	14.5±0.8	17.1±0.9	18.5±1.0	16.7±0.6
CMC	**28** 30 mg kg^{-1}	10	14.4±0.6	16.6±0.8	19.5±1.0	17.4±0.6
CMC	**28** 50 mg kg^{-1}	13	15.1±0.5	19.2±0.6*	22.5±0.9*	18.2±0.7
28 50 mg kg^{-1}	AM630 0.6 mg kg^{-1}	10	16.2±0.7	18.1±0.7	19.2±0.4^	17.6±0.6

* $P<0.05$ *vs.* CMC-treated mice; ^$P<0.05$ vs 28-treated mice. AM-630 and 28 were administered simultaneously.

Treatment with AM630, a selective CB_2 antagonist,[95] at the dose of 0.6 mg kg^{-1} p.o. partially reverted the antinociceptive effect of **28** in correspondence of its peak of efficacy.

The dose of AM630 employed (0.6 mg kg^{-1} p.o.) did not modify by itself mouse pain threshold in the presence of a thermal stimulus and represents the minimal dose able to reduce the antinociception induced by compound **28**

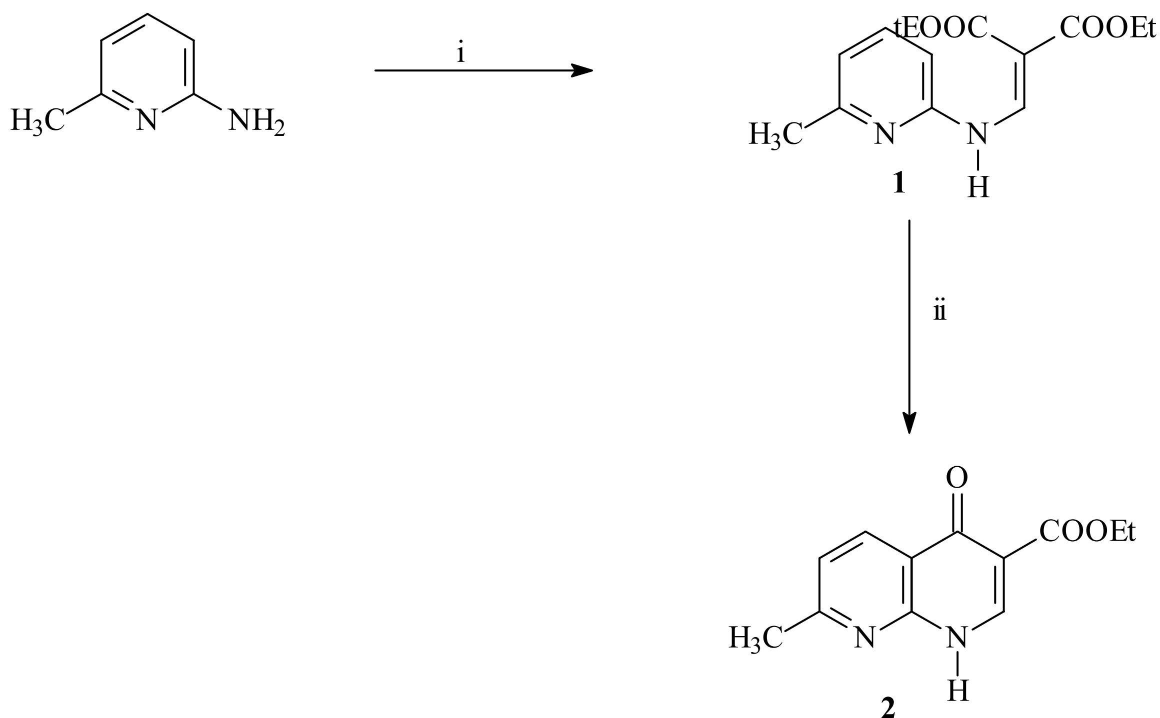
It is interesting to note this compound at the antinociceptive dose did not modify mouse motor coordination evaluated in the rotarod test (see Table 4).

Table 4. Effect of 28 on motor coordination in the mouse rota-rod test

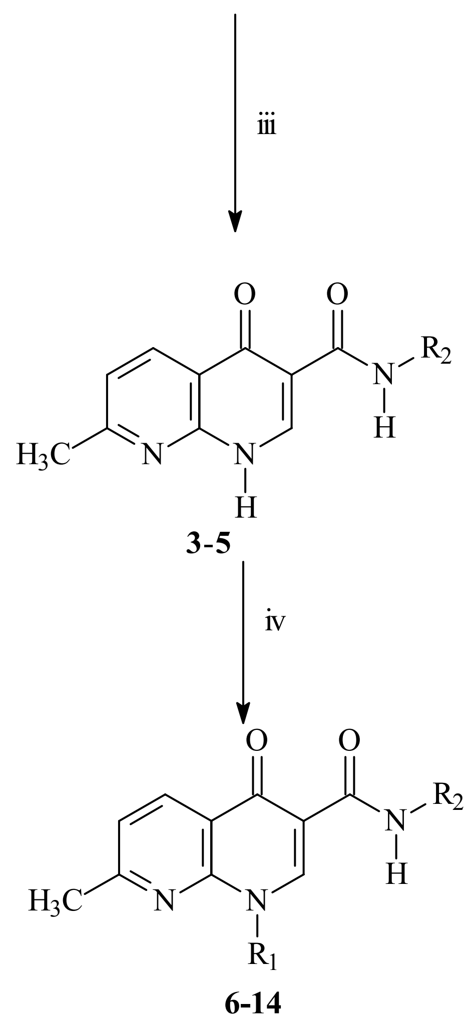
Treatment p.o.	N° of mice	N° of falls			
		Before treatment	After treatment		
			15 min	30 min	45 min
CMC	12	3.3±0.2	0.8±0.2	0.3±0.2	0.2±0.2
28 50 mg kg^{-1}	13	4.0±0.7	0.7±0.2	0.2±0.2	0.2±0.2

Scheme of synthesis

SCHEME 1

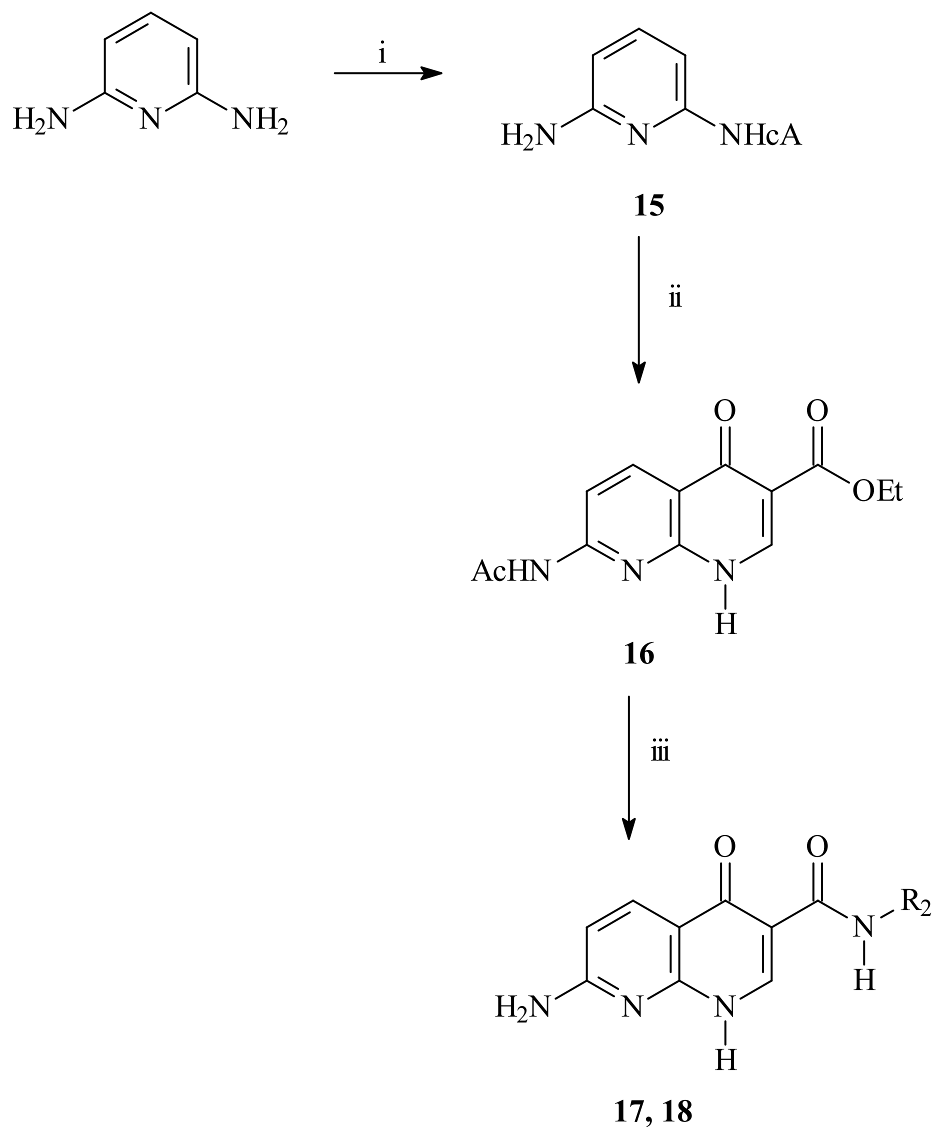


compd	R_1	R_2
3	H	4-methylcyclohexyl
4	H	cycloheptyl
5	H	cyclohexyl
6	benzyl	4-methylcyclohexyl
7	p-fluorobenzyl	4-methylcyclohexyl
8	o-fluorobenzyl	4-methylcyclohexyl
9	benzyl	cycloheptyl
10	p-fluorobenzyl	cycloheptyl
11	o-fluorobenzyl	cycloheptyl
12	1-ethyl-4-phenylpip	cyclohexyl
13	phenethyl	cyclohexyl
14	p-methoxybenzyl	cyclohexyl



Reagents and Conditions: (i) EMME, 110°C, 2h; (ii) Dowtherm A, 1h; (iii) R_2NH_2, 120°C, 24h; (iv) DMF, NaH, R_1Cl.

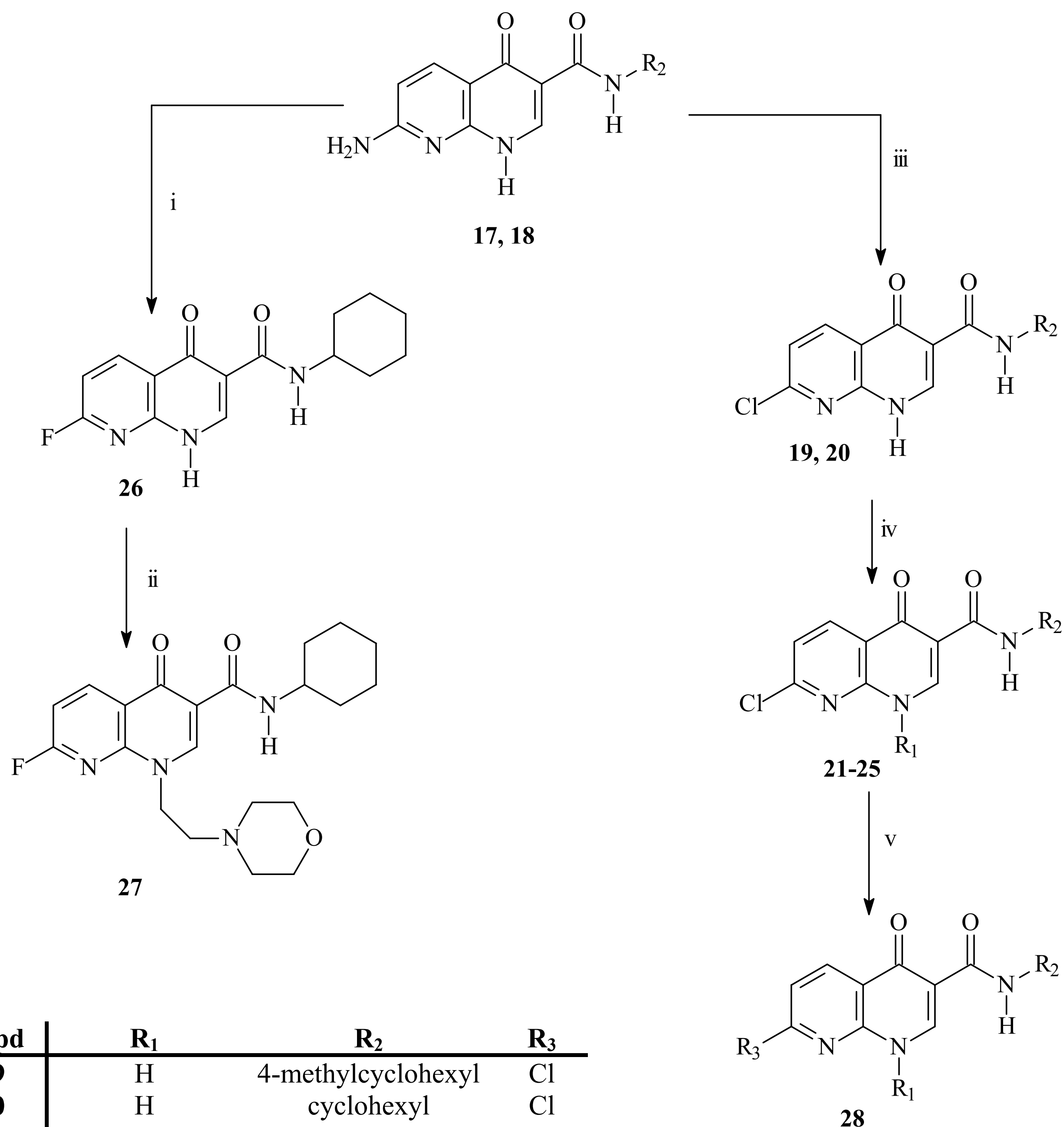
SCHEME 2



compd	R_2
17	4-methylcyclohexyl
18	cyclohexyl

Reagents and Conditions: (i) CH_3COCl, r.t. 2h; (ii) Dowtherm A, EMME, 200°C, 15 min. (iii) R_2NH_2, 120°C, 24h.

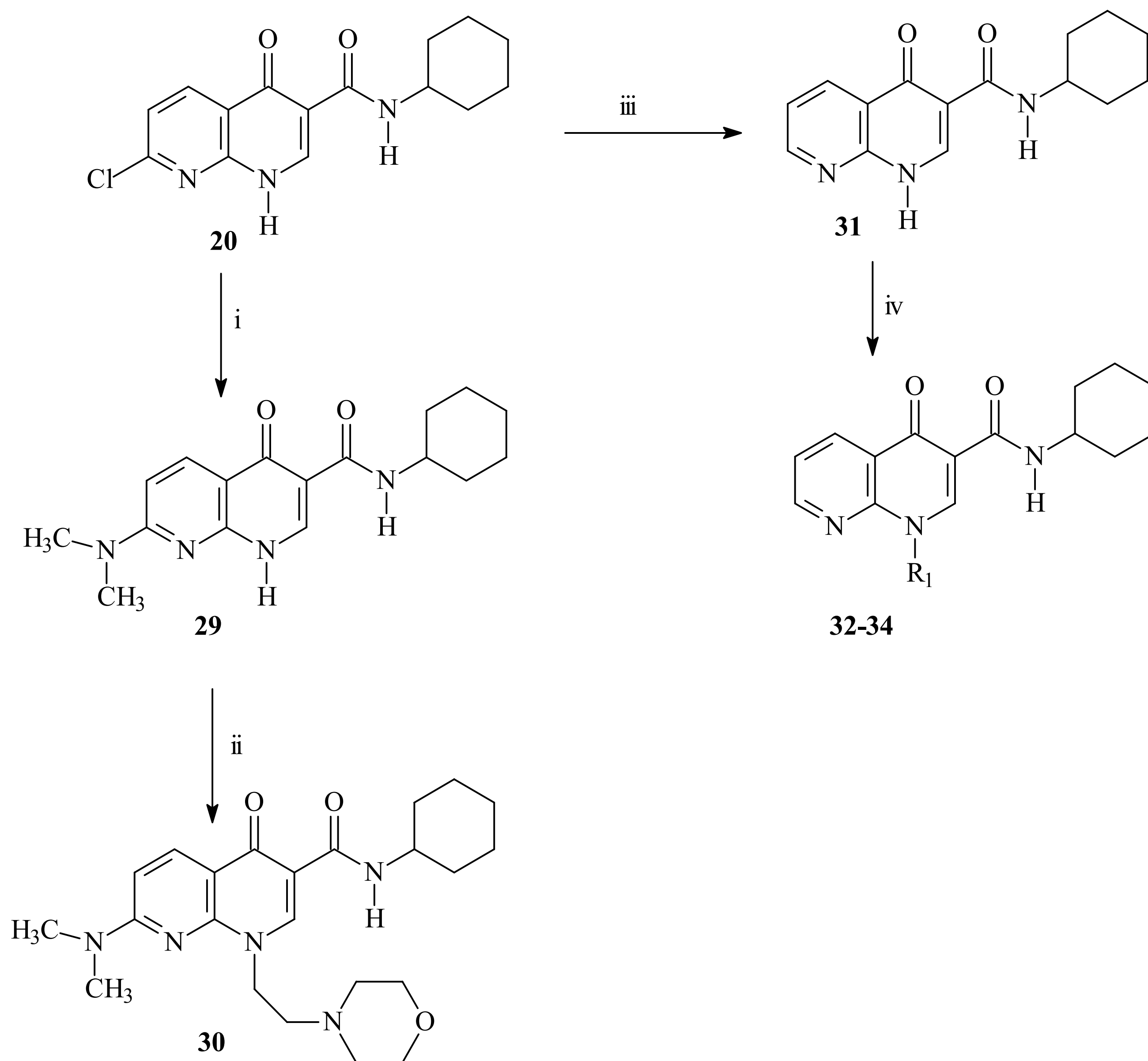
SCHEME 3



compd	R_1	R_2	R_3
19	H	4-methylcyclohexyl	Cl
20	H	cyclohexyl	Cl
21	ethylmorph	cyclohexyl	Cl
22	benzyl	cyclohexyl	Cl
23	p-fluorobenzyl	cyclohexyl	Cl
24	o-fluorobenzyl	cyclohexyl	Cl
25	ethylmorph	4-methylcyclohexyl	Cl
28	benzyl	cyclohexyl	OCH_3

Reagents and Conditions: (i) thetrafluoroboric acid, $NaNO_2$, r.t., 1h 30 min; (ii) DMF, NaH, 2-chloroethylmorpholine; (iii)$NaNO_2$, HCl, 40°C, 3h; (iv)DMF, NaH, R_1Cl; (v) CH_3ONa, 80°C, 5h 30 min.

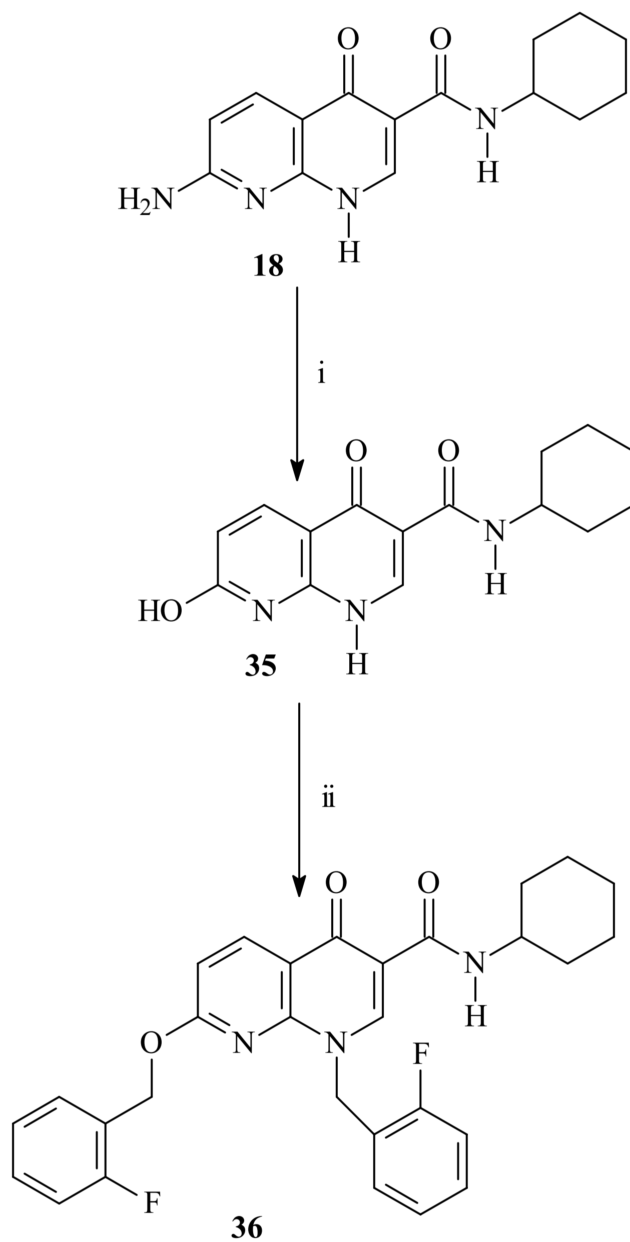
SCHEME 4



compd	R_1	R_2
32	p-fluorobenzyl	cyclohexyl
33	benzyl	cyclohexyl
34	ethylmorph	cyclohexyl

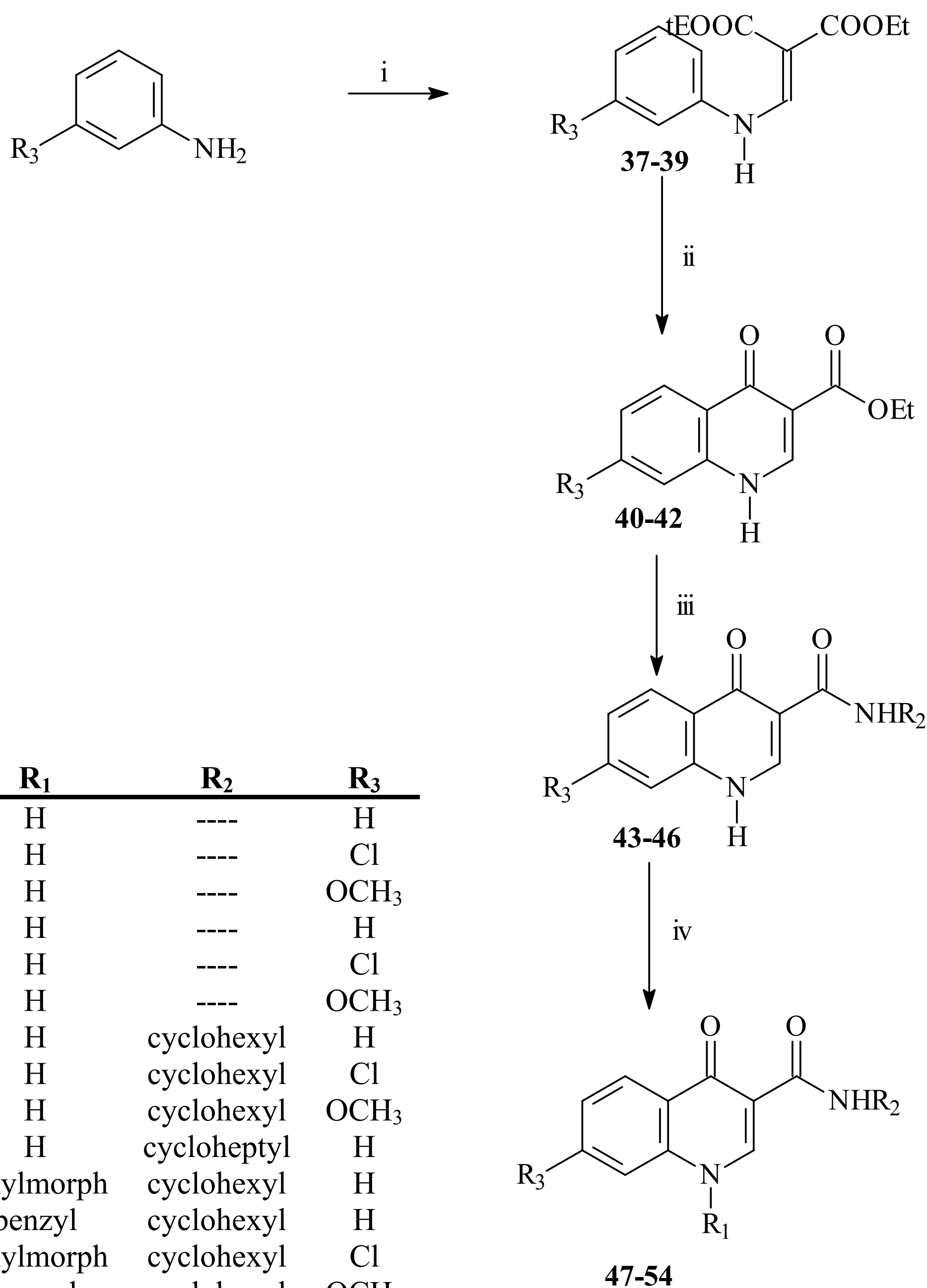
Reagents and Conditions: (i) dimethylamine,120°C, 24h; (ii) DMF, NaH, 2-chloroethylmorpholine; (iii) MeOH, H_2/Pd, r.t., 3h; (iv) DMF, NaH, R_1Cl.

SCHEME 5



Reagents and Conditions: (i) $NaNO_2$, H_2SO_4; (ii) DMF, NaH, o-fluorobenzylchloride

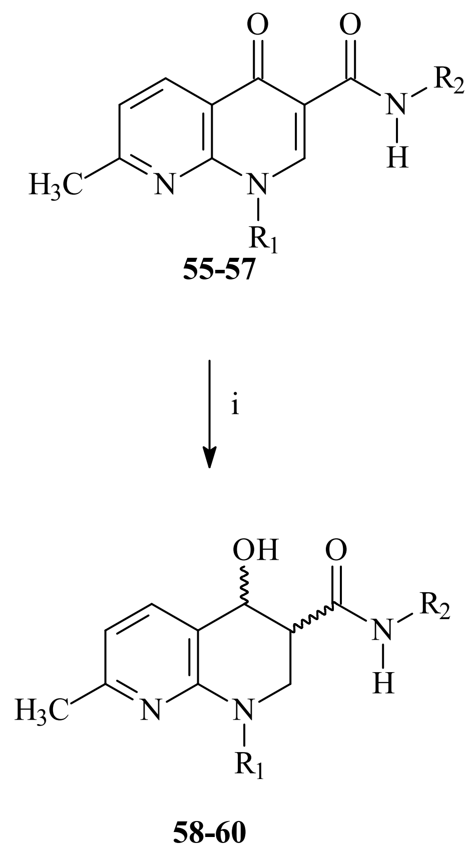
SCHEME 6



compd	R_1	R_2	R_3
37	H	----	H
38	H	----	Cl
39	H	----	OCH_3
40	H	----	H
41	H	----	Cl
42	H	----	OCH_3
43	H	cyclohexyl	H
44	H	cyclohexyl	Cl
45	H	cyclohexyl	OCH_3
46	H	cycloheptyl	H
47	ethylmorph	cyclohexyl	H
48	benzyl	cyclohexyl	H
49	ethylmorph	cyclohexyl	Cl
50	benzyl	cyclohexyl	OCH_3
51	ethylmorph	cyclohexyl	OCH_3
52	benzyl	cycloheptyl	H
53	ethylmorph	cycloheptyl	H
54	benzyl	cyclohexyl	Cl

Reagents and Conditions: (i) EMME, 130°C, 5h; (ii) Dowtherm A, reflux, 1h; (iii) R_2NH_2, 120°C, 24h; (iv) DMF, NaH, R_1Cl.

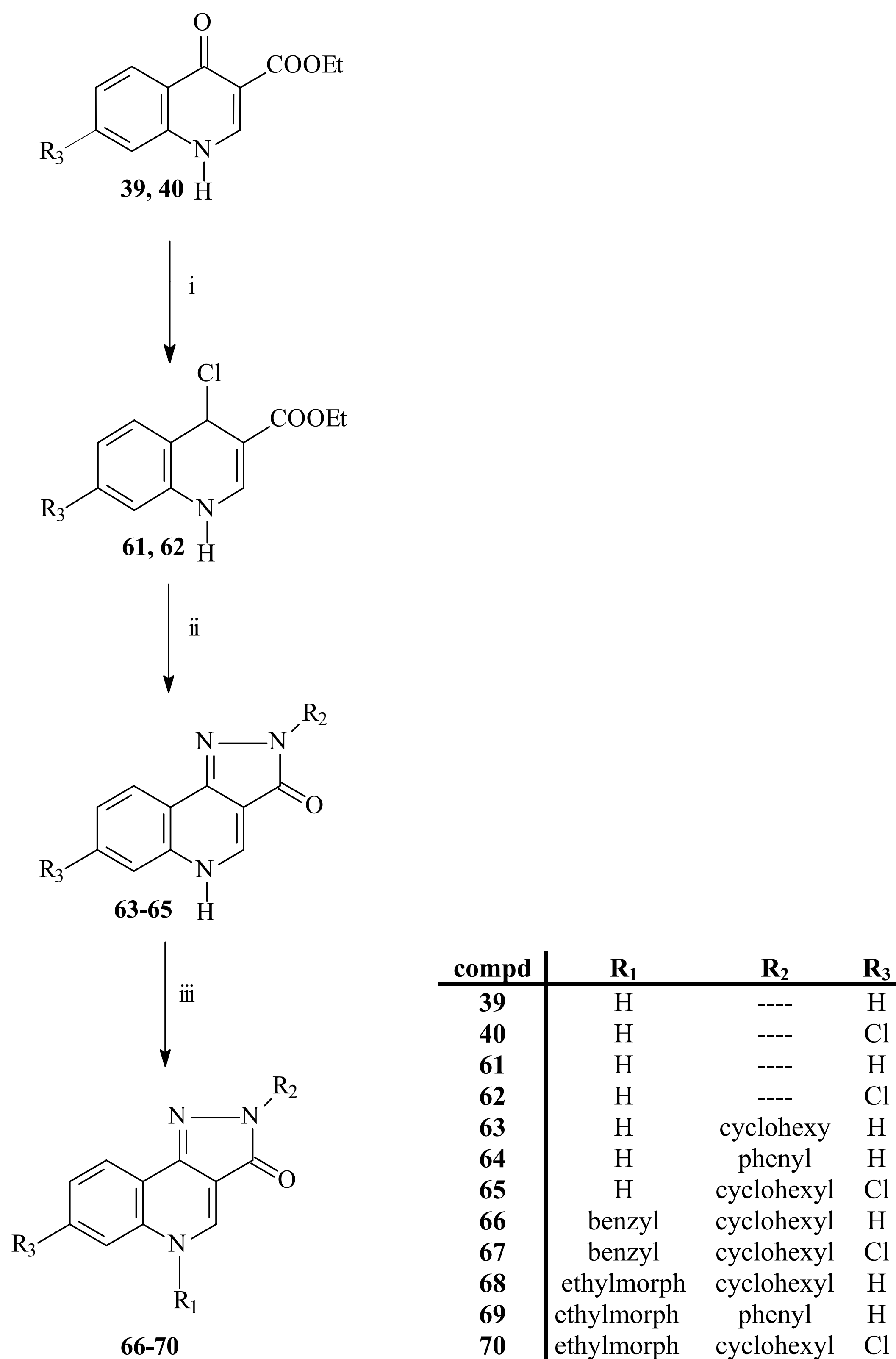
SCHEME 7



compd	R_1	R_2	R_3
55	o-fluorobenzyl	cyclohexyl	methyl
56	ethylmorph	4-methylcyclohexyl	methyl
57	benzyl	cyclohexyl	methyl
58	o-fluorobenzyl	cyclohexyl	methyl
59	ethylmorph	4-methylcyclohexyl	methyl
60	benzyl	cyclohexyl	methyl

Reagents and Conditions: (i) EtOH ass., $NaBH_4$.

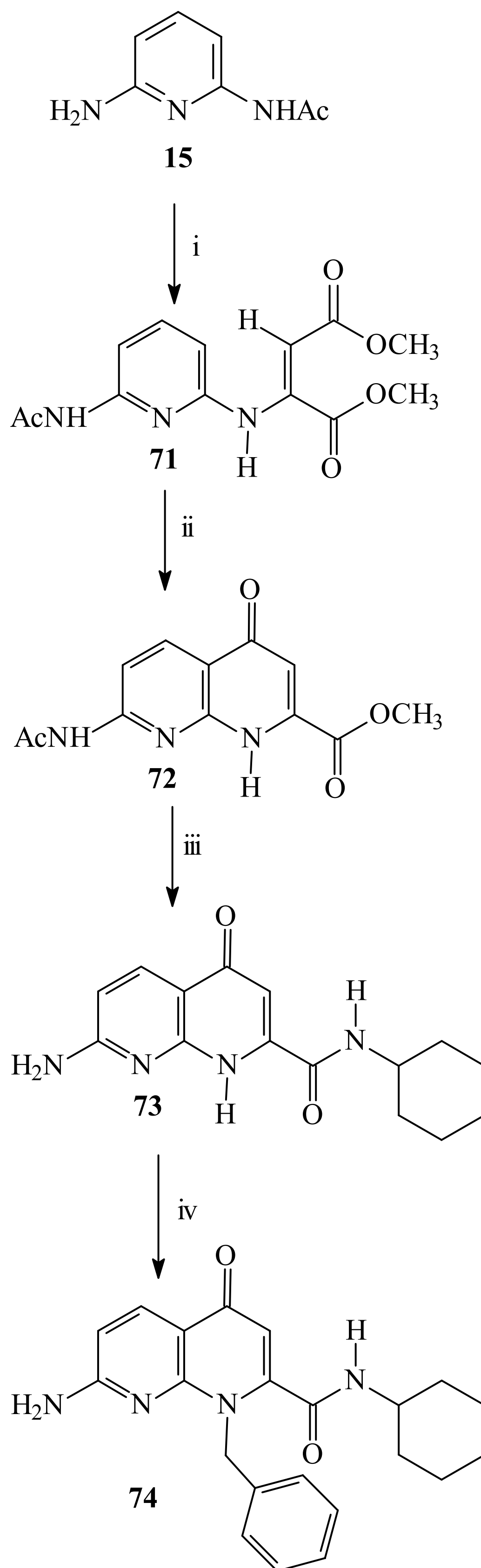
SCHEME 8



compd	R_1	R_2	R_3
39	H	----	H
40	H	----	Cl
61	H	----	H
62	H	----	Cl
63	H	cyclohexy	H
64	H	phenyl	H
65	H	cyclohexyl	Cl
66	benzyl	cyclohexyl	H
67	benzyl	cyclohexyl	Cl
68	ethylmorph	cyclohexyl	H
69	ethylmorph	phenyl	H
70	ethylmorph	cyclohexyl	Cl

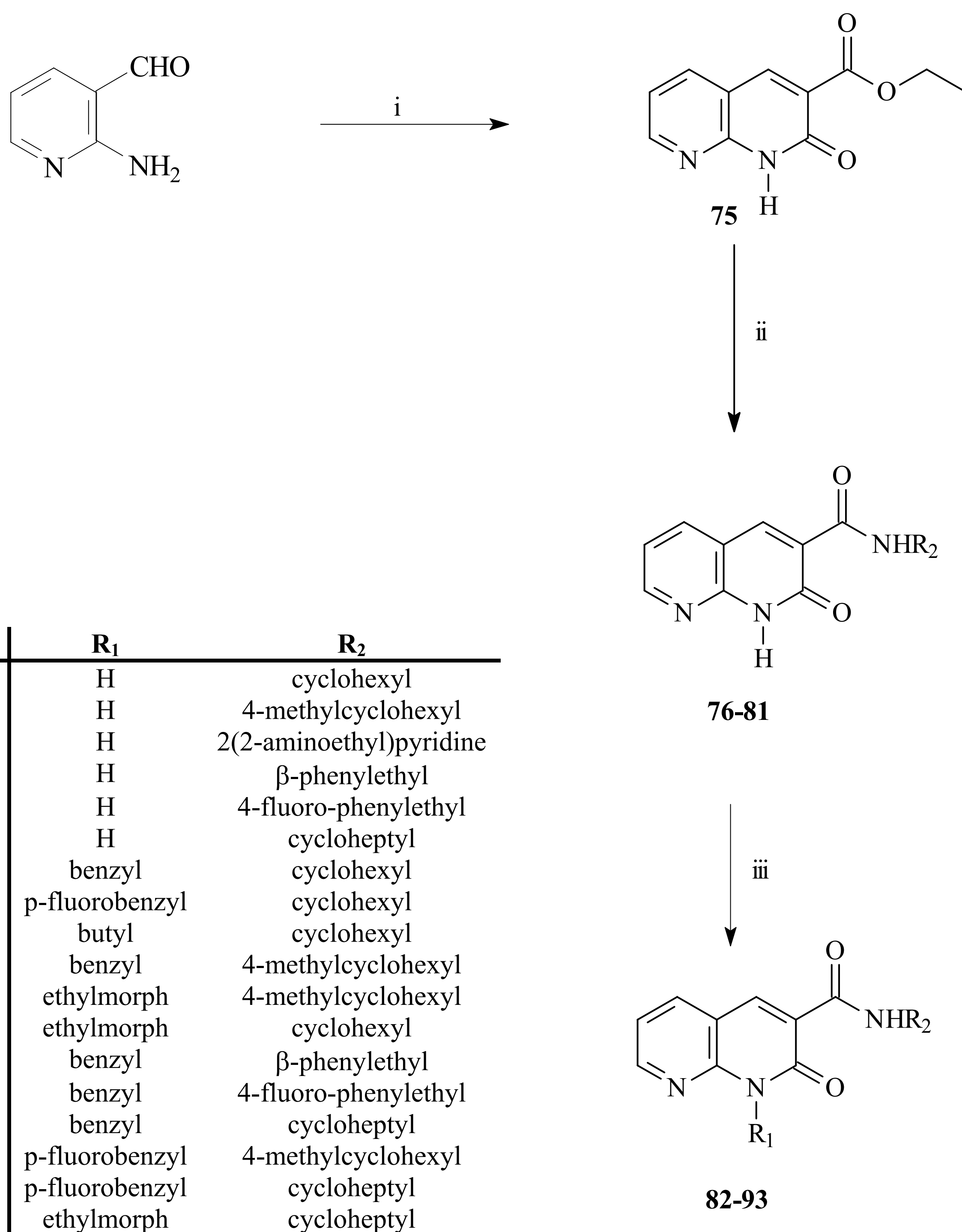
Reagents and Conditions: (i) $POCl_3$, 120°C, 14 min; (ii) R_2NHNH_2, p-xilene 150°C, 13h; (iii) DMF, NaH, R_1Cl.

SCHEME 9



Reagents and Conditions: (i) acethylendicarboxylic acid dimethyl ester, 180°C, 24h; (ii)Dowtherm A reflux, 1h; (iii) cyclohexylamine 120°C, 24h; (iv) DMF, NaH, benzylchloride.

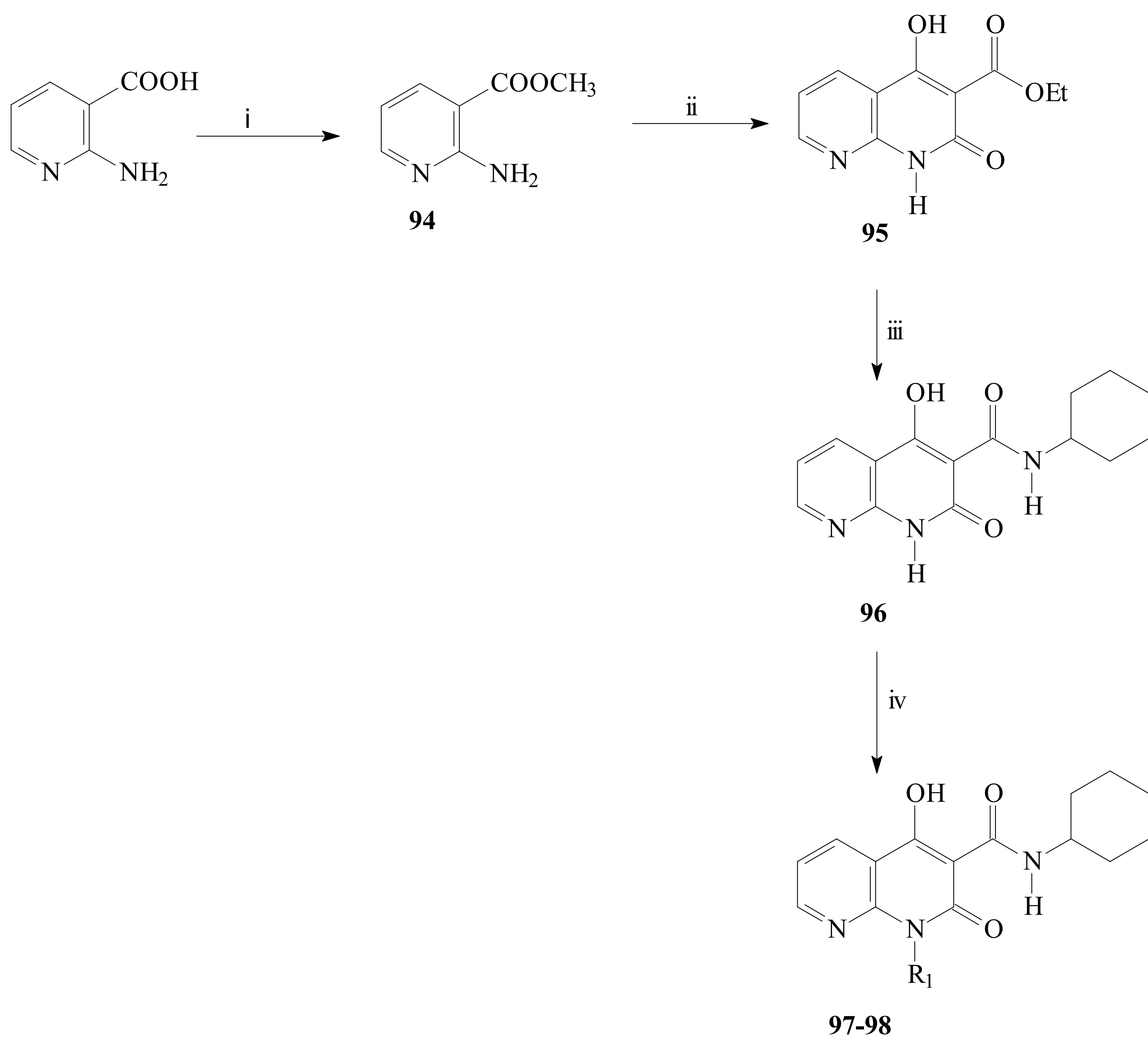
SCHEME 10



compd	R_1	R_2
76	H	cyclohexyl
77	H	4-methylcyclohexyl
78	H	2(2-aminoethyl)pyridine
79	H	β-phenylethyl
80	H	4-fluoro-phenylethyl
81	H	cycloheptyl
82	benzyl	cyclohexyl
83	p-fluorobenzyl	cyclohexyl
84	butyl	cyclohexyl
85	benzyl	4-methylcyclohexyl
86	ethylmorph	4-methylcyclohexyl
87	ethylmorph	cyclohexyl
88	benzyl	β-phenylethyl
89	benzyl	4-fluoro-phenylethyl
90	benzyl	cycloheptyl
91	p-fluorobenzyl	4-methylcyclohexyl
92	p-fluorobenzyl	cycloheptyl
93	ethylmorph	cycloheptyl

Reagents and Conditions: (i) EMME, 90°C, 24h; (ii) R_2NH_2, 120°C, 24h; (iii) DMF, NaH, R_1Cl.

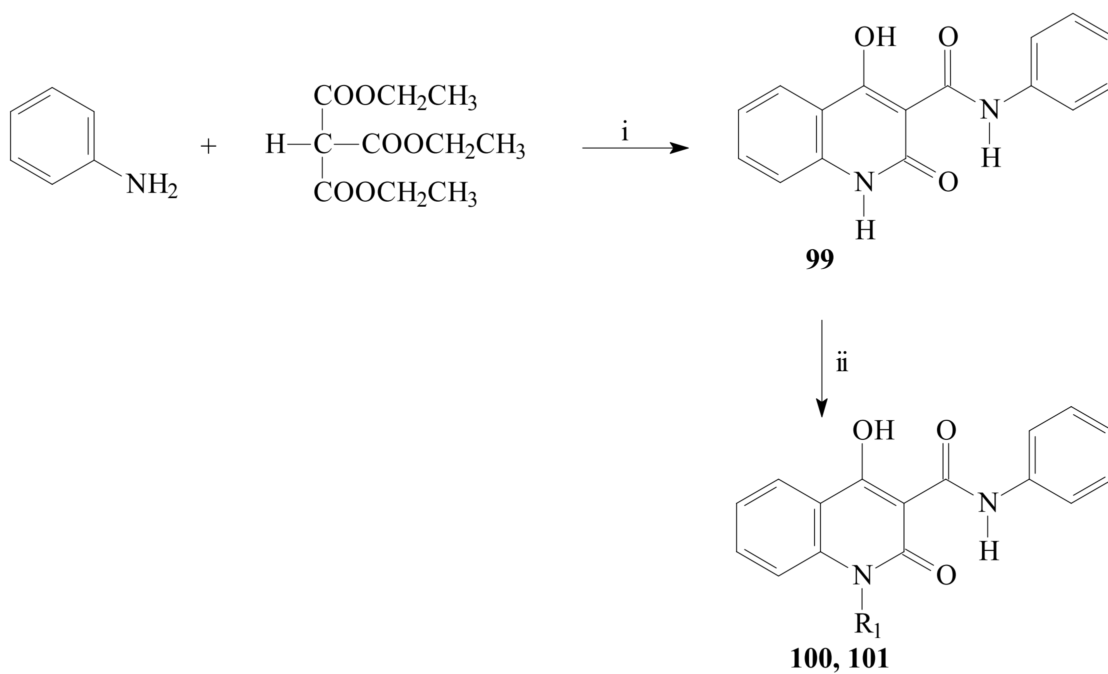
SCHEME 11



compd	R_1
97	Ethylmorph
98	benzyl

Reagents and Conditions: (i) MeOH, H_2SO_4, 80°C, 7h; (ii) EMME, Na, EtOH, reflux, 6h. (iii) cyclohexylamine 120°, 24h; (iv) DMF, NaH, R_1Cl, 50°C, 24h.

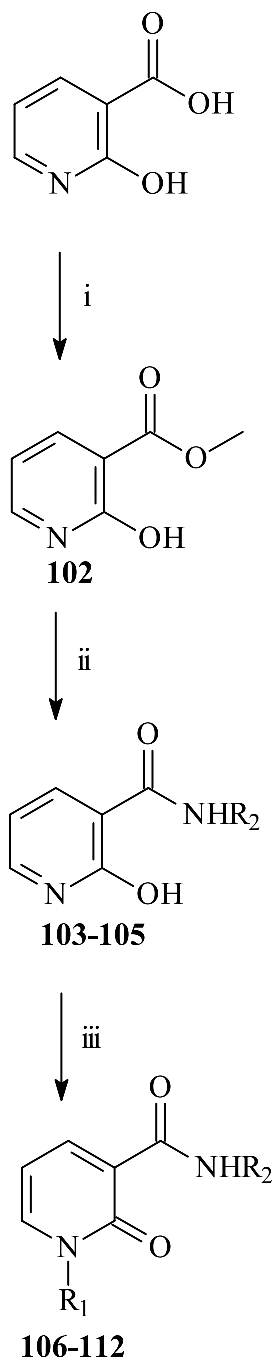
SCHEME 12



compd	R_1
100	Ethylmorph
101	benzyl

Reagents and Conditions: (i) MW, open vessel, 210°C, 15 min, 200W,;(ii) DMF, NaH, R_1Cl.

SCHEME 13



compd	R_1	R_2
103	H	cyclohexyl
104	H	cycloheptyl
105	H	4-methylcyclohexyl
106	benzyl	cyclohexyl
107	ethylmorph	cyclohexyl
108	p-fluorobenzyl	cycloheptyl
109	p-fluorobenzyl	cyclohexyl
110	benzyl	cycloheptyl
111	ethylmorph	cycloheptyl
112	p-fluorobenzyl	4-methylcyclohexyl

Reagents and Conditions: (i) MW, MeOh, H_2SO_4, 200W, 100psi, 80°C, 55 min; (ii) R_2NH_2, in MW for compound 103, in oil bath for compounds 104 and 105; (iii) DMF, NaH, R_1Cl, r.t..

Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra in Nujol mulls were recorded on an ATI Mattson Genesis series FTIR spectrometer. ^{1}H NMR spectra were recorded with a Bruker AC-200 spectrometer in δ units from TMS as an internal standard. Mass spectra were obtained with a Hewlett-Packard MS system 5988. Elemental analysis results (C, H, N) were within (0.4% of theoretical values and were performed on a Carlo Erba elemental analyzer model 1106 apparatus.

Experimental part scheme 1

2-(*N*-diethylmalonate)-amino-6-methylpyridine 1
A mixture of 0.1 mmol of 2-amino-6-methylpyridine and 0.1 mmol of EMME was heated at 110°C for 2 h. After cooling the mixture solidify to give the diethyl esther 1.
Yield: 98%; mp: 105-107° C;

ethyl-7-methyl-4-oxo-1,8-naphthyrydin-3-carboxylate 2
A mixture of 0.018 mol of 2-(*N*-diethylmalonate)-amino-6-methylpyridine and ml 25 of Dowtherm A was heated at reflux for 30'. After cooling the solid obtained was washed with petroleum ether and collected by filtration.
Yield: 67%; mp: 233-235°C; ^{1}H NMR: DMSO δ 8.44 (s, 1H, H_2), 8.38 (d, 1H, H_5), 7.35 (d, 1H, H_6), 4.20 (q, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.26 (t, 3H, CH_3).

General procedure for the synthesis of 7-methyl-1,8-naphthyridin-3-carboxyamide derivatives 3-5
A mixture of 1 mmol of 7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxylic acid ethyl ester and 10 mmol of the appropriate amine was heated in sealed tube at 120 °C for 24 h. After cooling, the reaction mixture was treated with ethyl ether to give a solid residue which was collected by filtration and purified by crystallization.

7-Methyl-*N*-(4-methylcyclohexyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 3
Yield 56%; mp 260–270 °C (cyclohexane); ^{1}H NMR: DMSO δ 10.55, 10.16 (2d, 1H, NH), 8.75, 8.74 (2s, 1H, H_2), 8.48, 8.43 (2d, 1H, H_5), 7.29, 7.25 (2d, 1H, H_6), 4.10 (m, 1H, $H_{1'}$), 3.70 (m, 1H, $H_{4'}$), 2.62 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 1.87–1.68 (m, 8H, cyclohexyl). Anal. $C_{17}H_{21}N_3O_2$ C, H, N.

***N*-Cycloheptyl-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamide 4**
Yield 70%; mp 270–272 °C (ethyl acetate); ^{1}H NMR: DMSO δ 9.93 (d, 1H, NH), 8.61 (s, 1H, H_2), 8.49 (d, 1H, H_5), 7.42 (d, 1H, H_6), 4.15 (m, 1H, CH), 2.62 (s, 3H, CH_3), 1.85–1.56 (m, 12H, cycloheptyl). Anal. $C_{17}H_{21}N_3O_2$ C, H, N.

***N*-Cyclohexyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 5**
Yield 85%; mp 302–304 °C (ethyl acetate); ^{1}H NMR: DMSO δ 10.31 (d, 1H, NH), 8.74 (s, 1H, H_2), 8.43 (d, 1H, H_5), 7.25 (d, 1H, H_6), 3.90 (brs, 1H, CH), 2.57 (s, 3H, CH_3), 1.81–1.06 (m, 10H, cyclohexyl). Anal. $C_{16}H_{19}N_3O_2$ C, H, N.

General Procedure for the Synthesis of N1-Substituted 1,8-Naphthyridine Derivatives 6-14
An amount of 1.2 mmol of NaH was added to a solution of 1 mmol of carboxamide derivatives **3-5** in 10 mL of dry *N,N*dimethylformamide. After 1 h, the appropriate chloride (1 mmol) was added and the mixture was stirred for 24 h at room temperature for compounds **6-11**, **12**, or at 50 °C for compounds **13**, **14**. The reaction mixture, after cooling was treated with water, and the precipitate formed was collected by filtration.

***N*-(4-Methylcyclohexyl)-1-benzyl-7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 6.**
Yield 80%; mp 239-241 °C (crystallized from ethyl acetate); MS *m/z* 389 (M+); ^{1}H NMR: *δ* 10.20, 9.75 (2d, 1H, NH), 9.08, 9.06 (2s, 1H, H_2), 8.52, 8.60 (2d, 1H, H_5), 7.48 (d, 1H, H_6), 7.33 (m, 5H, Ar), 5.80 (s, 2H, CH_2), 4.12, 3.69 (2m, 1H, CH), 2.63 (s, 3H, CH_3), 2.20-0.86 (m, 12H, cyclohexyl + CH_3). Anal. $C_{24}H_{27}N_3O_2$ C, H, N.

N-(4-Methylcyclohexyl)-1-(p-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamide 7.

Yield 66%; mp 167-169 °C (crystallized from *n*-hexane); MS *m/z* 407 (M+); ^{1}H NMR: δ 10.18, 9.77 (2d, 1H, NH), 9.12, 9.10 (2s, 1H, H_2), 8.59, 8.52 (2d, 1H, H_5), 7.50 (m, 3H, Ar + H_6), 7.16 (m, 2H, Ar), 5.77 (s, 2H, CH_2), 4.18, 3.70 (2m, 1H, CH), 2.65 (s, 3H, CH_3), 1.98-0.86 (m, 12H, cyclohexyl + CH_3). Anal. $C_{24}H_{26}FN_3O_2$ C, H, N.

N-(4-Methylcyclohexyl)-1-(o-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamide 8.

Yield 60%; mp 183-185 °C (crystallized from *n*-hexane); MS *m/z* 407 (M+); ^{1}H NMR: δ 10.18, 9.77 (2d, 1H, NH), 9.09, 9.07 (2s, 1H, H_2), 8.58, 8.52 (2d, 1H, H_5), 7.46 (d, 1H, H_6), 7.36-7.14 (m, 4H, Ar), 5.81 (s, 2H, CH_2), 4.15, 3.70 (2m, 1H, CH), 2.61 (s, 3H, CH_3), 2.00-0.86 (m, 12H, cyclohexyl + CH_3). Anal. ($C_{24}H_{26}FN_3O_2$) C, H, N.

N-Cycloheptyl-1-benzyl-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamide 9.

Yield 58%; mp 198-200 °C (crystallized from cyclohexane); MS *m/z* 389 (M+); ^{1}H NMR: δ 9.88 (d, 1H, NH), 9.07 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.48 (d, 1H, H_6), 7.33 (m, 5H, Ar), 5.79 (s, 2H, CH_2), 4.15 (m, 1H, CH), 2.64 (s, 3H, CH_3), 1.85-1.39 (m, 12H, cycloheptyl). Anal. $C_{24}H_{27}N_3O_2$ C, H, N.

N-Cycloheptyl-1-(p-fluorobenzyl)-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamide 10.

Yield 75%; mp 194-196 °C (crystallized from hexane); MS *m/z* 407 (M+); ^{1}H NMR: δ 9.91 (d, 1H, NH), 9.10 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.48 (m, 3H, Ar+H_6), 7.16 (m, 2H, Ar), 5.77 (s, 2H, CH_2), 4.15 (m, 1H, CH), 2.64 (s, 3H, CH_3), 1.85-1.39 (m, 12H, cycloheptyl). Anal. $C_{24}H_{26}FN_3O_2$ C, H, N.

N-Cycloheptyl-1-(o-fluorbenzyl)-7-methyl-1,8-naphthyridin-4(1H)-on-3-carboxamid 11.

Yield 63%; mp 188-189 °C (crystallized from cyclohexane); MS *m/z* 407 (M+); ^{1}H NMR: δ 9.90 (d, 1H, NH), 9.07 (s, 1H, H_2), 8.55 (d, 1H, H_5), 7.46 (d, 1H, H_6), 7.32-7.14 (m, 4H, Ar), 5.81 (s, 2H, CH_2), 4.15 (m, 1H, CH), 2.61 (s, 3H, CH_3), 1.85-1.39 (m, 12H, cycloheptyl). Anal. $C_{24}H_{26}FN_3O_2$ C, H, N.

N-Cyclohexyl-7-methyl-1-[2-(4-phenylpiperazin-1-yl)ethyl]-1,8-naphthyridin-4(1H)-on-3-carboxamide 12.

Purified by flash chromatography (ethyl acetate/hexane/triethylamine, 10:1:0.2), yield 0.120 g, 25%; mp 147-149 °C (crystallized from hexane); MS *m/z* 473 (M+); ^{1}H NMR: δ 9.87 (d, 1H, NH), 8.94 (s, 1H, H_2), 8.53 (d, 1H, H_5), 7.46 (d, 1H, H_6), 7.18 (m, 2H, Ar), 6.90-6.71 (m, 3H, Ar), 4.70 (m, 2H, CH_2), 3.80 (m, 1H, CH), 3.03 (m, 4H, piperazinyl), 2.73 (m, 2H, CH_2), 2.66 (s, 3H, CH_3), 2.60 (m, 4H, piperazinyl), 1.85-1.08 (m, 10H, cyclohexyl). Anal. $C_{28}H_{35}N_5O_2$ C, H, N

N-Cyclohexyl-7-methyl-1-phenethyl-1,8-naphthyridin-4(1H)-on-3-carboxamide 13.

Purified by flash chromatography (ethyl acetate/hexane, 2:3), yield 28%; mp 148-150 °C (crystallized from hexane); MS *m/z* 389 (M+); ^{1}H NMR: δ 9.86 (d, 1H, NH), 8.85 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.48 (d, 1H, H_6), 7.24 (m, 5H, Ar), 4.76 (m, 2H, CH_2), 3.90 (m, 1H, CH), 3.10 (m, 2H, CH_2), 2.69 (s, 3H, CH_3), 1.85-1.23 (m, 10H, cyclohexyl). Anal. $C_{24}H_{27}N_3O_2$ C, H, N.

***N*-Cyclohexyl-7-methyl-1-(4-methoxybenzyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 14.**

Purified by flash chromatography (ethyl acetate/hexane, 2: 1), yield 70%; mp 170-172 °C (crystallized from hexane); MS *m*/*z* 405 (M+); ^{1}H NMR: δ 9.90 (d, 1H, NH), 9.05 (s, 1H, H_2), 8.54 (d, 1H, H_5), 7.47 (d, 1H, H_6), 7.35 (d, 2H, Ar), 6.89 (d, 2H, Ar), 5.70 (s, 2H, CH_2), 3.80 (m, 1H, CH), 3.70 (s, 3H, OCH_3), 2.67 (s, 3H, CH_3), 1.90-1.30 (m, 10H, cyclohexyl). Anal. $C_{24}H_{27}N_3O_3$ C, H, N.

Experimental part scheme 2

2-acetylamino-6-aminopyridine 15.

To a mechanically stirred solution of 2 g (0.018 mol) of 2,6-diaminopyridine in 9 ml of dioxane was added at 5°C a solution of 0.43 ml (0.009 mol) of acethyl chloride and ml 1,5 of dioxane dropwise in 30 min.Succesfuld the reaction was stirring for 2 h at room temperature. The solid obtained was filtered and the dioxane obtained was evaporated to give a solid which was purificated by flash chromatography (ethyl acetate).

Yield: 34%; mp: 91-93° C; ^{1}H NMR: DMSO δ 7.71 (s, 1H, NH), 7.32 (m, 2H, Ar), 6.15 (d, 1H, Ar), 5.59 (s, 2H, NH_2), 2.11 (s, 3H, CH_3).

7-acetamido-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid ethyl ester 16

A mixture of 0.5 g of monoacetyl derivative **15** with ml 1 of EMME in 10 ml Dowtherm A at 200°C for 15 mins. After cooling the solid obtained was collected by filtration and washed with petroleum ether and ethanol to gave a 7-acetamido-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid ethyl ester **16.**

Yield: 78,55%; mp:298-300°C; ^{1}H NMR: DMSO δ 12.05 (d, 1H, NH), 10.80 (s, 1H, Ar), 8.45 (d, 1H, Ar), 8.13 (d, 1H, Ar), 4.22 (q, 2H, CH_2), 2.18 (s, 3H, CH_3), 1.26 (t, 3H, CH_3).

General procedure to the synthesis of the 7-Amino-*N*-substituted-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 17, 18.

A mixture of 1,8-naphthyridine-3-carboxylic acid ethyl ester **16**[81] (0.276 g, 1 mmol) and 10 mmol of suitable amine was heated in sealed tube at 120°C for 24 h. After cooling, the reaction mixture was treated with ethyl ether to give a solid residue, which was collected by filtration and purified by crystallization from ethyl acetate to obtain the compounds 17 and 18.

***N*-(4-Methylcyclohexyl)-7-amino-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 17.**

Yield: 83%; mp 198-200 °C; ^{1}H NMR: δ 10.45, 10.18 (2d, 1H, NH), 8.33, (s, 1H, H_2), 8.13 (d, 1H, H_5), 7.12 (s, 2H, NH_2), 6.55 (d, 1H, H_6), 3.80 (m, 1H, CH), 1.86-0.85 (m, 12H, cyclohexyl + CH_3). Anal. $C_{16}H_{20}N_4O_2$ C, H, N.

7-Amino-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 18.

Yield 79%; mp 275–278 °C (ethyl acetate). ^{1}H NMR: DMSO δ 11.80 (brs, 1H, OH), 10.14 (d, 1H, NH), 8.33 (s, 1H, H_2), 8.10 (d, 1H, H_5), 7.10 (s, 2H, NH_2), 6.55 (d, 1H, H_6), 3.90 (brs, 1H, CH), 1.84–1.29 (m, 10H, cyclohexyl). Anal. $C_{15}H_{18}N_4O_2$ C, H, N.

Experimental part scheme 3

_N_-Cyclohexyl-7-fluoro-1,8-naphthyridin-4(1_H_)-on-3-carboxamide 26.
Sodium nitrite (2.30 mmol) was added portionwise to a cooled solution (-5 °C) of 7-amino-1,8-naphthyridin-3-carboxamide 1 (1.05 mmol) in 8 mL of acqueous 50 % thetrafluoroboric acid. The mixture was stirred for 1h 30 min at r.t. and after cooling, was poured over crushed ice. The pH was adjusted to 9-10 with aqueous concentrated ammonium hydroxide. The solid obtained was collected by filtration, washed with water, and purified by flash chromatography (ethyl acetate).
Yield: 46%; mp >300°C MS m/z 289; ^{1}H NMR: DMSO δ 9.79 (d, 1H, NH), 8.75 (d, 1H, H_5), 8.65 (s, 1H, H_2), 7.31 (dd, 1H, H_6), 3.82 (br, 1H, CH), 1.86-1.22 (m, 10H, cyclohexyl). Anal. $C_{15}H_{16}FN_3O_2$ C, H, N.

General procedure to the synthesis of _N_-(4-Methylcyclohexyl) and N-cyclohexyl-7-chloro-1,8-naphthyridin-4(1_H_)-on-3-carboxamide 19, 20.
Sodium nitrite (0.34 g, 5.0 mmol) was added portion wise to a cooled solution (-5 °C) of 7-amino-1,8-naphthyridine- 3-carboxamide **17** and **18** (1.0 mmol) in 54.5 mL of concentrated hydrochloric acid. The mixture was stirred for 3 h at 40 °C and, after cooling, was poured over crushed ice. The pH was adjusted to 4-5 with aqueous concentrated ammonium hydroxide. The solid obtained was collected by filtration, washed with water, and purified by flash chromatography (ethyl acetate/hexane, 1:1) to obtain **19** and **20.**

7-chloro -_N_-(4-Methylcyclohexyl)-1,8-naphthyridin-4(1_H_)-on-3-carboxamide 19.
Yield 31%; mp 271-273 °C (crystallized from ethyl acetate); ^{1}H NMR: δ 10.00, 9.63 (2d, 1H, NH), 8.64 (m, 2H, H2 + H5), 7.62 (d, 1H, H6), 3.80 (m, 1H, CH), 1.97-1.00 (m, 9H, cyclohexyl), 0.89, 0.92 (2d, 3H, CH3). Anal. $C_{16}H_{18}ClN_3O_2$ C, H, N.

7-Chloro-_N_-cyclohexyl-1,8-naphthyridin-4(1_H_)-on-3-carboxamide 20.
Yield 52%; mp 266–268 °C. ^{1}H NMR: DMSO δ 9.80 (d, 1H, NH), 8.65 (s, 1H, H_2), 8.59 (d, 1H, H_5), 7.60 (d, 1H, H_6), 3.85 (m, 1H, CH), 1.95–1.31 (m, 10H, cyclohexyl). Anal. $C_{15}H_{16}ClN_3O_2$ C, H, N.

General Procedure for the Synthesis of N_1-Substituted 1,8-Naphthyridine Derivatives 27 and 21-25.
An amount of 1,2 mmol of NaH was added to a solution of 1 mmol of 7-fluoro **26** or 7-chloro derivatives **19**, **20** in 10 mL of dry N,N-dimethylformamide. After 1 h, the appropriate chloride (1 mmol) was added and the mixture was stirred for 24 h at 50 ° C for compound **21-24** and **27** or at room temperature for 24 h for **25.** The reaction mixture, after cooling in the was treated with water, and the precipitate formed was collected by filtration.

_N_-Cyclohexyl-7-fluoro-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1_H_)-on-3-carboxamide 27.
Yield: 71%; mp 171-173°C (crystallized from ethyl acetate), MS m/z 402; ^{1}H NMR: DMSO δ 10.14 (d, 1H, NH), 8.66 (s, 1H, H_2), 8.23 (d, 1H, H_5), 6.88 (d, 1H, H_6), 4.50 (t, 2H, NCH_2), 3.85 (br, 1H, CH), 3.52 (s, 4H, morpholine), 2.68 (t, 2H, CH_2N), 2.44 (s, 4H, morpholine), 1.85-1.22 (m, 10H, cyclohexyl). Anal.$C_{21}H_{27}FN_4O_3$ C, H, N.

7-Chloro-*N*-cyclohexyl-1-(2-morpholin-4-ylethyl)-1,8-naphthyridin-4(1*H*)-on-3 carboxamide 21.
Yield 70%; mp 188–190 °C. ^{1}H NMR: DMSO δ 9.80 (d, 1H, NH), 8.96 (s, 1H, H_2), 8.66 (d, 1H, H_5), 7.66 (d, 1H, H_6), 4.62 (t, 2H, CH_2N), 3.87 (m, 1H, CH), 3.47 (m, 4H, morpholine), 2.66 (t, 2H, CH_2N), 2.46 (m, 4H, morpholine), 1.88–1.23 (m, 10H, cyclohexyl). Anal. $C_{21}H_{27}N_4O_3Cl$ C, H, N.

***N*-Cyclohexyl-1-benzyl-7-chloro-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 22.**
Yield 70%; mp 258-260 °C (crystallized from hexane); MS *m/z* 395 (M+); ^{1}H NMR: δ 9.72 (d, 1H, NH), 9.12 (s, 1H, H_2), 8.66 (d, 1H, H_5), 7.67 (d, 1H, H_6), 7.32 (m, 5H, Ar), 5.73 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.85-1.32 (m, 10H, cyclohexyl). Anal. $C_{22}H_{22}ClN_3O_2$ C, H, N.

***N*-Cyclohexyl-7-chloro-1-(*p*-fluorbenzyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 23.**
Purified by flash chromatography (ethyl acetate/hexane, 5:6), yield 25%; mp 198-200 °C (crystallized from cyclohexane); MS *m/z* 413 (M+); ^{1}H NMR: δ 9.72 (d, 1H, NH), 9.14 (s, 1H, H_2), 8.65 (d, 1H, H_5), 7.68 (d, 1H, H_6), 7.44 (m, 2H, Ar), 7.18 (m, 2H, Ar), 5.71 (s, 2H, CH_2), 3.89 (m, 1H, CH), 1.90-1.17 (m, 10H, cyclohexyl). Anal. $C_{22}H_{21}ClFN_3O_2$ C, H, N.

***N*-Cyclohexyl-7-chloro-1-(*o*-fluorbenzyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 24.**
Yield 0.300 g, 73%; mp 198-200°C (crystallized from cyclohexane); MS *m/z* 413 (M+); ^{1}H NMR: δ 9.72 (d, 1H, NH), 9.11 (s, 1H, H_2), 8.65 (d, 1H, H_5), 7.67 (d, 1H, H_6), 7.41-7.12 (m, 4H, Ar), 5.76 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.98-1.32 (m, 10H, cyclohexyl). Anal. $C_{22}H_{21}ClFN_3O_2$ C, H, N.

***N*-(4′-Methylcyclohexyl)-7-chloro-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 25.**
Purified by flash chromatography (ethyl acetate/hexane, 1: 1), yield 30%; mp 191-193 °C (crystallized from cyclohexane); MS *m/z* 432 (M+); ^{1}H NMR: δ 10.20, 10.00 (2d, 1H, NH), 8.65, 8,61 (2s, 1H, H_2), 8.20, 8,18 (2d, 1H, H_5), 6.87 (d, 1H, H_6), 4.51 (m, 2H, CH_2), 4.00 (m, 1H, CH), 3.51 (m, 4H, morpholine), 2.67 (m, 2H, CH_2), 2.44 (m, 4H, morpholine), 1.89-0.76 (m, 12H, cyclohexyl + CH_3). Anal. $C_{22}H_{29}ClN_4O_3$ C, H, N.

***N*-Cyclohexyl-7-methoxy1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 28.**
A solution of sodium methylate 4.17 mmol, obtained by 4.17 mmol of sodium in 8 mL of methanol, was added to 0.18 mmol of 7-chloronaphtyridine **22** and heated for 5 h 30 min at 80°C. The solvent was evaporated in vacuo and the solid obtained was treated with water and collected by filtration, and purified by flash chromatography (AcOEt/Exane, 10:9)
Yield: 56%; mp 212-215 °C, MS m/z 391; ^{1}H NMR: DMSO δ 9.97 (d, 1H, NH), 9.04 (s, 1H, H_2), 8.46 (d, 1H, H_5), 7.33 (m, 5H, Ar), 6.97 (d, 1H, H_6), 5.74 (s, 1H, CH_2), 3.94 (s, 3H, OCH_3), 3.90 (m, 1H, CH), 1.86-1.23 (m, 10H, cyclohexyl). Anal $C_{23}H_{25}N_3O_3$, C, H, N.

Experimental part scheme 4

N-Cyclohexyl-7-(N,N-dimethylamine)-1,8-naphthyridin-4(1H)-on-3-carboxamide 29.
A mixture of 1 mmol of N-Cyclohexyl-7-cloro-1,8-naphthyridin-4(1H)-on-3-carboxylic acid ethyl ester **20** and 10 mmol of the dimethylamine was heated in sealed tube at 120°C for 24 h. After cooling, the reaction was treated with water to give a solid residue which was collected by filtration and purified by crystallization. Yield: 57,18%; mp 190-192°C (crystallized from ethyl acetate), MS m/z 314; ^{1}H NMR: DMSO δ 10.12 (d, 1H, NH), 8.36 (s, 1H, H_2), 8.18 (d, 1H, H_5), 6.86 (d, 1H, H_6), 3.80 (br, 1H, CH), 3.16 (s, 6H, $2CH_3$), 1.90-1.22 (m, 10H, cyclohexyl). Anal. $C_{17}H_{22}N_4O_2$ C, H, N.

***N*-Cyclohexyl-7-(*N,N*-dimethylamine)-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 30.**
An amount of 1,2 mmol of NaH was added to a solution of 1 mmol of *N,N*-dimethyl-1,8-naphthyridine-3-carboxamide derivative **29** in 10 mL of dry N,N-dimethylformamide. After 1 h, the appropriate chloride (1 mmol) was added and the mixture was stirred at 50° C for 48h**.** The reaction mixture, after cooling was treated with water, and the precipitate formed was collected by filtration.
Yield: 42,10%; mp 95-97°C (crystallized from ethyl acetate), MS m/z 427; ^{1}H NMR: DMSO δ 10.11 (d, 1H, NH), 8.66 (d, 1H, H_2), 8.23 (d, 1H, H_5), 6.87 (d, 1H, H_6), 4.50 (t, 2H, NCH_2), 3.80 (br, 1H, CH), 3.51 (s, 4H, morpholine), 3.17 (s, 6H, $2CH_3$), 2.67 (t, 2H, CH_2N), 2.44 (s, 4H, morpholine), 1.85-1.23(m, 10H, cyclohexyl). Anal $C_{23}H_{33}N_5O_3$ C, H, N.

***N*-Cyclohexyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 31.**
A solution of 7-chloronaphthyridine **20**[78] (0.40 g, 1.31 mmol) in methanol (20 mL) was submitted to hydrogenation in the presence of 10% Pd/C (0.04 g) at room pressure and temperature for 3 h.
The catalyst was filtered off, and the solvent was evaporated to dryness under reduced pressure to give a residual solid, which was purified by flash chromatography (ethyl acetate) and crystallized from hexane to give **31.**
Yield: 31%: mp 215-218 °C; ^{1}H NMR: δ 9.85 (d, 1H, NH), 8.84 (dd, 1H, H_7), 8.68 (s, 1H, H_2), 8.65 (dd, 1H, H_5), 7.55 (m, 1H, H_6), 3.85 (m, 1H, CH), 1.86-1.22 (m, 10H, cyclohexyl). Anal. $C_{15}H_{17}N_3O_2$ C, H, N.

General Procedure for the Synthesis of N_1-Substituted 1,8-Naphthyridine Derivatives 32-34.
An amount of 1,2 mmol of NaH was added to a solution of 1 mmol of *N*-Cyclohexyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide **31** in 10 mL of dry N,N-dimethylformamide. After 1 h, the appropriate chloride (1 mmol) was added and the mixture was stirred for 24 h at room temperature for compounds **32** and **33** or at 50°C for 48 h for compound **34**. The reaction mixture, after cooling in the was treated with water, and the precipitate formed was collected by filtration.

***N*-Cyclohexyl-1-(*p*-fluorbenzyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 32.**
Yield 0.185 g, 49%; mp 193-195 °C (crystallized from hexane); MS *m/z* 379 (M+); ^{1}H NMR: δ 9.80 (d, 1H, NH), 9.15 (s, 1H, H_2), 8.90 (dd, 1H, H_7), 8.67 (dd, 1H, H_5), 7.62 (m, 1H, H_6), 7.37 (m, 2H, Ar), 7.15 (m, 2H, Ar), 5.81 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.86-1.23 (m, 10H, cyclohexyl). Anal. $C_{22}H_{22}FN_3O_2$ C, H, N.

***N*-Cyclohexyl-1-benzyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 33.**

Yield 0.200 g, 55%; mp 181-183 °C (crystallized from hexane); MS *m/z* 361 (M+); ^{1}H NMR: δ 9.95 (d, 1H, NH), 9.12 (s, 1H, H_2), 8.90 (dd, 1H, H_7), 8.70 (dd, 1H, H_5), 7.70 (m, 1H, H_6), 7.29 (m, 5H, Ar), 5.84 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.86-1.30 (m, 10H, cyclohexyl). Anal. $C_{22}H_{23}N_3O_2$ C, H, N.

***N*-Cyclohexyl-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 34.** Yield 0.155 g, 40%; mp 142-144 °C (crystallized from hexane); MS *m/z* 384 (M+); ^{1}H NMR: δ 9.84 (d, 1H, NH), 8.98 (s, 1H, H_2), 8.90 (dd, 1H, H_7), 8.68 (dd, 1H, H_5), 7.60 (m, 1H, H_6), 4.66 (t, 2H, CH_2), 3.86 (m, 1H, CH), 3.49 (m, 4H, morpholine), 2.70 (m, 2H, CH_2), 2.44 (m, 4H, morpholine),1.85-0.82 (m, 10H, cyclohexyl). Anal. $C_{21}H_{28}N_4O_3$ C, H, N.

Experimental part scheme 5

***N*-Cyclohexyl-7-hydroxy-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 35.**
Sodium nitrite (0.55 g, 8.0 mmol) was added portionwise to a cooled solution (-10 °C) of 7-amino-1,8- naphthyridine-3-carboxamide **18**[78] (0.44 g, 1.6 mmol) in 7 mL of concentrated sulfuric acid. After standing for 4 h at room temperature, the mixture was poured over crushed ice and the pH was adjusted to 8 with aqueous concentrated ammonium hydroxide. The solid obtained was collected by filtration, washed with water, and purified by crystallization from toluene to obtain **35.**

yield 94%: mp 303-305 °C; ^{1}H NMR: δ 10.49 (d, 1H, NH), 8.45 (s, 1H, H_2), 8.10 (d, 1H, H_5), 6.25 (d, 1H, H_6), 3.78 (m, 1H, CH), 1.82-1.26 (m, 10H, cyclohexyl). Anal. $C_{15}H_{17}N_3O_3$ C, H, N.

***N*-Cyclohexyl-1-(*o*-fluorobenzyl)-7-(*o*-fluorobenzyloxy)-1,8-naphthyridin-4(1*H*)-on-3-carboxamide 36.**
NaH (0.05 g, 1.08 mmol, 50% in mineral oil) was added to a solution of 7-hydroxy-1,8 naphthyridine **35** (0.25 g, 0.87 mmol) in 6 mL of dry DMF. After 1 h, 2-fluorobenzyl chloride (0.125 g, 0.87 mmol) was added and the mixture was stirred for 3 days at 80 °C. After the mixture was cooled, water was added and the solid obtained was collected by filtration, purified by flash chromatography (ethyl acetate/hexane, 1:1), and crystallized from cyclohexane to give **36.**

yield 30%: mp 198-200 °C; MS *m*/*z* 503 (M+); ^{1}H NMR: δ 9.94 (d, 1H, NH), 9.03 (s, 1H, H_2), 8.52 (d, 1H, H_5), 7.47-7.10 (m, 8H, 2Ar), 7.03 (d, 1H, H_6), 5.80 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.32 (m, 10H, cyclohexyl). Anal. $C_{29}H_{27}F_2N_3O_3$ C, H, N.

Experimental part scheme 6

General procedure to the synthesis of ethyl-α-carboxy-β-subsituted-anilinacrilate 37-39.
A mixture of 1.22 mmol of the suitable aniline and 1.22 mmol of EMME was heated at 110°C for 2 h for the compound **37,** at 100°C for 3h 30 min for the compound **38** or at 130°C for 5 h for the compound **39**. After cooling the compound **37** was purified by flash chromatography (exane/ ethyl acetate 13:3). In the case of comound **39** the oil obtained was used without purification.

Ethyl- α-carboxy-β-anilinacrilate 37.
Yield: 97%; mp: 264-269°C; ^{1}H NMR: δ 11.00 (d, 1H, NH), 8.55 (d, 1H, CH), 7.38 (m, 2H, Ar), 7.17 (m, 3H, Ar), 4.30 (q, 4H, $2CH_2$), 1.35 (t, 6H, $2CH_3$). Anal. $C_{14}H_{17}NO_4$ C, H, N.

Ethyl- α-carboxy-β-m-chloro-anilinacrilate 38.
Yield: 98%; mp: 275-278°C; ^{1}H NMR: δ 11.10 (d, 1H, NH), 8.52 (d, 1H, CH), 7.40 (s, 1H, Ar), 7.10 (m, 3H, Ar), 4.36 (q, 4H, $2CH_2$), 1.30 (t, 6H, $2CH_3$).

Ethyl- α-carboxy-β-m-methoxy-anilinacrilate 39.
Yield: 97%; mp: 264-269°C; ^{1}H NMR: δ 11.00 (d, 1H, NH), 8.50 (d, 1H, Ar), 7.60 (s, 1H, CH), 7.28 (m, 1H, Ar), 6.68 (m, 3H, Ar), 4.30-4.21 (q, 4H, $2CH_2$), 3.83 (s, 3H, OCH_3) 1.33 (t, 6H, $2CH_3$). Anal. $C_{15}H_{19}NO_5$ C, H, N.

General procedure to the synthesis of 7-subsituted- quinoline-3-carboxylic acid ethyl ester 40-42
A mixture of 0.018 mol of suitable ethyl-α-carboxy-β-subsituted-anilinacrilate and ml 25 of Dowtherm A was heated at reflux for 1 h for the compounds **40**, **41** or for 30 min for the compound **42**. After cooling the solid obtained was washed with petroleum ether and collected by filtration.

Ethyl-4-oxo-quinolin-3-carboxylate 40.
Yield: 66%; mp: 270-271° C; ^{1}H NMR: δ 12.20 (br, 1H, NH), 8.54 (s, 1H, H_2), 8.15 (d, 1H, H_5), 7.02 (m, 3H, Ar), 4.22 (q, 2H, CH_2), 1.27 (t, 3H, CH_3).

Ethyl-7-chloro-4-oxo-quinolin-3-carboxylate 41.
Yield: 80%; mp: 240-243° C; ^{1}H NMR: δ 12.10 (br, 1H, NH), 8.50 (s, 1H, H_2), 8.10 (d, 1H, H_5), 7.08 (m, 2H, Ar), 4.20 (q, 2H, CH_2), 1.22 (t, 3H, CH_3)

Ethyl-7-methoxy-4-oxo-quinolin-3-carboxylate 42.
Yield: 70%; mp: 233-236° C; ^{1}H NMR: δ 12.00 (br, 1H, NH), 8.48 (s, 1H, H_2), 8.05 (d, 1H, H_5), 7.00 (m, 2H, Ar), 4.19 (q, 2H, CH_2), 3.60 (s, 3H, OCH_3), 1.26 (t, 3H, CH_3). Anal. $C_{13}H_{13}NO_4$ C, H, N.

General procedure to the synthesis of *N*-substituted-quinolin-4(1*H*)-on-3-carboxamide 43-46.
A mixture of 1 mmol of suitable quinoline-3-carboxylic acid ethyl ester **40**[83]**-42** and 10 mmol of opportune amine in a sealed tube was heated at 120 °C for 24 h. After cooling, the reaction mixture was treated with ethyl ether to give a solid residue, which was collected by filtration and purified by crystallization from ethyl acetate.

N-Cyclohexyl-quinolin-4(1H)-on-3-carboxamide 43

Yield 0.230 g, 88%; mp 112-114 °C; ^{1}H NMR: δ 10.10 (d, 1H, NH), 8.72 (s, 1H, H_2), 8.24 (d, 1H, Ar), 7.70 (m, 2H, Ar), 7.47 (m, 1H, Ar), 3.82 (m, 1H, CH), 1.86-1.31 (m, 10H, cyclohexyl). Anal. $C_{16}H_{18}N_2O_2$ C, H, N.

N-Cyclohexyl-7-chloroquinolin-4(1H)-on-3-carboxamide 44.

yield 0.275 g, 90%; mp 132-135 °C; ^{1}H NMR: δ 10.18 (d, 1H, NH), 8.72 (s, 1H, H_2), 8.21 (d, 1H, Ar), 7.69 (s, 1H, Ar), 7.40 (d, 1H, Ar), 3.81 (m, 1H, CH), 1.85-1.08 (m, 10H, cyclohexyl). Anal. ($C_{16}H_{17}ClN_2O_2$) C, H, N.

N-Cylohexyl-7-methoxyquinolin-4(1H)-on-3-carboxamide 45.

Yield: 52.70%; mp 211-214°C; (crystallization from ethyl acetate) MS m/z 300; ^{1}H NMR: DMSO δ 10.13 (d, 1H, NH), 8.64 (s, 1H, H_2), 8.13 (d, 1H, H_5), 7.09-7.03 (m, 2H, H_6+H_8), 3.87-3.75 (m, 4H, OCH_3+CH), 1.86-1.08 (m, 10H, cyclohexyl). Anal. $C_{17}H_{20}N_2O_3$ C,H,N.

N-Cycloheptyl-quinolin-4(1H)-on-3-carboxamide 46.

Yield: 77.55%; MS m/z 284; ^{1}H NMR: $CDCl_3$ δ 10.55 (d, 1H, NH), 8.45 (d, 1H, Ar), 7.71 (m, 2H, Ar), 7.49 (m,1H, Ar), 4.32 (m, 1H, NCH), 2.10-1.62 (m, 12H, cycloheptyl). Anal $C_{17}H_{20}N_2O_2$ C,H,N.

General Procedure for the Synthesis of N_1-Substitutedquinoline Derivatives 47-54.

NaH (4.36 mmol, 50% in mineral oil) was added to a solution of *N*-substituted quinoline-3-carboxamide (0.92 mmol) in 9.2 mL of dry DMF at room temperature, in the case of derivatives **44** or **45** the solution was heated at 50°C. After 1 h, 4-(2-chloroethyl)-morpholine hydrochloride or benzyl chloride (0.92 mmol) was added, and the mixture was stirred for 24 h, at 50 °C for compounds **47**, **49** and **50-54** or at 80 °C for compound **48**. After the mixture was cooled (3-5 °C), the addition of water caused the precipitation of the title compounds, which were purified by crystallization in the cases of **47-50**, and **52-54,** or by flash chromatography for **51**.

N-Cyclohexyl-1-(2-morpholin-4-ylethyl)-quinolin-4(1H)-on-3-carboxamide 47.

Yield 0.180 g, 50%; mp 169-170 °C (crystallized from ethyl acetate); MS *m/z* 383 (M+); ^{1}H NMR: δ 10.04 (d, 1H, NH), 8.80 (s, 1H, H_2), 8.34 (d, 1H, Ar), 7.90 (m, 2H, Ar), 7.52 (m, 1H, Ar), 4.58 (m, 2H, CH_2), 3.90 (m, 1H, CH), 3.49 (m, 4H, morpholine), 2.65 (m, 2H, CH_2), 2.42 (m, 4H, morpholine), 1.88-1.22 (m, 10H, cyclohexyl). Anal. ($C_{22}H_{29}N_3O_3$) C, H, N.

N-Cyclohexyl-1-benzylquinolin-4(1H)-on-3-carboxamide 48.

Yield 62%; mp 239-240 °C (crystallized from hexane); MS *m/z* 360 (M+); ^{1}H NMR: δ 10.05 (d, 1H, NH), 8.97 (s, 1H, H_2), 8.54 (d, 1H, Ar), 7.61-7.15 (m, 8H, Ar), 5.48 (s, 2H, CH_2), 3.98 (m, 1H, CH), 2.02-1.44 (m, 10H, cyclohexyl). Anal. ($C_{23}H_{24}N_2O_2$) C, H, N.

N-Cyclohexyl-7-chloro-1-(2-morpholin-4-ylethyl)-quinolin-4(1H)-on-3-carboxamide 49.

Yield 52%; mp 229-231 °C (crystallized from ethyl acetate); MS *m/z* 417 (M+). ^{1}H NMR: δ 10.00 (d, 1H, NH), 8.80 (s, 1H, H_2), 8.35 (d, 1H, Ar), 8.05 (m, 1H, Ar), 7.60 (m, 1H, Ar), 4.50 (s, 2H, CH_2), 3.80 (m, 1H, CH), 3.47 (m, 4H, morpholine), 2.45 (m, 6H, CH_2 + morpholine), 1.40-1.05 (m, 10H, cyclohexyl). Anal. ($C_{22}H_{28}ClN_3O_3$) C, H, N.

***N*-Cyclohexyl-1-benzyl-7-methoxyquinolin-4(1*H*)-on-3-carboxamide 50.**
Yield: 66.66%; mp 126-129°C (crystallized from exane), MS m/z 390 ; ^{1}H NMR: DMSO δ 10.22 (d, 1H, NH), 8.99 (d, 1H, H_2), 8.33 (d, 1H, H_5), 7.36-7.08 (m, 7H, H_6+H_8+Ar), 5.75 (s, 1H, CH), 3.78 (s, 3H, OCH_3), 1.86-1.22 (m, 10H, cyclohexyl). Anal. $C_{24}H_{26}N_2O_2$ C, H, N.

***N*-Cyclohexyl-7-methoxy-1-(2-morpholin-4-yl-ethyl)-quinolin-4(1*H*)-on-3-carboxamide 51.**
Yield: 22 %; mp 145-148 °C,(flash chromatography AcOEt/ MeOH/ Exane,10:1.5:3) MS m/z 413 ; ^{1}H NMR: DMSO δ 10.10 (d, 1H, NH), 8.71 (s, 1H, H_2), 8.23 (d, 1H, H_5), 7.21(s, 1H, H_8) 7.18 (d, 1H, H_6), 4.55 (t, 2H, NCH_2), 3.94 (s, 3H, OCH_3), 3.80 (br, 1H, CH), 3.52 (m, 4H, morpholine), 2.67 (t, 2H, CH_2N), 2.48 (m, 4H, morpholine), 1.85-1.25 (m, 10H, cyclohexyl). Anal. $C_{23}H_{31}N_3O_4$ C, H, N.

***N*-Cycloheptyl-1-benzylquinolin-4(1*H*)-on-3-carboxamide 52.**
Yield: 50.10%; mp 203-206°C (crystallized from ethyl acetate), MS m/z 374 ; ^{1}H NMR: $CDCl_3$ δ 10.10 (d, 1H, NH), 8.95 (s, 1H, H_2), 8.54 (d, 1H, Ar), 7.65-7.15 (m, 8H, Ar), 5.48 (s, 2H, NCH_2), 4.24 (br, 1H, CH), 2.02-0.90 (m, 12H, cycloheptyl). Anal. $C_{24}H_{26}N_2O_2$ C, H, N.

***N*-Cycloheptyl-1-(2-morpholin-4-yl-ethyl)-quinolin-4(1*H*)-on-3-carboxamide 53.**
Yield: 63.34%; mp 158-160 °C,(crystallization from ethyl acetate) MS m/z 397 ; ^{1}H NMR: $CDCl_3$ δ 10.03 (d, 1H, NH), 8.79 (s, 1H, H_2), 8.53 (d, 1H, H_5), 7.75 (d, 1H, H_8) 7.52 (m, 2H, H_6+H_7), 4.36 (s, 2H, NCH_2), 3.84 (m, 1H, CH), 3.70 (m, 4H, morpholine), 2.81 (s, 2H, CH_2N), 2.52 (m, 4H, morpholine) 2.02-0.84 (m, 12H, cycloheptyl). Anal. $C_{23}H_{31}N_3O_3$ C, H, N.

***N*-Cyclohexyl-1-benzyl-7-chloroquinolin-4(1*H*)-on-3-carboxamide 54.**
Yield 62%; mp >300°C (crystallized from hexane); MS *m/z* 360 (M+); ^{1}H NMR: δ 8.48 (s, 1H, H2), 7.93 (d, 1H, Ar), 7.35-7.28 (m, 7H, Ar), 4.54 (s, 2H, CH_2), 3.48 (m, 1H, CH), 2.08-0.98 (m, 10H, cyclohexyl). Anal. $C_{23}H_{23}N_2O_2$ Cl C, H, N.

Experimental part scheme 7

General Procedure for the Preparation of N1-Substituted 4-Hydroxy-7-methyl-1,2,3,4-tetrahydro-1,8-naphthyridin-3-carboxamides 58-60.

$NaBH_4$ (0.30 g, 8 mmol) was added to a solution of the appropiate 7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives[78] (0.38 mmol) in absolute ethanol (7.5 mL), and the mixture was stirred at room temperature for 12h. The organic solvent was evaporated from the reaction mixture under reduced pressure to obtain a residue, which was treated with H_2O. In the cases of **58** and **60**, the solid precipitate obtained was collected by filtration and purified by crystallization from hexane, whereas for **59** the mixture was extracted with chloroform, the organic solution was dried ($MgSO_4$) and evaporated to dryness under reduced pressure, and the crude solid was purified by crystallization from cyclohexane.

***N*-Cyclohexyl-1-(*o*-fluorbenzyl)-4-hydroxy-7-methyl-1,2,3,4-tetrahydro-1,8-naphthyridin-3-carboxamide 58.**

Yield 87%; MS *m/z* 397 (M+); ^{1}H NMR: δ 7.82 (m, 1H, NH), 7.40 (d, 1H, H_5), 7.30-7.08 (m, 4H, Ar), 6.44 (d, 1H, H_6), 5.63 (d, 1H, OH), 4.95-4.69 (m, 3H, CH_2 + H_4), 3.56 (m, 1H, CH), 3.30 (m, 2H, $2H_2$), 2.23 (s, 3H, CH_3), 1.68-1.11 (m, 11H, cyclohexyl+H_3). Anal. $C_{23}H_{28}FN_3O_2$ C, H, N.

4-Hydroxy-*N*-(4-methylcyclohexyl)-1-(2-morpholin-4-ylethyl)-7-methyl-1,2,3,4-tetrahydro-1,8-naphthyridin-3-carboxamide 59.

Yield 61%; MS *m/z* 416 (M+).^{1}H NMR: δ 7.81 (m, 1H, NH), 7.34 (d, 1H, H_5), 6.35 (d, 1H, H_6), 5.55 (m, 1H, OH), 4.61 (m, 1H, H_4), 3.78 (m, 1H, CH), 3.57-3.33 (m, 8H, morpholine + NCH_2 + $2H_2$), 2.45 (m, 6H, morpholine + CH_2), 2.22 (s, 3H, CH_3), 1.70-1.05 (m, 13H, cyclohexyl + CH_3 + H_3). Anal. $C_{23}H_{36}N_4O_3$ C, H, N.

***N*-Cyclohexyl-1-benzyl-4-hydroxy-7-methyl-1,2,3,4-tetrahydro-1,8-naphthyridin-3-carboxamide 60.**

Yield 52%; MS *m/z* 379 (M+); ^{1}H NMR: δ 7.48 (d, 1H, H_5), 7.31 (m, 5H, Ar), 6.48 (m, 1H, H_6), 6.00 (d, 1H, OH), 4.92 (m, 3H, CH_2 + H_4), 3.74 (m, 1H, CH), 3.40 (m, 2H, $2H_2$), 2.57 (m, 1H, H_3), 2.38 (s, 3H, CH_3), 1.84-0.83 (m, 10H, cyclohexyl). Anal. $C_{23}H_{29}N_3O_2$ C, H, N

Experimental part scheme 8

General procedure for the synthesis of 4-chloro 7-substituted-quinolin-3-carboxylate 61, 62.

A mixture of 1 mmol of suitable 4-hydroxyquinoline-3-carboxylic acid ethyl ester **39, 40** and 2 mmol of phosphoryl trichloride was stirred at 120°C for 15 min. After cooling the reaction mixture was poured over crushed ice and the pH was adjusted to 8-9 with concentrated ammonium hydroxide. The solid obtained was collected by filtration.

4-chloroquinoline-3-carboxylate 61.

Yield: 72.90%; mp: 43-44°C; ^{1}H NMR: DMSO δ 11.03 (d, 1H, NH), 9.00 (s, 1H, H_2), 8.57 (s, 1H, Ar), 8.11 (d,1H, Ar), 7.72-7.51 (m, 2H, Ar), 4.21 (q, 2H, CH_2), 1.36 (t, 3H, CH_3). Anal: $C_{12}H_{11}NO_2Cl$ C, H, N,.

4,7-dichloroquinoline-3-carboxylate 62.

Yield: 87.44%; ^{1}H NMR: DMSO δ 10.23 (d, 1H, NH), 9.18 (s,1H, H_2), 8.38 (d,1H, H_5), 8.23 (s, 1H, H_8), 7.89 (d, 1H, H_6), 4.23 (q, 2H, CH_2), 1.38 (t, 3H, CH_3). Anal: $C_{12}H_{10}NO_2Cl_2$ C, H, N,.

General Procedure for the Synthesis of 2-Substituted-2,5-dihydroxy-3H-pyrazol-[4,3-c]quinoline 63-65.

A mixture of 1 mmol of 4-cloroquinoline-3-carboxylic acid ethyl ester **61** or **62**[85], 1 mmol of cyclohexyhydrazine hydrochloride or phenylhydarzine and 3 mmol of triethylamine in 10 ml of *p*-xilene was stirred at 150 °C for 12h 30 min. The precipitate formed was collected by filtration, treated with water and purified by cristallization.

2-Cyclohexyl-2,5-dyhydroxy-3H-pyrazol-[4,3-c]quinoline 63.

Yield:76.42%, mp 198-202°C (crystallized from exane) MS m/z 276; ^{1}H NMR: $CDCl_3$ δ 9.35 (d, 1H, NH), 8.77 (s, 1H, H_2), 8.35 (d, 1H, H_5), 7.88 (d, 1H, H_8), 7.34 (m, 2H, H_6+H_7), 4.66 (br, 1H, CH), 2.07-1.26 (m, 10, cyclohexyl). Anal. $C_{16}H_{17}N_3O$ C, H, N.

2-phenyl-2,5-dyhydroxy-3H-pyrazol-[4,3-c]quinoline 64.

Yield: 75.35%; mp: >300°C; ^{1}H NMR: DMSO δ 9.20 (d, 1H, NH), 8.73 (s, 1H, H_2), 8.21 (d, 2H, Ar), 7.70-7.34 (m, 6H, Ar), 7.18 (m, 1H, H_7). Anal. $C_{16}H_{11}N_3O$ C, H, N.

2-Cyclohexyl-7-chloro-2,5-dyhydroxy-3H-pyrazol-[4,3-c]quinoline 65.

Yield:80.05%, mp > 300°C (crystallized from DMF) MS m/z 300; ^{1}H NMR: $CDCl_3$ δ 9.23 (d, 1H, NH), 8.73 (s, 1H, H_2), 8.27 (d, 1H, H_5), 7.86 (d, 1H, H_8), 7.46 (m, 1H, H_6), 4.36 (br, 1H, CH), 2.05-1.26 (m, 10, cyclohexyl). Anal. $C_{16}H_{16}N_3OCl$ C, H, N.

General Procedure for the Synthesis of N_1-Substituted 2,5-dihydroxy-3H-pyrazol-[4,3-c]quinoline Derivatives 66-70.

An amount of 1,2 mmol of NaH (50% in mineral oil) was added to a solution of 1 mmol of **63** or **64** or of 7-chloroquinoline derivative **65** in 10 mL of dry N,N-dimethylformamide. After 1 h, the appropriate chloride (1 mmol) was added and the mixture was stirred for 24 h at room temperature for compound **66**, **67** or at 50°C for compound **68**, **69** or at 70°C for **70**. The reaction mixture, after cooling was treated with water, and the precipitate formed was collected by filtration.

5-Benzyl-2-cyclohexyl-2,5-dihydroxy-3H-pyrazol-[4,3-c]quinoline-3-one 66.
Yield: 53,85%; mp 202-206°C (crystallized from exane), MS m/z 357; ^{1}H NMR: DMSO δ 9.2 (s, 1H, H_2), 8.27 (d, 1H, H_5), 7.91 (d, 1H, H_8), 7.64-7.40 (m, 7H, Ar+H_6+H_7), 5.79 (s, 2H, CH_2), 4.45 (br, 1H, CH), 1.98-1.09 (m, 10H, cyclohexyl). Anal. $C_{23}H_{23}N_3O$ C, H, N.

5-Benzyl-7-chloro-2-cyclohexyl-2,5-dihydroxy-3H-pyrazol-[4,3-c]quinoline-3-one 67.
Yield: 59.03%; mp 185-188°C (crystallized from ethyl acetate), MS m/z 390; ^{1}H NMR: $CDCl_3$ δ 9.05 (s, 1H, H_2), 8.37 (d, 1H, H_5), 8.01 (d, 1H, H_8), 7.53-7.40 (m, 6H, Ar+H_6), 5.64 (s, 2H, CH_2), 4.45 (br, 1H, CH), 2.10-1.27 (m, 10H, cyclohexyl). Anal. $C_{23}H_{22}N_3OCl$ C, H, N.

2-Cyclohexyl-2,5-dihydroxy-5-(2-morpholin-4-yl-ethyl)-3H-pyrazol-[4,3-c]quinoline-3-one 68.
Yield: 44.53%; mp 118-122°C (crystallized from ethyl acetate), MS m/z 380; ^{1}H NMR: DMSO δ 9.25 (d, 1H, NH), 8.89 (s, 1H, H_2), 8.41 (d, 1H, H_5), 8.12 (d, 1H, H_8), 7.82-7.72 (m, 2H, H_6+H_7), 4.96 (br, 1H, CH), 4.51 (s, 2H, CH_2), 3.37-3.33 (m, 4H, morpholine), 2.79 (t, 2H, CH_2), 2.56-2.50 (m, 4H, morpholine), 2.06-1.08 (m, 10H, cyclohexyl). Anal. $C_{22}H_{28}N_4O_2$ C, H, N.

2-phenyl-2,5-dihydroxy-5-(2-morpholin-4-yl-ethyl)-3H-pyrazol-[4,3-c]quinoline-3-one 69.
Yield: 31.64%; mp 233-237°C (crystallized from ethyl acetate), MS m/z 374; ^{1}H NMR: DMSO δ 8.78 (s, 1H, H_2), 8.21 (d, 2H, H_5+ H_8), 7.75-7.41 (m, 6H, Ar+H_6), 7.17 (m, 1H, H_7), 4.58 (t, 2H, CH_2), 3.52 (m, 4H, morpholine), 2.71 (t, 2H, CH_2),2.50 (m, 4H, morpholine). Anal. $C_{22}H_{22}N_4O_2$ C, H, N.

2-Cyclohexyl-7-chloro-2,5-dihydroxy-5-(2-morpholin-4-yl-ethyl)-3H-pyrazol-[4,3-c]quinoline-3-one 70.
Yield: 33.94%; mp > 300°C (crystallized from ethyl acetate), MS m/z 413; ^{1}H NMR: $CDCl_3$ δ 8.35 (s, 1H, H_2), 8.30 (d, 1H, H_5), 7.56 (s, 1H, H_8), 7.46 (d, 1H, H_6), 4.40 (m, 1H, NCH), 4.31 (t, 2H, NCH_2), 3.73-3.69 (m, 4H, morpholine), 2.82 (t, 2H, CH_2N), 2.54 (m, 4H, morpholine), 2.06-1.23 (m, 10H, cyclohexyl). Anal. $C_{22}H_{27}N_4O_2Cl$ C, H, N.

Experimental part scheme 9

Dimethyl-N-[(6-acetylamino)-2-pyridin-yl]-aminofumarate 71.
A mixture of 5.28 mmol of 2-acetylamino-6-aminopyridine and 5.28 mmol of with acetylendicarboxylic acid dimethyl ester in 27 ml of methanol was heayted at 80°C for 24h. The solution was concentrated and cooling in ice. The solid obtained was collected by filtration to give the dimethyl ester **71**.
Yield: 65%; mp: 125-130°C; IR: 1736.99 cm^{-1} (C=O).

7-aminoacetyl-4-oxo-1,8-naphthyridin-2-carboxylic acid methyl ester 72.
A mixture of 5.1 mmol of Dimethyl-N-[(6-acetylamino)-2-pyridin-yl]-aminofumarate in 30 ml of Dowtherm A was heated at reflux for 30 min. After cooling the solid obtained was washed with petroleum ether and collected by filtration, to gave the 2-methylester **72** purified by flash-chromatography on silica gel eluiting with AcOEt/MeOH 10:1.
Yield: 65%; mp: 125-130°C; ^{1}H NMR: DMSO δ 11.81 (s, 1H, NH), 10.69 (s, 1H, OH), 7.99 (d, 1H, H_5), 6.51 (m, 2H, Ar), 3.91 (s, 3H, OCH_3), 2.21 (s, 3H, CH_3). Anal $C_{12}H_{11}N_3O_4$, C, H, N,.

7-amino-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-2-carboxamide 73.
A mixture of 1,8-naphthyridine-2-carboxylic acid methyl ester (1 mmol) and 10 mmol of cyclohexylamine amine was heated in a sealed tube at 120°C for 24h. After cooling, the reaction mixture was treated with ethyl ether to give a solid residue, which was collected by filtration and purified by crystallization from ethyl acetate.
Yield 87 %; mp 185–188 °C. ^{1}H NMR: DMSO δ 8.63 (d, 1H, NH), 7.90 (d, 1H, H_5), 6.90 (br, 2H, NH_2), 6.53 (d, 1H, H_3), 6.49 (d, 1H, H_6), 3.73 (br, 1H, CH), 1.98–1.20 (m, 10H, cyclohexyl).

7-amino-1-benzyl-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-2-carboxamide 74.
An amount of 1,2 mmol of NaH was added to a solution of 1 mmol of 7-amino-*N*-cyclohexyl-1,8-naphthyridin-4(1*H*)-on-2-carboxamide in 10 mL of dry N,N-dimethylformamide. After 1 h, the benzyl chloride (1 mmol) was added and the mixture was stirred for 24 h at 50° C. The reaction mixture, after cooling was treated with water, and the precipitate formed was collected by filtration and purified by flash cromatogrphy.(ethyl acetate/ exane 1:1)
Yield 40 %; mp 204-207 °C. ^{1}H NMR: $CDCl_3$ δ 8.33 (d, 1H, NH), 8.22 (d, 1H, H_5), 7.72(s, 1H, H_3), 7.52-7.39 (m, 5H, Ar), 6.77 (d, 1H, H_6), 5.34 (s, 2H, NCH_2), 5.12 (br, 2H, NH_2) 3.93 (br, 1H, CH), 2.26–1.20 (m, 10H, cyclohexyl). Anal. $C_{22}H_{24}N_4O_2$, C, H, N.

Experimental part scheme 10

ethyl-2-oxo-1,8-naphthyrydin-3-carboxylate 75.
A solution of 1 g (8,19 mmol) of 2-aminopyridin-3-carboxaldehyde, 1,85 ml of ethyl malonate and few drops of piperidine was heated at 120°C for 20h. Afetr cooling the precipitate was treated with ethyl ether to give the compound **75** as a yellow solid, cristallized from ethyl acetate.
Yield: 76%; mp 182-185°C; ^{1}H NMR: δ 8.60 (dd, 1H, H_7), 8.50 (s, 1H, H_4), 8.26 (dd, 1H, H_5), 7.29 (dd, 1H, H_6), 4.32-4.22 (q, 2H, CH_2), 1.29 (t, 3H, CH_3).

General procedure to the synthesis of *N*-substituted-1,8-naphthyridin-2(1H)-on-3-carboxamide 76-81.
A mixture of 1 mmol of ethyl ester and 10 mmol of the appropriate amine in a sealed tube was heated at 120°C for 24h. After cooling, the reaction mixture was treated with ethyl ether to give a solid residue which was collected by filtration and purified by crystallization from ethyl acetate (**76** and **77**) or from ethanol (**78- 81**).

***N*-Cyclohexyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 76.**
Yield 88%; mp 277-280 °C; ^{1}H NMR: δ 9.70 (d, 1H, NH), 8.85 (s, 1H, Ar), 8.66 (dd, 1H, Ar), 8.42 (dd, 1H, Ar), 7.36 (dd, 1H, Ar), 3.85 (m, 1H, CH), 1.89-1.27 (m, 10H, cyclohexyl). Anal. $C_{15}H_{17}N_3O_2$ C, H, N.

***N*-(4-methylcyclohexyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide 77.**
Yield 87%; mp 158-161 °C; ^{1}H NMR: δ 10.0, 9.60 (2d, 1H, NH), 8.85 (s, 1H, Ar), 8.66 (dd, 1H, Ar), 8.42 (dd, 1H, Ar), 7.36 (m, 1H, Ar), 4.16, 3.80 (2m, 1H, CH), 2.00-1.05 (m, 9H, $4CH_2$+CH), 0.92, 0.89 (2d, 3H, CH_3). Anal. $C_{16}H_{19}N_3O_2$ C, H, N.

***N*-2(2-aminoethyl)pyridin-1,8-naphthyridin-2(1H)-on-3-carboxamide 78.**
Yield 82%; mp >300 °C; ^{1}H NMR: δ 9.78 (t, 1H, NH), 8.85 (s, 1H, Ar), 8.65 (dd, 1H, Ar), 8.41 (dd, 1H, Ar), 7.72 (dd, 1H, Ar). 7.39-7.24 (m, 4H, Ar), 3.74 (q, 2H, CH_2), 3.01 (t, 2H, CH_2). Anal. $C_{16}H_{14}N_4O_2$ C, H, N.

***N*-(β-phenylethyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide 79.**
Yield 87%; mp 270-272°C; ^{1}H NMR: δ 9.17 (t, 1H, NH), 8.86 (s, 1H, Ar), 8.65 (dd, 1H, Ar), 8.40 (dd, 1H, Ar), 7.39-7.25 (m, 6H, Ar), 3.59 (m, 2H, CH_2), 2.85 (t, 2H, CH_2). Anal. $C_{17}H_{15}N_3O_2$ C, H, N.

***N*-(4-fluoro-phenylethyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide 80.**
Yield 84%; mp >300°C; ^{1}H NMR: δ 9.68 (t, 1H, NH), 8.85 (s, 1H, Ar), 8.65 (d, 1H, Ar), 8.40 (d, 1H, Ar), 7.39-7.28 (m, 3H, Ar), 7.17-7.07 (m, 2H, Ar), 3.58 (m, 2H, CH_2), 2.85 (t, 2H, CH_2). Anal. $C_{17}H_{14}N_3O_2$ F C, H, N.

***N*-Cycloepthyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 81.**
yield 71%; mp 200-204°C ^{1}H NMR: δ 9.75 (t, 1H, NH), 8.84 (s, 1H, Ar), 8.65 (dd, 1H, Ar), 8.40 (dd, 1H, Ar), 7.35 (dd, 1H, Ar). 4.10(brs, 1H, CH), 1.90-1.45 (m, 12H, cycloepthyl).

General Procedure for the Synthesis of N_1-Substituted1,8-naphthyridin-2(1H)-on-3-carboxamide 82-93.
NaH (0.10 g, 2.00 mmol, 50 % in mineral oil) was added to a solution of suitable 1,8-naphthyridine-2(1H)-on-3-carboxamide derivatives **76, 77** and **79-81** (0.81 mmol) in 6.4 mL of dry DMF. After 1h, the suitable chloride (0.81 mmol) was added and the mixture was stirred for 24 h at room temperature (**82-84,** and **85, 87-91**) or at 50 °C (**86** and **92**) or at 70°C (**93**). After cooling, water was added and the solid obtained was collected by filtration and was purified by crystallization from hexane (**82**, **84, 85** and **93**) or from ethyl acetate (**87** and **88**) or ethanol (**89**) or by flash chromatography [**86**, **92** (ethyl acetate: methanol, 10:0.5)].

***N*-Cyclohexyl-1-benzyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 82.**
Yield: 61%; mp 154-156 °C; ^{1}H NMR: δ 9.58 (d, 1H, NH), 8.96 (s, 1H, Ar), 8.76 (dd, 1H, Ar), 8.54 (dd, 1H, Ar), 7.45 (dd, 1H, Ar), 7.27-7.20 (m, 5H, Ar), 5.72 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.89-1.30 (m, 10H, cyclohexyl). Anal. $C_{22}H_{23}N_3O_2$ C, H, N.

***N*-Cyclohexyl-1-(*p*-fluorbenzyl)-1,8-naphtyridin-2(1H)-on-3-carboxamide 83.**
Yield: 8.44%; mp 202-205°C; ^{1}H NMR: δ 9.56 (d,1H, NH) 8.95 (s, 1H, Ar), 8.77 (dd, 1H, Ar), 8.53 (dd, 1H, Ar), 7.47 (dd, 1H, Ar), 7.36-7.06 (m, 4H, Ar), 5.68 (s, 2H, NCH_2), 3.80 (br, 1H, CH), 1.88-1.22 (m, 10H, $5CH_2$). Anal. $C_{22}H_{20}N_3O_2F$ C, H, N.

***N*-Cyclohexyl-1-butyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 84.**
Yield: 55%; mp 152-154 °C; ^{1}H NMR: δ 9.69 (d, 1H, NH), 8.88 (s, 1H, Ar), 8.79 (dd, 1H, Ar), 8.49 (dd, 1H, Ar), 7.45 (dd, 1H, Ar), 4.47 (t, 2H, CH_2), 3.82 (m, 1H, CH), 1.89-0.88 (m, 17H, cyclohexyl + $2CH_2$ + CH_3). Anal. $C_{19}H_{25}N_3O_2$ C, H, N.

***N*-(4-Methylcyclohexyl)-1-benzyl-1,8-naphtyridin-2(1H)-on-3-carboxamide 85.**
Yield: 75%; mp 171-174°C. ^{1}H NMR: δ 9.84, 9.47 (2s, 1H, NH), 8.97 (s, 1H, Ar), 8.77 (dd, 1H, Ar), 8.57 (dd, 1H, Ar), 7.51 (m, 2H, Ar), 7.30 (m, 4H, Ar), 5.73, 5.78 (2s, 2H, CH_2), 4.10, 3.75 (2m, 1H, CH), 1.94-0.99 (m, 9H, $4CH_2$ + CH), 0.94,0.89 (2d, 3H, CH_3). Anal. $C_{23}H_{25}N_3O_2$ C, H, N.

***N*-(4-Methylcyclohexyl)-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide 86.**
Yield: 33%; mp 142-144 °C; ^{1}H NMR: δ 9.90, 9.52 (2s, 1H, NH), 8.90 (d, 1H, Ar), 8.79 (d, 1H, Ar), 8.50 (dd, 1H, Ar), 7.45 (dd, 1H, Ar), 4.65 (m, 2H, CH_2), 4.00, 3.75 (2m, 1H, CH), 3.51 (m, 4H, morpholine), 2.56 (m, 6H, morpholine + CH_2), 0.92, 0.88 (2d, 3H) 1.90-0.87 (m, 9H,). Anal. $C_{22}H_{30}N_4O_3$ C, H, N.

***N*-Cyclohexyl-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide 87.**
Yield: 56%; mp 148-152 °C; ^{1}H NMR: δ 9.80 (d, 1H, NH), 8.88 (s, 1H, Ar), 8.78 (dd, 1H, Ar), 8.50 (dd, 1H, Ar), 7.45 (m, 1H, Ar), 4.64 (t, 2H, CH_2), 3.90 (m, 1H, CH), 3.51 (m, 4H, morpholine), 2.57 (m, 6H, morpholine), 1.89-1.22 (m, 10H, $5CH_2$). Anal. $C_{21}H_{28}N_4O_3$ C, H, N.

***N*-(β-phenylethyl)-1-benzyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 88.**
Yield: 98%; mp 219-222°C; ^{1}H NMR: δ 9.64 (t, 1H, NH), 8.96 (s,1H, Ar), 8.77 (d, 1H, Ar), 8.53 (d, 1H, Ar), 7.47 (dd, 1H, Ar), 7.39-7.09 (m, 10H, Ar), 5.72 (s, 2H, CH_2), 3.59 (m, 2H, CH_2), 2.86 (tr, 2H, CH_2). Anal. $C_{24}H_{21}N_3O_2$ C, H, N.

***N*-(4-fluoro-phenylethyl)-1-benzyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 89.**
Yield: 87%; mp 182-185°C; ^{1}H NMR: δ 9.72 (brs, 1H, NH), 8.93 (s, 1H, Ar), 8.78 (dd, 1H, Ar), 8.43 (dd, 1H, Ar), 7.49-7.23 (m, 8H, Ar), 7.11-7.02 (m, 2H, Ar), 5.82 (s, 2H, CH_2) 3.69 (m, 2H, CH_2), 2.95 (tr, 2H, CH_2). Anal. $C_{24}H_{21}N_3O_2$ C, H, N.

***N*-Cycloheptyl-1-benzyl-1,8-naphthyridin-2(1H)-on-3-carboxamide 90.**
Yield: 88%; mp 171-175°C; ^{1}H NMR: δ 9.62 (d, 1H, NH), 8.95 (s, 1H, Ar), 8.76 (dd, 1H, Ar), 8.53 (dd, 1H, Ar), 7.46 (dd, 1H, Ar), 7.30-7.20 (m, 5H, Ar), 5.72 (s, 2H, CH_2), 4.09 (m, 1H, CH), 1.90-0.85 (m, 12H, cycloheptyl). Anal. $C_{25}H_{25}N_3O_2$ C, H, N.

***N*-(4-Methylcyclohexyl)-1-(*p*-fluorbenzyl)-1,8-naphtyridin-2(1H)-on-3-carboxamide 91.**
Yield 74 %; mp 143-147°C; ^{1}H NMR: δ 9.85, 9.43 (2d, 1H, NH), 8.95, 8.97 (2s, 1H, Ar), 8.77 (dd, 1H, Ar), 8.52 (dd, 1H, Ar), 7.47 (dd, 1H, Ar), 7.31 (m, 2H, Ar), 7.10 (m, 2H, Ar) 5.72, 5.69 (2s, 2H, CH_2), 4.10, 3.70 (2m, 1H, CH), 1.94-0.99 (m, 9H, $4CH_2$ + CH), 0.92, 0.88 (2d, 3H, CH_3).

Cycloheptyl-1-(*p*-fluorbenzyl)-1,8-naphtyridin-2(1H)-on-3-carboxamide 92.
Yield: 97.04%; mp 160-163°C; ^{1}H NMR: δ 9.78 (d, 1H, NH); 8.88 (s, 1H, Ar); 8.75 (dd, 1H, Ar); 8.12 (m, 3H, Ar); 7.48 (dd, 1H, Ar); 6.94 (m, 2H, Ar); 5.78 (s, 2H, CH_2); 4.10 (m, 1H, CH); 1.88-1.01 (m, 12H, cycloheptyl).

***N*-Cycloheptyl-1-(2-morpholin-4-yl-ethyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide 93.**
Yield: 64%; mp 154-158 °C; ^{1}H NMR: δ 9.77 (d, 1H, NH), 8.88 (s, 1H, Ar), 8.68 (dd, 1H, Ar), 8.08 (dd, 1H, Ar), 7.28 (dd, 1H, Ar), 4.78 (tr, 2H, CH_2), 4.10 (m, 1H, CH), 3.70 (m, 4H, morpholine), 2.78-2.65 (m, 6H, 4Hmorpholine+CH_2), 2.05-0.85 (m, 12H, cycloheptyl). Anal. $C_{22}H_{30}N_4O_3$ C, H, N.

Experimental part scheme 11

Methyl-2-amino-3-pyridinecarboxylate 94.
A mixture of 250 mg (1.80 mmol) of 2-aminonicotinic acid and 1.8 ml of concentrated H_2SO_4 in 3.6 ml of CH_3OH was heated at 80°C for 7h. After cooling the CH_3OH was removed by evaporation under reduced pressure and the remaining mixture was neutralized with the solution of $NaHCO_3$ (pH 7-8). The precipitate thus formed was collected by filtration.
Yield: 88%; mp 79-82°C; ^{1}H NMR: DMSO δ 8.20 (dd, 1H, Ar), 8.05 (dd, 1H, Ar), 7.12 (br, 2H, NH_2), 6.63 (dd, 1H, Ar), 3.80 (s, 3H, OCH_3). Anal. $C_7H_8N_2O_2$ C, H, N.

Ethyl-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxilate 95.
A solution of Na (0.22 g, 9.87 mmol) in anhydrous ethanol (8.23 mL) was added to 1.58 g of diethylmalonate (9.87 mmol) and the mixture was stirred at room temperature. After 5 min, the methyl-2-amino-nicotincarboxilate (0.5 g, 3.29 mmol) was added and the mixture was heated under reflux for 6h. The cooled mixture was diluted with 8.23 mL of water and then acidified with concentrated HCl. The precipitate thus formed was collected by filtration and crystallized from ethanol.
Yield 58%; mp 145-148 °C; ^{1}H NMR: δ 11.30 (br, 1H, NH), 8.53 (m, 1H, Ar), 8.30 (m, 1H, Ar), 7.22 (m, 1H, Ar), 4.24 (q, 2H, CH_2), 1.23 (t, 3H, CH_3) . Anal. $C_{11}H_{10}N_2O_4$ C, H, N.

***N*-Cyclohexyl-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxamide 96.**
A mixture of ethyl-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxilate **95** (0.20 g, 0.85 mmol) and cyclohexylamine (0.97 mL, 10 mmol) in a sealed tube was placed in the microwave CEM 2000 using one-step procedure: 20 min. at 140°C at 100 psi (1 psi= 6.89 KPa). After cooling, the reaction mixture was treated with ethyl ether to give a solid which was collected by filtration and crystallized from ethyl acetate.
Yield 90%; mp 253-255 °C; ^{1}H NMR: δ 10.04 (br, 1H, NH), 8.79 (m, 1H, Ar), 8.46 (m, 1H, Ar), 7.31 (dd, 1H, Ar), 3.97 (br, 1H, CH), 2.01-0.86 (m, 10H, cyclohexyl) . Anal. $C_{15}H_{17}N_3O_3$ C, H, N.

General procedure to the synthesis of N_1-Substituted-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxamide 97, 98.
To a solution of *N*-Cyclohexyl-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxamide 74 (0.20 g, 0.69 mmol) in 6.96 mL of dry DMF, NaH (3.42 mmol, 50 % in mineral oil) was added. After 1h, 4-(2-chloroethyl)-morpholine hydrochloride or benzyl chloride (0.69 mmol) was added and the mixture was stirred for 24h at 50°C. After cooling, in the case of 75 the solvent was evaporated in vacuo and the semisolid obtained was treated with water and collected by filtration. For **97**, the reaction mixture was treated with water and then the precipitate formed was collected by filtration.

***N*-Cyclohexyl-1-(2-morpholin-4-yl-ethyl)-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxamide 97.**
yield 17%; mp 247-250 °C (crystallized from ethyl acetate). ^{1}H NMR: δ 10.20 (d, 1H, NH), 8.80 (d, 1H, H_5), 8.40 (d, 1H, H_7), 7.40 (m, 1H, H_6), 4.52 (t, 2H, CH_2), 3.90 (m, 1H, CH), 3.52 (m, 4H, morpholine), 2.50 (m, 6H, morpholine+CH_2), 1.88-1.22 (m, 10H, cyclohexyl). Anal. $C_{21}H_{28}N_4O_4$ C, H, N.

***N*-Cyclohexyl-1-benzyl-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridin-3-carboxamide 98.**

yield 18%; mp 143-145 °C (crystallized from $CHCl_3$). ^1H NMR: δ 10.20 (d, 1H, NH), 8.76 (d, 1H, H_5), 8.48 (d, 1H, H_7), 7.44 (m, 1H, H_6), 7.26 (m, 5H, Ar), 5.61 (s, 2H, CH_2), 3.85 (m, 1H, CH), 1.87-1.22 (m, 10H, cyclohexyl). Anal. $C_{22}H_{23}N_3O_3$ C, H, N.

Experimental part scheme 12

***N*-Phenyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 99.**

A solution of aniline (0.24 mL, 2.69 mmol) and triethylmethancarboxilate (1.83 mL, 8.61 mmol) was placed in the microwave CEM 2000 (open vessel) using one-step procedure: 15 min. at 210 °C at 200 psi (1 psi= 6.89 KPa). After cooling, the reaction mixture was treated with petroleum ether to give a pure solid which was collected by filtration.

Yield 27%; mp 193-195 °C; ^1H NMR: δ 12.10 (s, 1H, NH), 8.00 (d, 1H, Ar), 7.67 (m, 3H, Ar), 7.37 (m, 5H, Ar). Anal. $C_{16}H_{12}N_2O_3$ C, H, N.

General procedure to the synthesis of N_1-Substituted-4-hydroxy-2-oxo-1,2-dihydro quinoline-3-carboxamide 100, 101.

To a hot solution (50 °C) of *N*-Phenyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide **99** (0.12 g, 0.42 mmol) in 4.18 mL of dry DMF, NaH (2.11 mmol, 50 % in mineral oil) was added. After 1h, 4-(2-chloroethyl)-morpholine hydrochloride or benzyl chloride (0.42 mmol) was added and the mixture was stirred for 24 h at room temperature **100** or at 50 °C **101**. After cooling, in the case of **100** the reaction mixture was treated with water and then the precipitate formed was collected by filtration. For **101** the solvent was evaporated in vacuo and the semisolid obtained was treated with water and collected by filtration.

***N*-Phenyl-4-hydroxy-1-(2-morpholin-4-yl-ethyl)-2-oxo-1,2-dihydroquinoline-3-carboxamide 100.**

Yield 51%; mp 173-175 °C (crystallized from ethanol); ^1H NMR: δ 8.13 (d, 1H, Ar), 7.85 (m, 1H, Ar), 7.67 (d, 2H, Ar), 7.41 (m, 3H, Ar), 7.20 (m, 2H, Ar), 4.43 (t, 2H, CH_2), 3.56 (m, 4H, morpholine), 2.50 (m, 8H, morpholine+CH_2). Anal. $C_{22}H_{23}N_3O_4$ C, H, N.

1-Benzyl-4-hydroxy-*N*-phenyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 101.

yield 11%; mp 195-197 °C (crystallized from ethyl acetate); ^1H NMR: δ 8.19 (d, 1H, Ar), 7.73-7.20 (m, 13H, Ar), 5.61 (s, 2H, CH_2). Anal. $C_{23}H_{18}N_2O_3$ C, H, N.

Experimental part scheme 13

Methyl 2-oxo-1,2-dihydro-3-pyridinecarboxylate 102.
A mixture of 750 mg (5,40 mmol) of 1,2-dihydro-2-oxo-3-pyridinecarboxylic acid (2-hydroxynicotinic acid) and 5,4 ml of concentrated H_2SO_4 in 10,8 ml of CH_3OH was heated in microware (CEM) to 80°C for 55 minute (power 200W, pressure 100psi, stirring on). After cooling the CH_3OH was removed by evaporation under reduced pressure. The remaining mixture was neutralized with the solution of $NaHCO_3$ (pH 7-8). The desired compound was extracted with methylene chloride by the mixture. The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to give **102** as a white solid (748 mg), which was recrystallizated from toluene.
Yield: 91%; mp 150-154°C; ^{1}H NMR: DMSO δ 8.08 (d, 1H, H_4), 7,68 (d, 1H, H_6), 6,29 (t, 1H, H_5), 3,39 (s, 1H, OH).

General Procedure for the Synthesis of 2-oxo-1,2-dihydro-3-pyridinecarboxamide derivatives 103-105.
A mixture of 1,0 mmol of methyl 2-oxo-1,2-dihydro-3-pyridinecarboxylic acid and 10,0 mmol of suitable amide, were heated in microwave to 140°C for 60 minute (power 200W, pressure 100psi, stirring on) for amide **103,** and were heated at 120°C in oil bath for 15 h for amide **104** or heated at 150°C for 72 h for amide **105**. After cooling the reactions were treated with ethyl ether to give **103-105** as solids residue, which were collected by filtration and purified by crystallization (crystallized from ethyl acetate for compounds **103** and **104**) and purified by flash chromatography for compound **105.** (petroleumether/ethylacetate 1:1).

2-oxo-1,2-dihydro-3-pyridinecarboxycyclohexylamide 103.
Yield: 65%; mp 195-198°C; ^{1}H NMR: DMSO δ 9,87 (d, 1H, NH), 8,35 (d, 1H, H_4), 7,73 (d, 1H, H_6), 6,50 (t, 1H, H_5), 3,80 (m, 1H, NCH), 1,85-1,29 (m, 10H, cyclohexyl).

2-oxo-1,2-dihydro-3-pyridinecarboxycyclohepthylamide 104.
Yield: 67,87%; mp 138-145°C; ^{1}H NMR: DMSO δ 9,88 (d, 1H, NH), 8,32 (d, 1H, H_4), 7,71 (d, 1H, H_6), 6,48 (t, 1H, H_5), 3,99 (m, 1H, NCH), 2,10-1,48 (m, 12H, cycloheptyl).

2-oxo-1,2-dihypyridinecarboxy-4-methylcyclohexylamide 105.
Yield: 63,52 %; mp 248-250° C; ^{1}H NMR: DMSO δ 10.03; 9.08 (2d, 1H, NH); 8.33 (d, 1H, H_3); 7.74 (d, 1H, H_5); 6.42 (t, 1H, H_4); 4.05; 3.65 (2s, 1H, NCH); 2.00 – 0.92 (m, 12H, cyclohexyl + CH_3).

General Procedure for the Synthesis of N_1-Substitutedpyridine Derivatives 106-111
An amount of 1,2 mmol of NaH (50% in mineral oil) was added to a solution of 1 mmol of **103-105** in 10 mL of dry N,N-dimethylformamide. After 1 h, the appropriate chloride (1 mmol) was added and the mixture was stirred for 2,5 h at r.t.. The reaction mixture, after cooling was concentrated under reduced pressure and the residue was treated with H_2O and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, collected by filtration, and evaporated under reduced pressure. The oily obtained was purified by flash chromatography (exane/ethyl acetate 10:5) for compounds **106, 108** and **110.** For the compound **107, 109** and **111** the mixture was concentrated under reduced pressure and the residue was treated with H_2O to give a white solid which was collected by filtration and purified by crystallization from hexane.

1-benzyl-2-oxo-3-pyridinecarboxycyclohexylamide 106.
Yield: 43%; mp 65-68°C; ^1H NMR: $CDCl_3$ δ 9.75 (d, 1H, NH), 8.37 (d, 1H, H_4), 8.20 (d, 1H, H_6), 7.57-7.17 (m, 5H, Ar), 6.59 (t, 1H, H_5), 5.26 (s, 2H, NCH_2), 3.98 (m, 1H, NCH), 1.84-1.28 (m, 10H, cyclohexyl).

1-[4-(2-cloroethyl)morpholine]- 2-oxo-3-pyridinecarboxycyclohexylamide 107.
Yield: 51%; mp 94-98°C; ^1H NMR $CDCl_3$ δ 9.74 (d, 1H, NH), 8.55 (dd, 1H, H_6), 7.53 (dd, 1H, H_4), 6.42 (t, 1H, H_5), 4.13 (t, 2H, NCH_2), 4.01-3.99 (m, 1H, NCH), 3.70 (t, 4H, morpholine), 2.73 (t, 2H, NCH_2), 2.52 (t, 4H, morpholine), 2.02-1.27 (m, 10H, cyclohexyl).

1-[4-fluoro-benzyl]-2-oxo-3-pyridinecarboxycyclohexylamide 108.
Yield: 19%; mp 120-125°C; ^1H NMR: $CDCl_3$ δ 9.71 (d, 1H, NH), 8.56 (d, 1H, H_4), 7.50 (d, 1H, H_6), 7.31 (t, 4H, Ar), 6.43 (t, 1H, H_5), 5.21 (s, 2H, NCH_2), 3.99 (m, 1H, NCH), 2.03-1.23 (m, 10H, cyclohexyl).

1-[4-fluoro-benzyl]-2-oxo-3-pyridinecarboxycycloheptylamide 109.
Yield: 73,68%;m.p. 118-120° C; ^1H NMR: $CDCl_3$ δ 9.77 (s, 1H, NH), 8.57 (d, 1H, H_4), 7.50 (d, 1H, H_6), 7.29 (t, 4H, Ar), 6.43 (t, 1H, H_5), 5.22 (s, 2H, NCH_2), 4.18 (m, 1H, NCH), 2.01-0.90 (m, 12H, cycloheptyl).

1-benzyl-2-oxo-3-pyridinecarboxycycloheptylamide 110.
Yield: 38%; ^1H NMR: $CDCl_3$ δ 9.80 (d, 1H, NH), 8.54 (d, 1H, H_4), 7.46 (d, 1H, H_6), 7.33 (t, 5H, Ar), 6.41 (t, 1H, H_5), 5.24 (s, 2H, CH_2), 4.18-4.14 (m, 1H, NCH), 2.03-1.27 (m, 12H, cycloheptyl).

1-[4-(2-cloroethyl)morpholine]- 2-oxo-3-pyridinecarboxycycloheptylamide 111.
Yield: 43.33%;m.p. 40-45° C; ^1H NMR: $CDCl_3$ δ 9.90 (d, 1H, NH), 8.53 (d, 1H, H_4), 7.52 (d, 1H, H_6), 6.40 (t, 1H, H_5), 4.12 (t, 2H, NCH_2), 4.01-3.99 (m,1H, NCH), 3.68 (t, 4H, morpholine), 2.73 (t, 2H, NCH_2), 2.53 (t, 4H, morpholine), 2.02-1.27 (m, 12H, cycloheptyl).

1-[4-fluoro-benzyl]-2-oxo-3-pyridinecarboxy-4-methylcyclohexylamide112.
An amount of 0,250 g (5,48 mmol) of NaH was added to a solution of 0,640 g (2,74 mmol) of compound **105** in 37,29 ml of DMF. After 2 h 0,328 ml (0,396 g; 2,74 mmol) of 4-fluro-benzyl chloride was added and the mixture was stirred for 24 h at room temperature. The mixture was concentrated under reduced pressure and the residue was treated with H_2O to give **112** as yellow oil. The compound was purified by chromatography using Petroleum ether / AcOEt (1 : 4).
Yield: 20.33%; mp: 33 – 35° C; ^1H NMR: $CDCl_3$ 9.98; 9.59 (2d, 1H, NH), 8.57 (d, 1H, H_4), 7.52 (m, 1H, Ar), 7.34 (m, 2H, Ar), 7.12 (m, 2H, Ar), 6.41 (m, 1H, Ar), 5.22 (d, 2H, NCH_2), 4.23; 3.92 (2d, 1H, NCH), 2.03 – 1.22 (m, 12H, cyclohexyl + CH_3).

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92. Male Swiss albino mice (24–26 g) from Morini (San Polo d'Enza, Italy) were used. Ten mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 22 ± 1 _C with a 12 h light/dark cycle, light at 7 a.m. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Mice were placed inside a stainless steel container, which was set thermostatically at 52.5 ± 0.1 _C in a precision water bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch before and 15, 30, 45, and 60 min after administration of analgesic drugs. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted to avoid injury. No sign of tissue injury was observed up to 45 s. Ten mice per group were tested.
93. The apparatus consisted of a base platform and a rotating rod with a diameter of 3 cm and a non-slippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. Those mice scoring less than 3 and more than 6 falls in the pretest were rejected (20%). The performance time was measured before (pretest) and 15, 30, and 45 min after the beginning of the test.Ten mice per group were tested.

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