Università degli Studi di Ferrara

DOTTORATO DI RICERCA IN

"SCIENZE FARMACEUTICHE"

CICLO XXVI

COORDINATORE Prof. Stefano Manfredini

Discovery of new CB_2 cannabinoid receptor full agonists

Settore Scientifico Disciplinare

CHIM/08

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Anni 2011/2013

INDEX

Appendixes

APPENDIX I

Publications list

1. *Pier Giovanni Baraldi, Giulia Saponaro, Allan R. Moorman, Romeo Romagnoli, Delia Preti, Stefania Baraldi, Emanuela Ruggiero, Katia Varani, Martina Targa, Fabrizio Vincenzi, Pier Andrea Borea, and Mojgan Aghazadeh Tabrizi*. 7-Oxo-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamides as Selective CB_2 Cannabinoid Receptor Ligands: Structural Investigations around a Novel Class of Full Agonists *J. Med. Chem*. **2012**, 55, 6608−6623.

2. *Mojgan Aghazadeh Tabrizi, Pier Giovanni Baraldi, Giulia Saponaro, Allan Moorman, Romeo Romagnoli, Delia Preti, Stefania Baraldi, Emanuela Ruggiero, Cristina Tintori, Tiziano Tuccinardi, Fabrizio Vincenzi, Pier Andrea Borea, and Katia Varani*. Discovery of 7-Oxo-pyrazolo[1,5-a]pyrimidine-6-carboxamides as Potent and Selective CB_2 Cannabinoid Receptor Inverse Agonists. *J. Med. Chem.*, **2013**, *56* (11), 4482–4496.

3. *Pier Giovanni Baraldi, Giulia Saponaro, Delia Preti, Stefania Baraldi, Emanuela Ruggiero, Pier Andrea Borea, Mojgan Aghazadeh Tabrizi*. 7-oxo-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamides as selective CB_2 cannabinoid receptor ligands: structural investigation around a novel class of full agonists. *Chimica e l'Industria* (Milan, Italy) **2013**, 95, (4), 118-120.

Posters and oral presentations

1. Pier Giovanni Baraldi, Emanuela Ruggiero, and Mojgan Aghazadeh Tabrizi. *New Synthesis of Diazepino[3,2,1-*ij*]quinoline and Pyrido[1,2,3-*de*]quinoxalines via Addition-elimination followed by Cycloacylation.*. Berlin, Germany.
   September 2-6, 2012
   22nd International Symposium on Medicinal Chemistry

2. Pier Giovanni Baraldi, Giulia Saponaro, Delia Preti, Stefania Baraldi, Emanuela Ruggiero, Pier Andrea Borea, Mojgan Aghazadeh Tabrizi. *7-Oxo-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamides as Selective CB_2 Cannabinoid Receptor Ligands: Structural Investigation around a Novel Class of Full Agonists.*
   Ferrara 17-12-2012
   XII Giornata della Chimica dell'Emilia Romagna

3. Emanuela Ruggiero, Mojgan Aghazadeh Tabrizi, Pier Giovanni Baraldi, Giulia Saponaro, Pier Andrea Borea. *Design, synthesis and evaluation of new pyrazolopyridine-5-carboxamide derivatives as CB_2-receptor partial agonists.*
   Bologna 18-12-2013
   XIII Giornata della Chimica dell'Emilia Romagna

APPENDIX II

Abbreviation list

$(5\text{-HT})_2$	5-hydroxytryptamine
2-AG	2-arachidonoyl glycerol
2-AGE	2-arachidonoylglyceryl ether
AD	Alzheimer's disease
AEA	anandamide
AIMP	acid-induced muscle pain
ALS	amyotrophic lateral sclerosis
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CB	cannabinoid
CBD	cannabidiol
CDI	carbonyldiimidazole
CHO	chinese hamster ovary
CNS	central nervous system
COX-2	cyclooxygenase-2
DAG	diacylglycerol
DAGL	DAG lipase
DEEM	diethyl ethoxymethylene malonate
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
eCB	endocannabinoid
ECS	endocannabinoid system
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EMT	endocannabinoid membrane transporter
FAAH	fatty acid amide hydrolase-1
FDA	Food and Drug Administration
GABA	γ-aminobutyric acid
GPCR	G protein-coupled receptor
HBTU	*o*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate

HD	Huntington's disease
HOBt	1-hydroxybenzotriazole
MAGL	monoacylglycerol lipase
MAPK	mitogen-activated protein kinase
MS	multiple sclerosis
NADA	*N*-arachidonoyldopamine
NA-lysoPE	*N*-arachidonyllisophosphatidylethanolamine
NAPE	*N*-arachidonoyl-phosphatidyl-ethanolamine
NAPE-PLD	*N*-acyl-phosphatidylethanolamine-selective phosphodiesterase
NBS	*N*-bromosuccinimide
ODA	*cis*-9,10-octadecanoamide
PA	Parkinson's disease
PD	phosphatidic acid
PI	phosphoinositides
PLA_2	type-A_2 phospholipase
PLC	phospholipase C
PPA	polyphosphoric acid
PPAR	peroxisome proliferator-activated receptor
SAR	structure–activity relationship
STZ	streptozotocin
TEA	triethylamine
THC	tetrahydrocannabinol
THF	tetrahydrofuran
TLC	thin-layer chromatography
TRP	transient receptor potential
VIRODHAMINE	*O*-arachidonoylethanolamine

Introduction

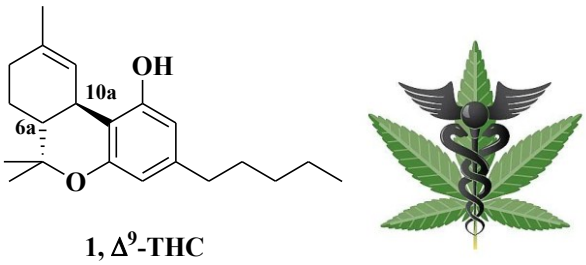
1.1 HISTORY

Marijuana has been used for centuries as a recreational drug, because of its property to alter sensory perception and cause euphoria. Furthermore, its application as a medicine has been recorded for over five thousand years as for malaria, constipation, rheumatic pain and female disorders. Actually, some of the earliest hemp evidence have been traced in rope imprints on broken Chinese pottery, which date back to 10000 B.C. Chinese people used the hemp plant for their clothes, their writing materials, their "confrontation" with evil spirits, and in the treatment of pain and diseases. Consequently, they were the first recorded people to experience marijuana's peculiar psychotropic effects. Evidence of its use have been found also in India, where Cannabis were adopted for the treatment of numerous diseases, such as dysentery, loss of appetite, poor digestion. Around 500 B.C., marijuana spread in Northern Europe, basing on an urn containing cannabis leaves and seeds, unearthed near Berlin. Cannabis was introduced to modern western medicine by W.B. O'Shaughnessy, professor at the University of Calcutta. In 1839, after validating many of its applications, he documented its analgesic properties in the treatment of rheumatism, and as a remedy for severe convulsions. At the same time, a French psychologist J. J. Moreau de Tours suggested cannabis use to treat mental illness, so that the interest in medical cannabis spread from Europe to America and many formulations started to be prepared, such as analgesic pills and sedative tablets (**Fig. 1**).[1]

Fig. 1 Cannabis preparations in the early XX century

Beginning in the late 1960s, many academic studies have been carried out on marijuana, which demonstrated that cannabis effects, both psychotropic and therapeutic, are due to its hundreds natural components, which are classified on the basis of their chemical structure. The most psychoactive component of the hemp plant is tetrahydrocannabinol (THC), which is characterized by a terpenophenolic core, and is the parent compound of a major group, called cannabinoids.

Initially, the strong hydrophobic nature of this compounds suggested that marijuana elicited its effects by a non-specific perturbation of cell membranes rather than by a specific interaction with selective binding sites. The first important finding that ultimately led to a rejection of this theory was the identification by Gaoni and Mechoulam in 1964 of the chemical structure of the (-)-*trans*-Δ^9-THC (**1**, **Fig. 2**).[2] In particular, they established the exact geometry through X-ray crystallography and NMR analysis, which led them prove that, in THC-type cannabinoids, activity resides exclusively in the (-)isomers with the 6a*R*, 10a*R* stereochemistry. This represented the starting point for the development of a whole range of synthetic analogous in the 1970s, that were used as tools to investigate cannabinoid system.[3]



1, Δ^9-THC

Fig. 2 Chemical structure of Δ^9-THC

1.2 CANNABINOID RECEPTORS

Studies of the biological effects of THC and its synthetic derivatives revealed a drug-receptor interactions. Indeed, in 1990, Δ^9-THC led to the identification of the first cannabinoid receptor, which was labelled as CB_1. Three years later, in 1993, a second cannabinoid receptor was identified, and consequently named CB_2.[4]

CB_1 and CB_2 receptors are members of the superfamily of G protein-coupled receptors (GPCRs) that inhibit adenylyl cyclase and activate mitogen-activated protein kinase

(MAPK) by coupling with $G_{i/o}$ proteins. They present structural omology of 44%, which turns into 68%, considering just the transmembrane domains (**Fig. 3**).[5]

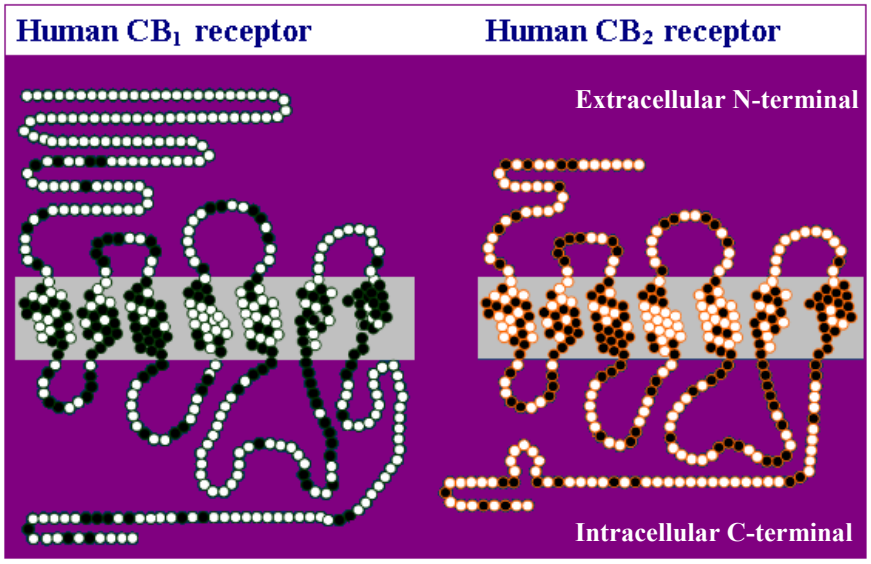


Fig. 3 CB_1/CB_2 receptor homology

CB_1 receptors are distributed abundantly and heterogeneously within the central nervous system (CNS), where they mediate inhibition of the release of different excitatory and inhibitory neurotransmitters. A high density of CB_1 receptors can be found in the hippocampus, some olfactory regions, caudate, putamen, accumbens nucleus, substantia nigra, globus pallidus. Moreover, they are also present on some peripheral neurones as well as in certain nonneuronal tissues, like reproductive system. Given the distribution, their activation can affect processes such as cognition and memory, can alter the control of motor function, and induce signs of analgesia and hypothermia. However, it is worth mentioning that CB_1 receptors are not widely express in the brain areas involved in respiration and heart rate, reason why cannabinoid drugs are not lethal. Nevertheless, their activation is widely associated with psychotropic effects observed with non selective cannabinoids, thus limiting the therapeutic potential. CB_1 receptor presents unique properties: it has been preserved throughout evolution, considering that human, rat and mouse CB_1 receptors have 97–99% amino acid sequence identity. This data reflect the important functions played by endocannabinoids in cell and system physiology. Another remarkable characteristic of the CB_1 receptors is their high expression in the brain: actually, it is the most abundant GPCR in the brain, with densities about 50 fold above those of classical neurotransmitters.

Otherwise, CB_2 was initially identified as a peripherally restricted receptor, predominantly expressed in cells of the immune system, especially in monocytes, macrophages, B-cells and T-cells and, when activated, could modulate immune cell migration and cytokine release both outside and within the brain. However, subsequent data showed that it is also expressed in both the central and the peripheral nervous systems, especially in microglial cells. It is likely to be involved in inflammation-associated pathologies, such as human multiple sclerosis, osteoarthritis, rheumatoid arthritis, Crohn's disease and inflammatory pain.[3]

Notwithstanding, further studies proved that many cannabinoid effects cannot be attributed exclusively to the CB_1 and CB_2 receptors activation. For example, peripherally mediated antihyperalgesic activity persist in CB_1- and CB_2-gene knockout animals.[6] These findings have led to the suggestion of novel cannabinoid receptors. Actually, in 1999, an orphan GPCR was isolated and named GPR55, with low sequence identity with CB receptors (< 15%). It is expressed in the brain, in the vascular endothelium and in the immune system. And, it can bind a wide range of endogenous, natural and synthetic cannabinoid ligands. Yet, its mechanism of action remains uncertain.[7] In addition, application of cannabinoids seems to generate a slow inward current in some of the transient receptor potential (TRP) channels, including TRPV and TRPA.[8]

1.3 ENDOCANNABINOID SYSTEM

The characterization of cannabinoid receptors opened the way for the identification of the respective endogenous ligands, named endocannabinoids (eCBs), which chemical structures are depicted in **Fig. 4**.

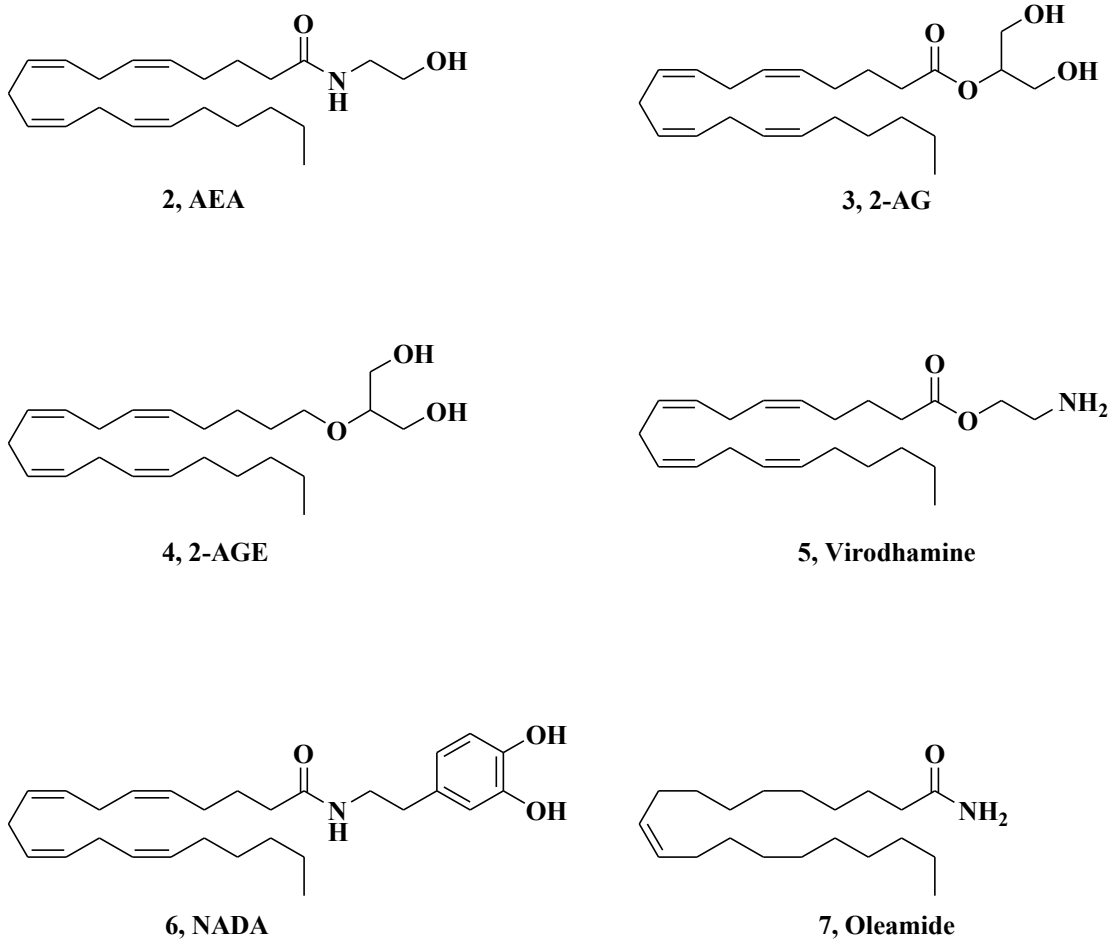


Fig. 4 Chemical structures of endocannabinoids

The first endogenous ligand for these receptors was isolated in 1992 and it was named anandamide (**2**, AEA). It showed the same physiological and pharmacological properties of natural and synthetic cannabinoids, such as analgesia, motor depression, catalepsy. Three years later, a second endogenous intermediate was found to interact with CB receptors, 2-arachidonoylglycerol (**3**, 2-AG). Following, other endogenous compounds that may also bind cannabinoid receptors have been discovered and each of them turned out to be an arachidonic acid derivative: 2-arachidonoylglyceryl ether (noladin ether, **4**, 2-AGE), *O*-arachidonoylethanolamine (**5**, virodhamine), *N*-arachidonoyldopamine (**6**, NADA,) and *Cis*-9,10-octadecanoamide (oleamide, **7**, ODA).

Endogenous cannabinoids, along with CB receptors and the enzymatic pathways for the synthesis, transport and degradation of these ligands, constitute the endocannabinoid system (ECS).

Unlike classical neurotransmitters, eCBs are not stored in the cells but produced mainly *on demand* by stimulus-dependent cleavage of membrane phospholipids precursors. In particular, AEA is released from *N*-arachidonoyl-phosphatidyl-ethanolamine (NAPE), through an *N*-acyl-phosphatidylethanolamine-selective phosphodiesterase (NAPE-PLD). AEA acts as a retrograde messenger at presynaptic cannabinoid receptors (CB_1), where it regulates neurotransmitter release through its second transduction systems, mainly Ca^{2+}. It is also involved in the neuromodulation of major transmitter systems, including dopamine, at postsynaptic cells, where it regulates synaptic plasticity through its modulation of potassium (K^+) channels. It is also involved in the neuromodulation of major transmitter systems at postsynaptic cells, where it regulates synaptic plasticity through modulation of K^+ channels and MAPK. Moreover, AEA is capable of interacting with other targets, such as the TRPV1 receptor and the peroxisome proliferator-activated receptors (PPARs).[9] Recently, it has been suggested a different biosynthetic pathway, independent from NAPE-PLD, but involving a type-A_2 phospholipase (PLA_2), which hydrolyzes NAPE, releasing fatty acids and *N*-arachidonyllisophosphatidylethanolamine (NA-lysoPE), an additional precusor of AEA. On the other hand, 2-AG is produced from the hydrolysis of diacylglycerols (DAGs) by DAG lipases (DAGLs) specific for *sn-1* position. The molecules of DAG, which must possess an esteric function at *sn-2* position, can be produced from the hydrolysis either of phosphoinositides (PI), catalyzed by a Ca^{2+}-dependent phospholipase C (PLC), or of phosphatidic acid (PA) catalyzed by a specific Ca^{2+}-dependent PA hydrolase.[10]

Subsequently, eCBs are transported in both directions through cell membrane by diffusion or selective transport by the putative endocannabinoid membrane transporter (EMT): a process that is fast rate, temperature-dependent, saturable, bidirectional and selective. In the extracellular space they can interact with cannabinoid receptors or be internalized and degraded. Endocannabinoid signalling is closed by a two-step process that includes transport into cells and hydrolysis by two specific enzymatic systems. AEA is metabolized by fatty acid amide hydrolase-1 (FAAH) in arachidonic acid and ethanolamine, while 2-AG is hydrolized by monoacylglycerol lipases (MAGLs) into arachidonic acid and glycerol. FAAH is a 64kDa integral membrane protein, which displays optimal activity at alkaline pH (about 9). It is composed by 597 amino acids and is rich in glycine and serine residues. Immunohistochemical studies confirmed that

the expression of this enzyme in different brain areas is directly correlated to CB_1 receptor density.[11] X-ray crystallography studies helped determine the enzyme structure and, consequently, led to the identification of binding sites. FAAH, unlike other amidases, does not use catalytic serine-histidine-aspartate triad, but it presents a serine-serine-lysine sequence, in which Ser^{241} acts as a nucleophile, Ser^{217} stabilizes the negative charge and the Lys^{142} acts as a base.[12] MAGL is a 33 kDa protein that belongs to the serine hydrolase family and presents a typical catalytic triad made of Ser^{122}, His^{269} and Asp^{239}. It is highly expressed in those areas of the brain rich in CB_1 receptors.[13]

Moreover, recent experimental studies confirmed that AEA and 2-AG can function as substrate for cyclooxygenase-2 (COX-2) and some lipoxygenases. Actually, COX-2 efficiently oxygenates 2-AG and AEA into prostaglandin ethanolamides and prostaglandin glycerol esters respectively (**Fig. 5**).[14]

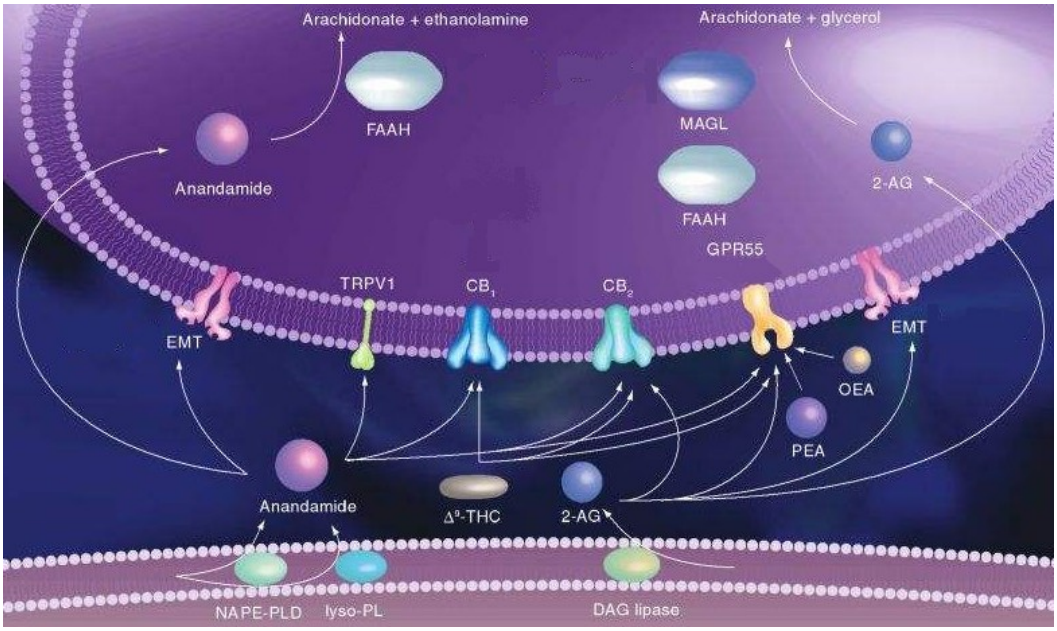


Fig. 5 Cannabinergic transmission[15]

AEA and 2-AG exhibit different binding properties and intrinsic activity at CB receptors. Actually, AEA behaves as a partial agonist at both receptors, with higher affinity for type-1 receptor. Alternatively, 2-AG is a full agonists at both receptor, but displays lower affinity than AEA.[16]

2-AGE is an ether-derivative of 2-AG, which binds mostly to CB_1 and very weakly to CB_2 receptor. However, it has been demonstrated an opposite effect, because it can induce the inhibition of μ-opioid receptor through CB_2 receptor activation. It is also capable of activating PPAR-α receptor and presents more stability *in vivo* than both

AEA and 2-AG, which are rapidly hydrolyzed. Virodhamine acts as a partial agonist on CB_1 receptors, even though it can be an antagonist of this receptor at higher concentrations. It also acts as full agonist on CB_2 receptor and can activate PPAR-α receptor and GPR55. NADA is an endogenous ligand of CB_1 and TRPV1 receptors. It can act as either an endovanilloid or endocannabinoid, depending on which one it interacts with. Moreover, it is able to activate PPAR-γ. Finally, Oleamide acts as a full agonist of cannabinoid receptors with higher selectivity for the CB_1 receptor.[9]

1.4 CANNABINOID RECEPTOR LIGANDS

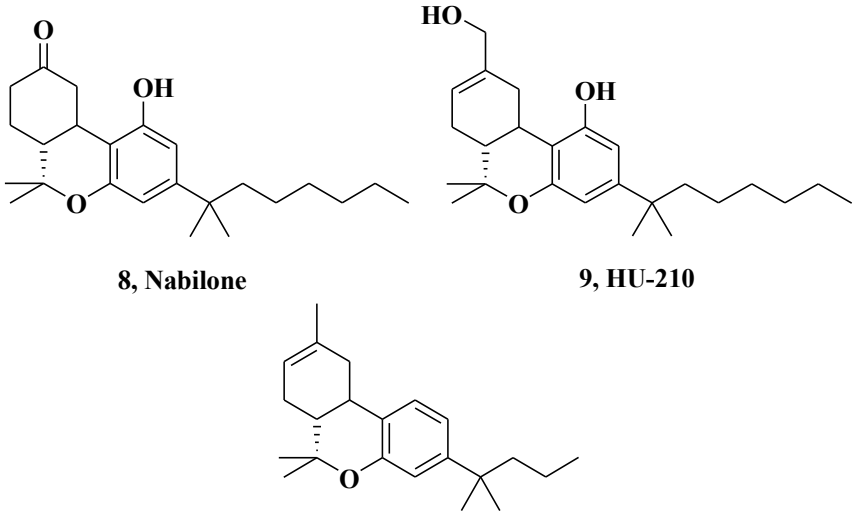
The ubiquitous distribution of the ECS correlates with its role as a modulator of multiple physiological processes. Synthetically produced compounds which display affinity to CB receptors may act either as agonists, enhancing the activity of eCBs, or as antagonists, preventing the binding of eCBs, and consequently inhibiting the activity of the ECS. Agonistic behaviour can be exploited for a wide set of applications: hypnotic, analgesic, antiemetic, antiasthmatic, antihypertensive, immunomodulatory, anti-inflammatory, neuroprotective and so on. On the other hand, cannabinoid receptor antagonism might be exploited in conditions with enhanced eCBs signalling, as drugs and alcohol addiction status.[17, 18] Anyhow, it is possible to modulate ECS transmission and metabolism also by targeting the biosynthetic and hydrolytic enzymatic pathways (FAAH and MAGL inhibitors) or acting on the putative EMT.

1.4.1 Agonists

According to the International Union of Pharmacology[3], cannabinoid receptor agonists can be classified in classical and non classical cannabinoids, aminoalkylindoles and eicosanoids (eCBs). Classical cannabinoids are defined by the characteristic tricyclic terpenophenolic moiety of the parent compound **1**, Δ^9-THC, and are also called phytocannabinoids. Compound **1** is a nonselective agonist that binds to both CB receptors, with a similar affinity of approximately 40 nM.[19] It displays a wide range of therapeutically interesting effects, such as analgesia, anti-inflammatory, immunosuppressive, anticonvulsive, antiemetic, and orexigenic properties, albeit its employment has been restrained because of its psychotropic effects and drug abuse. The commercially available derivative of THC, known as Marinol®, is currently approved in the United States for treating nausea and vomiting in patients receiving chemotherapy. Its synthetic analogue Nabilone (**8**, **Fig. 6)**, marketed as Cesamet®, was approved in 1985 by the Food and Drug Administration (FDA) for treatment of chemotherapy-induced nausea and vomiting and for chronic pain management. Cannabidiol (CBD, **11, Fig. 7**), on the other hand, lack the terpenophenolic core, and actually is a non-psychotropic phytocannabinoid, that displayed strong antioxidant, neuroprotective, anti-ischemic and antiinflammatory effects. Interestingly, CBD displays very low affinity towards CB recpetors, whereon it acts as an antagonists. Surprisingly, emerging data from several studies has led to the recognition that CBD couples positively with the

putative new cannabinoid receptor GPR55 and, moreover, it can activate some ionotropic receptors, like TRPV and TRPA.[8] Its commercially available formulation, Sativex®, is an oromucosal spray which delivers a 1:1 ratio of CBD and THC and it can be prescribed for neuropathic and oncological pain.

Starting from THC, different substitutions on the critical positions of the tricyclic scaffold (C-1, C-3, C-9) have been made, that led to the synthesis of numerous compounds. The most representative ones are described in **Fig. 6**.



8, Nabilone **9, HU-210**

10, JWH-133

Fig. 6 Chemical structures of Classical cannabinoids

Synthetic cannabinoid HU-210 (**9**) displayed significantly enhanced affinity for both CB receptors, producing many of the same pharmacological effects as **1**. Moreover, it has been demonstrated that the removal of C-1 hydroxyl group enhances the affinity for CB_2 rceptor.[18] Actually, in the *des*-hydroxyl series, compound **10**, known as JWH-133, showed 200 fold higher activity towards type-2 CB receptor, making it one of the most potent of the series.

Modifications of the terpenophenolic nucleus generated the so-called non classical cannabinoids.

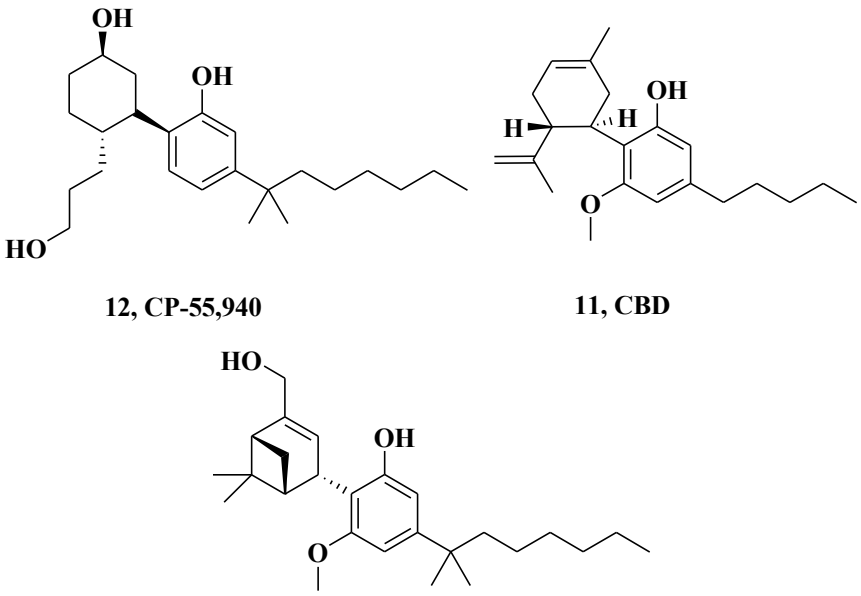


Fig. 7 Chemical structures of Nonclassical cannabinoids

This class of molecules include bicyclic or tricyclic analogues of THC that possess a replacement of the pyranyl ring or its entire loss. The most representative compound in this series (**Fig. 7**) is **12**, CP-55,940, which behaves as a full agonist with high affinity for both CB_1 and CB_2 receptors. Its radiolabelled [^{3}H] isotope is among the most widely employed reference compound in the characterization of cannabinoid ligands. Compound **13**, named HU-308, exhibited excellent selectivity for the CB_2 receptor (>440 fold) and showed anti-inflammatory and peripheral analgesic activity in a formalin-induced inflammation model in mice.[20]

In the early 1990s, it has been identified the first novel class of totally synthetic cannabinoid derivatives, charachterized by an aminoalkylindole core. These molecules present an indole scaffold substituted by a lipophilic aroyl group at C-3 and an aminoalkyl side chain at N-1 (**Fig. 8**).

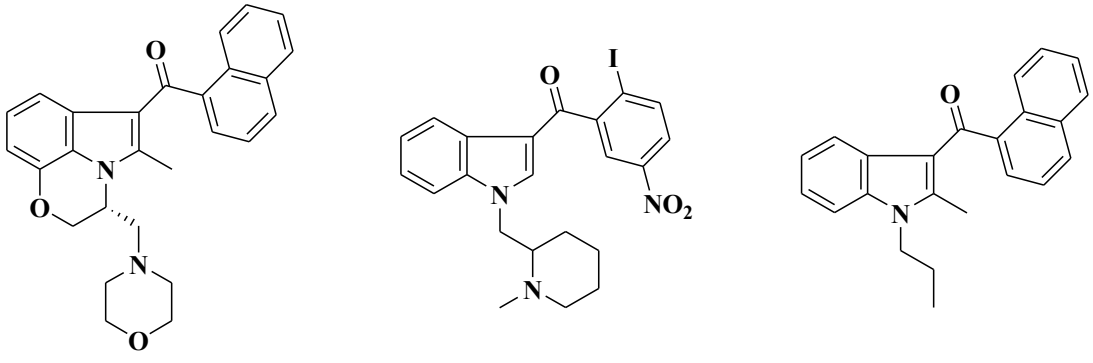
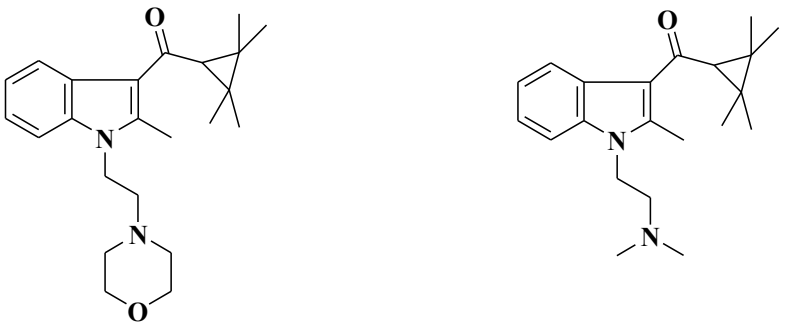


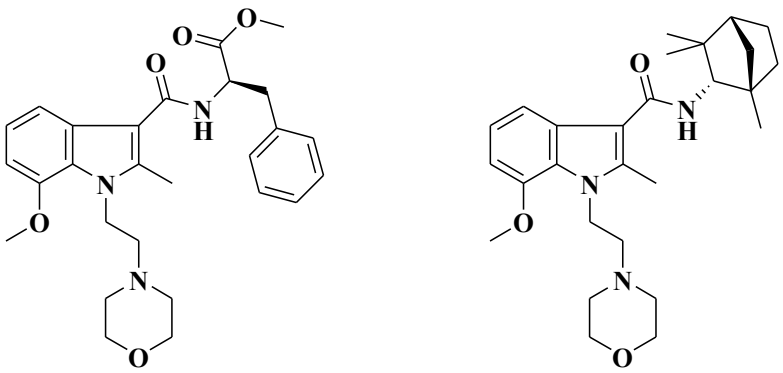
Fig. 8 Chemical structures of Aminoalkylindole derivatives

Compound **14**, labelled as WIN55212-2, is one of the most studied synthetic cannabinoids in the series and has been widely utilized, as [^{3}H] isotopic form, for pharmacological investigations in cannabinoid research.[21] The aminoalkylindole series is probably the most studied among the synthetic cannabinoids. Actually, various compounds in this group are noteworthy, like **15**, AM1241, which displays notable CB_2 selectivity (SI > 80). Collectively, the indolic cannabinoids presented small substituents at C-2 of the indole core, because larger groups usually led to a loss in activity. Compound **16**, JWH-015, also selective for the CB_2 subtype, showed also immunosuppressive properties.

Regarding C-3 position, although many of the synthetic cannabinoids have generally favoured 3-naphthoyl or 3-aroyl groups, a distinct chemical series carrying a tetramethyl cycloalkyl ketone has been developed, which displayed an interesting pharmacological profile. Compound **17** (A-796260) is a notable example, which revealed receptor affinity and selectivity about 192-fold over CB_1 receptor. Subsequently, extensive SAR studies have been carried out on the cycloalkyl ketone series, wherein side chain variations at the N-1 position led to potent and highly selective CB_2 agonists (**18**, **Fig. 9**).[22]



17, A796260 **18**

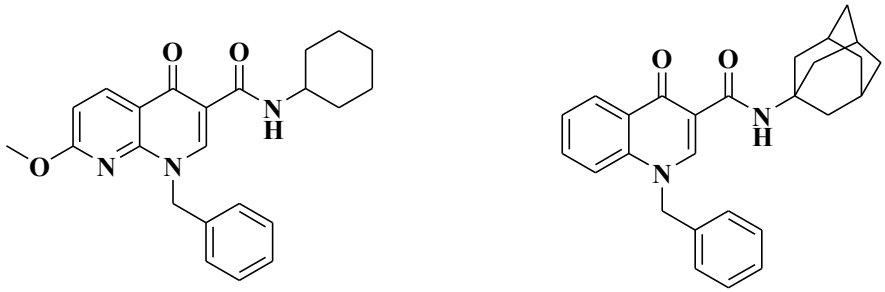


19 **20**

Fig. 9 Cycloalkyl ketone and Carboxamide derivatives

Afterwards, substitution of the ketone moiety with a bulky amide residue led to the formation of new indole-derived cannabinoid receptor ligands (**19**-**20**).[23] Compound **19** displayed an excellent CB_2 agonist behaviour, suggesting that a carboxamide residue could be involved in receptor binding.

Actually, several independent groups reported CB_2 selective 6,6-fused heterocyclic modulators which maintained the amide function. A series of naphthyridines (**21**) and quinolines (**22**) were synthesized and analyzed for their cannabinoid activity (**Fig. 10**).[24] Compound **22** provided the best pharmacological profile, with great affinity and 51-fold higher selectivity against CB_1 receptor. These novel series demonstrated significant analgesic effects in a mouse formalin test of acute peripheral and inflammatory pain.



21 **22**

Fig. 10 Naphtyridine and Quinoline derivatives as CB_2-receptor agonists

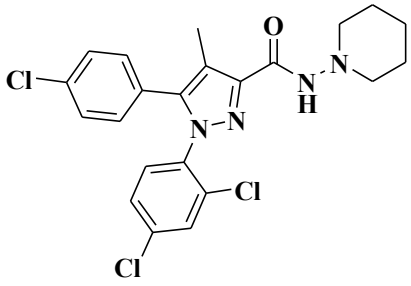
1.4.2 Antagonists

The developing of CB receptors antagonists aims to attenuate eCBs tone in those pathologies whose etiology involves high ECS activation. These medical conditions include eating disorders, especially obesity and associated cardiometabolic problems and drug addiction. To date, numerous CB antagonists have been synthesized, which are characterized by different chemical nucleus and are depicted in **Fig. 11**.

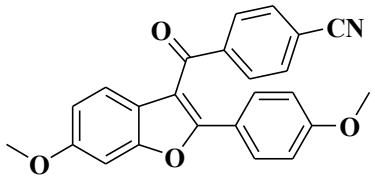
Specifically, the first CB_1 receptor anatagonist **23**, SR141716A, known as Rimonabant (Acomplia®), presents a diarylpyrazole structure. It binds with significantly higher affinity to CB_1 rather than CB_2 receptors, and lacks significant affinity for non-cannabinoid receptors. It behaves as an inverse agonist and showed clear clinical efficacy for the treatment of obesity and alcohol and tobacco addiction status.

LY320135 (**24**) presents a benzofuranic nucleus. It displays lower affinity to CB_1, compared to Rimonabant, but greater affinity to muscarinic, 5-hydroxytryptamine $(5\text{-HT})_2$, histamine, dopamine receptors and adrenoreceptors, at different concentrations.

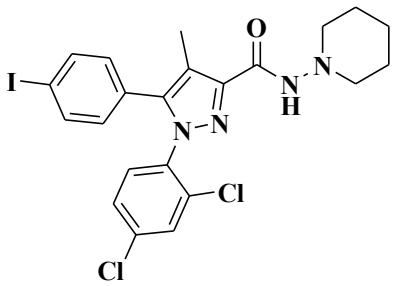
AM251 (**25**) and AM281 (**26**) are both characterized by a dyarylpyrazole core, like Rimonabant. They showed respectively three and eight times less potency than Rimonabant and both compounds proved higher affinity for CB_1 than CB_2 receptors.[25] Moving to CB_2-receptor antagonists, they could have therapeutic potential in inflammatory disorders, arthritis and autoimmune diseases. However, their development is too limited to analyze their pharmacological profile. The most important known CB_2-receptor antagonists are the diarylpyrazole **27** (SR144528) and the aminoalkylindole 6-iodopravadoline **28**, labelled as AM630. (**Fig. 10**). Compound **27** showed great selectivity towards CB_2 receptor in both *in vitro* and *in vivo* assays, with a SI higher than 700. Likewise, AM630 is a potent and selective compound which behaves as an inverse agonist for CB_2, with a K_i of 32.1 nM, and displays a selectivity index over 165.[26]



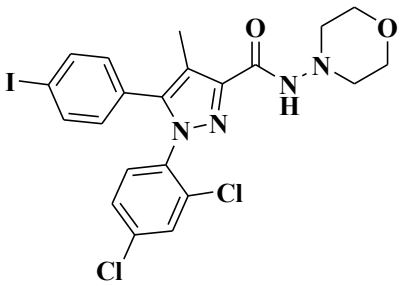
23, Rimonabant



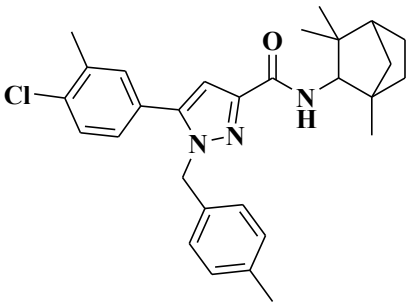
24, LY320135



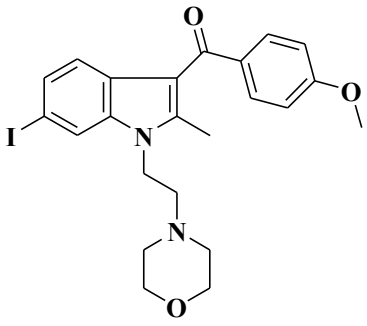
25, AM251



26, AM281



27, SR144528



28, AM-630

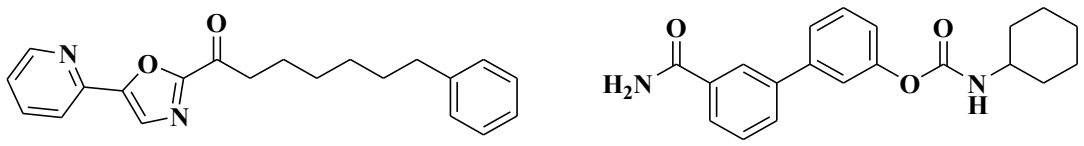
Fig. 11 Chemical structures of the most representative CB receptors antagonists

1.4.3 FAAH inhibitors

An attractive alternative to the preparation of CB receptors ligands is the development of FAAH inhibitors (**Fig. 12**), which raise the endogenous levels of eCBs by blocking their hydrolysis. This approach may avoid the undesired side effects of a conventional drug-receptor interaction.

To date, a remarkable series of potent, selective, and efficacious FAAH inhibitors have been disclosed and classified in reversible and irreversible compounds, basing on their mechanism of action. Many of them have been exploited as a tool to characterize the enzyme, define its mechanism of fatty acid amide hydrolysis, and validate it as a therapeutic target for the treatment of pain and inflammation.

The first class is characterized mostly by a α-ketoheterocycle scaffold that binds to FAAH by reversible hemiketal formation with a serine residue of the active site. However, additional classes of reversible FAAH inhibitors have been reported later, including substituted (thio)hydantoins and imidazolidinediones, oxime, enol carbamates, benzothiazoles, benzoxazoles, arylboronic acids, selected sulfonamides, cyclic ureas and lactams. The most representative reversible FAAH inhibitor is compound **29**, OL-135, which is reversible, competitive and selective. It produces analgesic, antinociceptive and anti-inflammatory activity in various preclinical animal models. However, much research groups focused on developing irreversible covalent FAAH inhibitors, in order to achieve long acting pharmacological activity in vivo. Carbamate-based inhibitors are the most studied class of irreversible inhibitors. Its lead compound is **30** (URB597), which binds to the active site and induces a carbamylation of Ser[241], inactivating the enzyme. Analogously to reversible inhibitors, also the irreversible class has been increased with a lot of different compounds: actually, research in this field is still growing.[27]



29, OL-135 **30, URB597**

Fig. 12 Chemical structures of the most representative FAAH inhibitors

1.5 THERAPEUTIC TARGETS

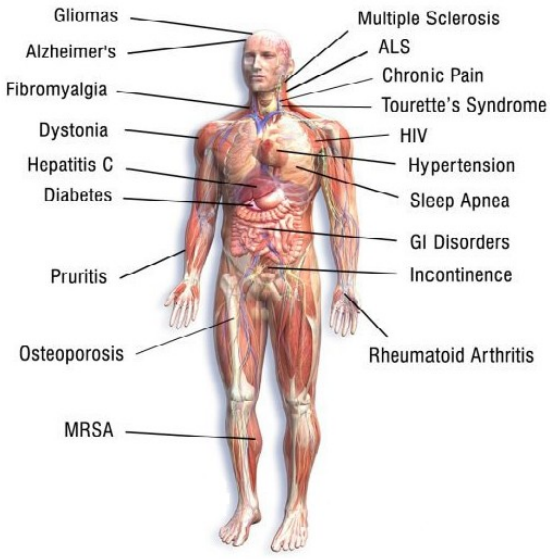


Fig. 13 Therapeutic targets of cannabinoids

As introduced previously in this work, *Cannabis* and its derivatives have always showed great potential as therapeutic agents (**Fig. 13**). In the last decades, scientists primarily studied cannabis ability to alleviate various disease symptoms, such as the nausea associated with cancer chemotherapy. Today, research is directed towards the potential role of cannabinoids, investigating their property to moderate autoimmune disorders, such as multiple sclerosis (MS), rheumatoid arthritis, and inflammatory bowel disease, as well as their role in the treatment of neurological disorders, that is to say Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). Moreover, many cannabinoids produce inhibition of pain responses and can be useful in management of glaucoma, bronchial asthma, inflammatory disorders and cancer.[28]

Some of the most widespread uses of cannabis derivatives are analyzed below.

1.5.1 Nausea and vomiting

The development of chemotherapy drugs has improved cancer treatment. However, this kind of compounds present remarkable side effects, such as nausea and vomiting, which may last for several days. These symptoms determine a reduction of patient compliance, interfering with therapy success. Since the discovery of the ECS, considerable researches confirm that cannabinoids serve as effective anti-emetics. In ferret and shrew models, the site of action has been identified in the emetic area of the brainstem and the dorsal vagal complex. It is has been confirmed that their anti-emetic properties are mediated by CB_1 receptors; actually, CB_1 antagonists SR-141716 and AM251 respectively potentiated the strength of toxin-induced vomiting in animal models. Yet, also CBD, which does not possess affinity for type-1 cannabinoid receptor, can produces anti-emetic effects. Consequently, further researches need to be carried on about this property.[29]

1.5.2 Neurodegeneration and neuroprotection

Cannabinoids showed neuroprotective and anti-proliferative properties. Recently, many research groups have demonstrated the involvement of ECS in the protection against acute or chronic brain damage. In particular, phytocannabinoids, synthetic and endogenous cannabinoids showed neuroprotection both *in vitro* and *in vivo* cytotoxic models of ischemia and head trauma. In addition, cannabinoids are also neuroprotective in several chronic neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), ALS, AD and MS, which present excitotoxicity, mitochondrial dysfunction, inflammation and oxidative stress. There are few molecular mechanisms underlying these properties. Actually, they include some processes not mediated by CB receptors directly, but involving *N*-methyl-D-aspartate (NMDA) receptor antagonism, and others that are definitely mediated by either CB_1 or CB_2 receptors, which are able to reduce glutamate release, calcium influx and inflammation, while on the other hand, can stimulate γ-aminobutyric acid (GABA) activity.[30]

1.5.3 Appetite and obesity

Cannabis ability to stimulate appetite has been known all along its widespread. Recent experiments using synthetic or natural cannabinoid agonists have confirmed the role of the ECS as a modulator of food intake. However, the molecular mechanism is still far from being understood, and consequently its elucidation is the cornerstone of many researches. Based on these findings, dronabinol was approved by FDA in 1992 for the treatment of AIDS-related anorexia, a syndrome which occurs in about 18% of AIDS patients and which decreases the quality of life and the chance of recovery.

Interestingly, the first potent and selective CB_1 cannabinoid antagonist, Rimonabant, was able to reverse the hyperphagia induced by Δ^9-THC and also determined changes in ingestive behaviours when administered alone. These results suggested to exploit cannabinoid antagonists for the treatment of eating disorders and, above all, obesity. Moreover, based on preliminary pharmacological studies, CB_1 antagonists may also be useful for the treatment of alcoholism, and in smoking cessation. Actually, clinical studies with Rimonabant have already shown increased tobacco-smoking abstinence as well as prevention of the secondary weight gain often associated with smoking cessation.

1.5.5 Pain

Pain is a weakening condition that responds poorly to available therapies. It is generally associated with nerve injury, toxic insults and disease states. Consequently, drug discovery efforts have been directed toward the identification on novel analgesic targets. The role of cannabinoids in pain modulation has been systematically studied since the XIX century, although the mechanism underlying the analgesic effects was barely understood until the characterization of CB receptors. Actually, cannabinoids suppress allodynia and hyperalgesia in different neuropathic pain models, through CB_1 and CB_2 specific mechanism. These studies have not only provided a detailed understanding of the network of neural and inflammatory cells, but, moreover, have led to the comprehension of the physiological role of eCBs in pain regulatory circuits. Nevertheless, the psychoactive effects mediated by central CB_1-activation, represent an important boundary in pharmacotherapies. It follows that novel approaches to minimize unwanted side effects, such as selectively targeting CB_2-receptor, represent a valuable direction for future evaluations.

1.5.6 Cancer

The therapeutic effects of cannabinoids on some cancer-related conditions, such as emesis, nausea, depression, muscle tension, insomnia, chronic pain and loss of appetite, have been widely investigated. The anti-neoplastic activity of Δ^9-THC and its derivatives was first observed in the early 1970s, but no further investigations were performed on this topic until recently.

Several natural (THC and CBD), synthetic (WIN-55, 212-2 and HU-210) and endogenous cannabinoids (AEA and 2-AG) produce antiproliferative effects on a wide spectrum of tumour cells.[31] More importantly, cannabinoid administration to nude mice delays the growth of various tumours, including lung carcinomas, gliomas, thyroid epitheliomas, skin carcinomas and lymphomas.

It has been proved that eCBs play a protective role in the early stages of tumour development. In a recent study, indeed, AEA and the corresponding biosynthetic precusors levels have been analyzed in various human tumours. In most cases, cancer tissues presented higher levels of AEA compared to the healthy tissues. As evidence, several tumour cell lines demonstrated the same peculiarities: the ability to synthesize eCBs, the expression of CB receptors and the expression of their biosynthetic enzymes,

confirming the involvement of ECS in the control of cell proliferation. Moreover, it has been demonstrated that AEA and 2-AG inhibit the cell proliferation in breast cancer and prostate cancer, through CB_1 stimulation. Subsequent findings have than reported the inhibitory action of cannabinoids on the growth of glioma cell lines, thyroid epitheliomas, and mast cell lymphomas. More recent studies have focused the attention on the effect of eCBs in angiogenesis and formation of metastases. In conclusion, the use of cannabinoid receptor ligands might not only slow down the growth of tumours via multiple mechanisms, but at the same time also attenuate the related disorders. However, many efforts still need to be done in this research area.[32]

Research Purposes and Objectives

To date, CB_2 receptor agonists have gained attention as potential therapeutic targets in the management of numerous diseases. It is necessary to achieve selectivity towards this receptor subtype in order to avoid the characteristic central side effects, mediated by CB_1-activation.

The purpose of this project has been the design, the synthesis and the biological evaluation of new CB_2-selective agonists.

We designed a new chemical structure (**Fig.14**) that included the primary characteristics of the most active and selective CB_2-receptor agonists (**14**, **22)**. Initially, we decided to prepare a series of diazepinoquinoline (**46-52**) and pyridoquinoxaline (**53-56**) derivatives, basing on their easy preparation. Actually, we optimized the synthetic routes, obtaining the formation of the tricyclic scaffold in just three steps.[33] Then, we synthesized different lipophilic carboxamide derivatives, in order to value the influence of carboxamide moiety on the receptor affinity.

The novel synthesized compounds have been evaluated in receptor binding assays, using [^{3}H]CP,55-940 as radioligand. The preliminary obtained data were discouraging, giving affinity values higher than 150 nM.

Consequently, we needed to improve the molecular core, thus we designed a new hybrid chemical structure defined by a oxazinoquinoline scaffold (**Fig. 15**).

The first synthesized examples of this novel series (**69-80**) showed really promising results (**Table 2**) which led to explore the novel nucleus, by investigating the effect of different substitutions at the crucial positions (C-2, C-3, C-6, C-8, C-9, C-10) on the affinity for CB receptors.[34]

The novel oxazinoquinoline derivatives were tested in competition binding assays towards both rat and human CB receptors. Affinity values (K_i, nM) were used to calculate the selectivity of these novel compounds versus CB_2 receptors. The ligands were also examined in cAMP assays on hCB_2 (Chinese hamster ovary) CHO cells, in order to evaluate the inhibition of the adenylyl cyclase activity and consequently the potency values (IC_{50}, nM).

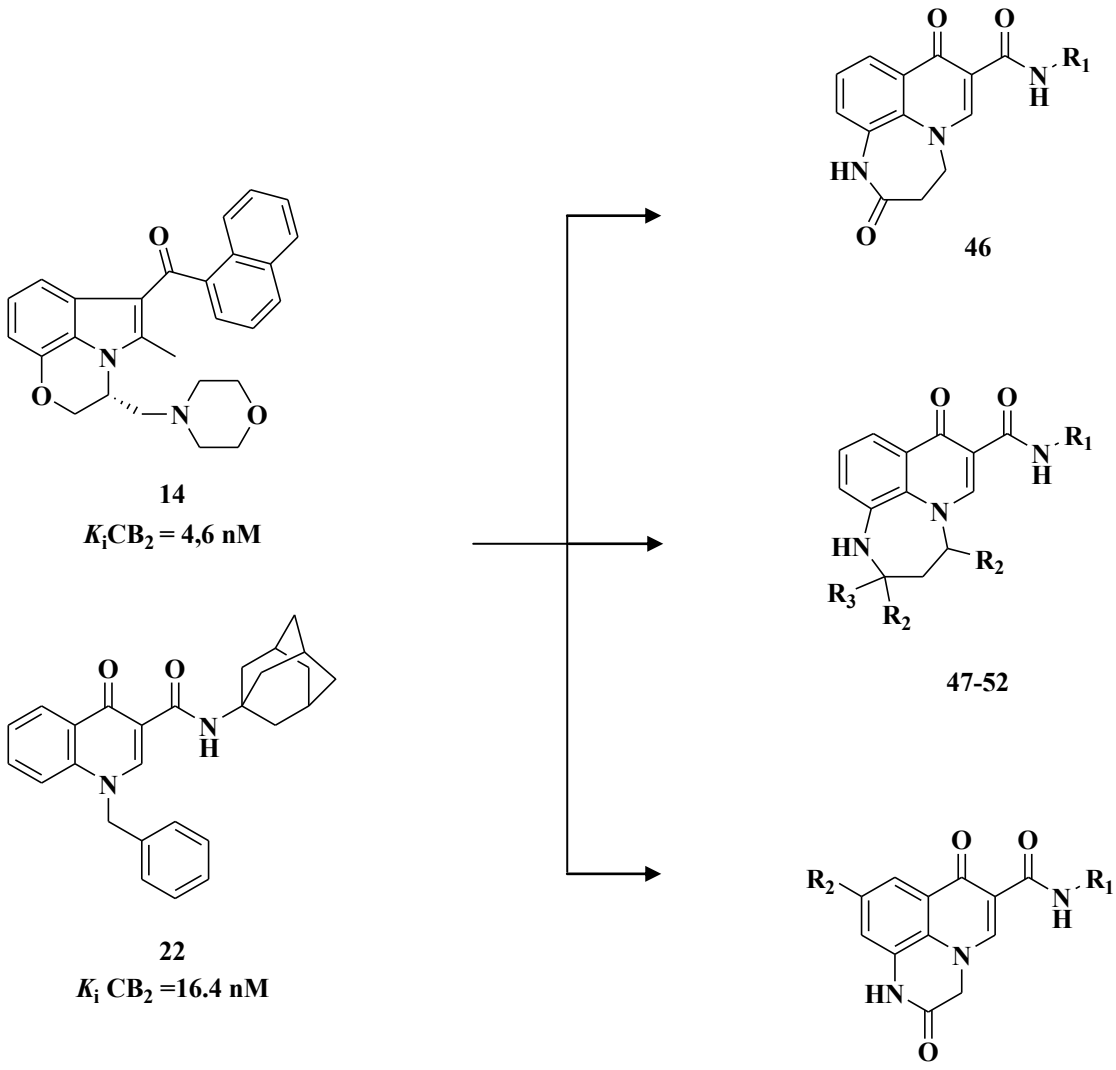
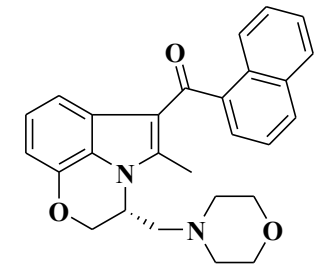
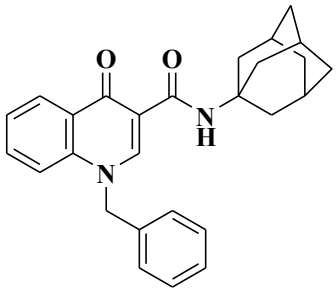


Fig. 14 Rational design of diazepinoquinoline and quinoxaline derivatives.
Baraldi, P.G.; Ruggiero, E.; Agahazadeh Tabrizi, M. *J. Het. Chem.* 2014, 51, 101-105



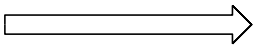
14

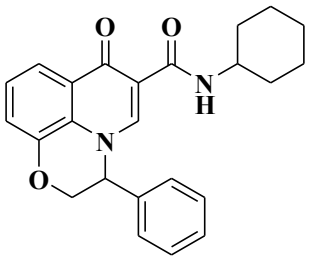
$K_i CB_2$ = 4,6 nM



22

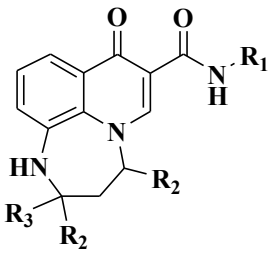
K_i CB_2 =16.4 nM





70

K_i CB_2 = 2.52 nM



47-52

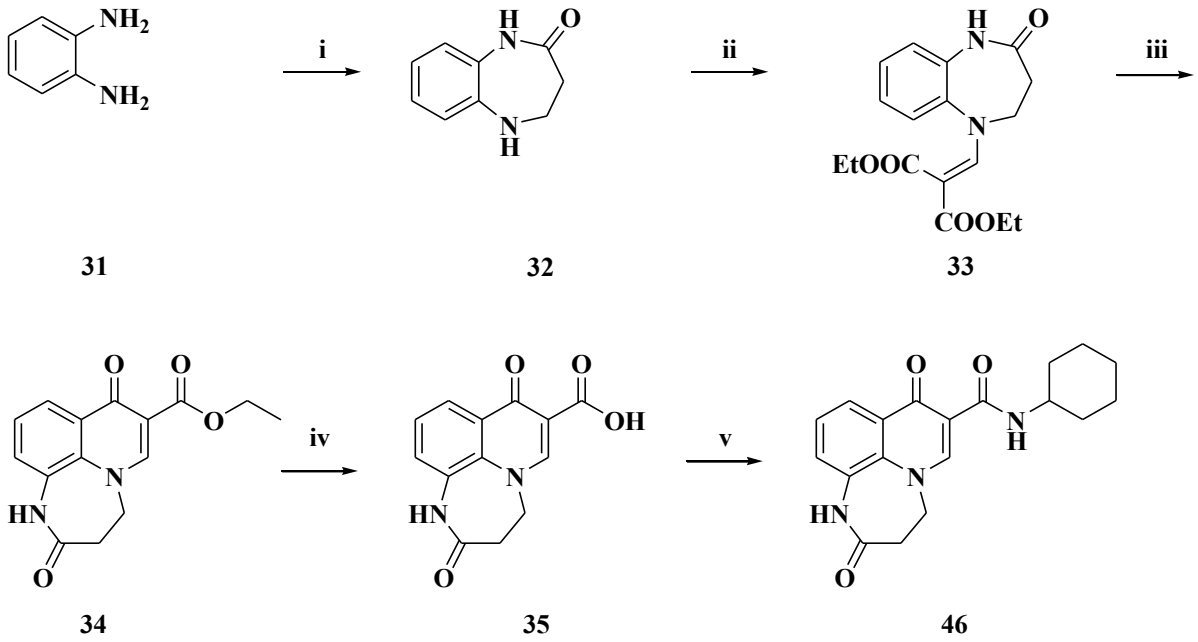
K_i CB_2 > 150 nM

Fig. 15 Rational design of oxazinoquinoline compounds
Baraldi, P.G.; et al. *J. Med. Chem.* 2012, 55, 6608-6623

Development of diazepinoquinoline and pyridoquinoxaline derivatives

The 2,8-dioxo-1,2,3,4-tetrahydro-8*H*[1,4]diazepino[3,2,1-*ij*]quinoline-7-carboxamide **46** was prepared as described in **Scheme 1**. Starting *o*-phenylenediamine **31** was condensed with acrylic acid to yield the benzodiazepin-2-one **32** in 68% yield.[35] Following reaction with diethyl ethoxymethylene malonate (DEEM) at 140 °C led to the formation of the methylenemalonate derivative **33** in 90% yields. Cyclization of **33** in polyphosphoric acid (PPA) provided the tricyclic derivative **34** in 95% yields. After hydrolysis of the ethyl ester functionality of compound **34** in acidic conditions, the resulting carboxylic acid **35** was converted into the target carboxamide **46** under peptide coupling conditions.

Scheme 1[a]

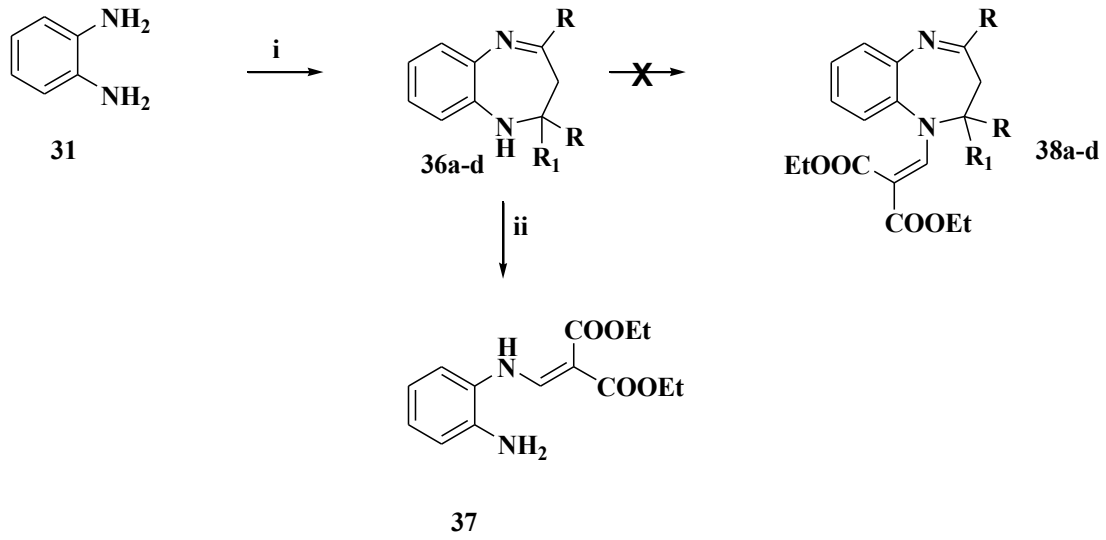


[a] *Reagents and conditions*:
(i) acrylic acid, HCl conc., H_2O, 70 °C, 3 h;
(ii) DEEM, 140 °C, 2 h;
(iii) PPA, 140 °C, 1 h;
(iv) HCl 20%, CH_3COOH, 100 °C, 4h;
(v)cyclohexylamine, DIEA, HBTU, DMF, rt, 16 h.

The synthesis of diazepinoquinoline derivatives **47-52** was initially realized as outlined in **Scheme 2**. 2,3-dihydro-1*H*-benzo[*b*][1,4]diazepines **36a-d** were prepared by heating *o*-phenylenediamine **31** with appropriate ketones in the presence of 2 mol% *N*-bromosuccinimide (NBS) as a catalyst, which mechanism is depicted in **Fig. 16**.[36] Subsequent addition-elimination reaction with DEEM did not afforded **38a-d** as

expected, but determined hydrolysis of the iminic carbon nitrogen double bond, yielding diethyl 2-[(2-aminophenylamino) methylene]malonate **37**.

Scheme 2



[a] *Reagents and conditions*:
(i) appropriate ketone, 10mol % NBS, 40 °C, 4h;
(ii) DEEM, 140 °C, 2 h.

The alternative route to prepare the tricyclic scaffold is shown in **Scheme 3**. Sodium borohydride ($NaBH_4$) was used to reduce the carbon nitrogen double bond of compounds **36a-d** in 75-80% yield.[37, 38] Saturation of prochiral imine **36c** provided a mixture of *syn/anti* diastereomers (3:1) that were separated by column chromatography on silica gel to furnish the *syn*-isomer **38c** as major diastereomer. The *syn*-conformation was confirmed by NMR studies, such as NOEDIFF experiments, that showed the presence of NOE enhancements between H-3 (δ = 2.39 ppm) and H-4 (δ = 4.43 ppm) protons. Moreover, the saturation of H-4 (δ = 4.43 ppm) protons did not reveal NOE with the cyclopentyl hydrogens.

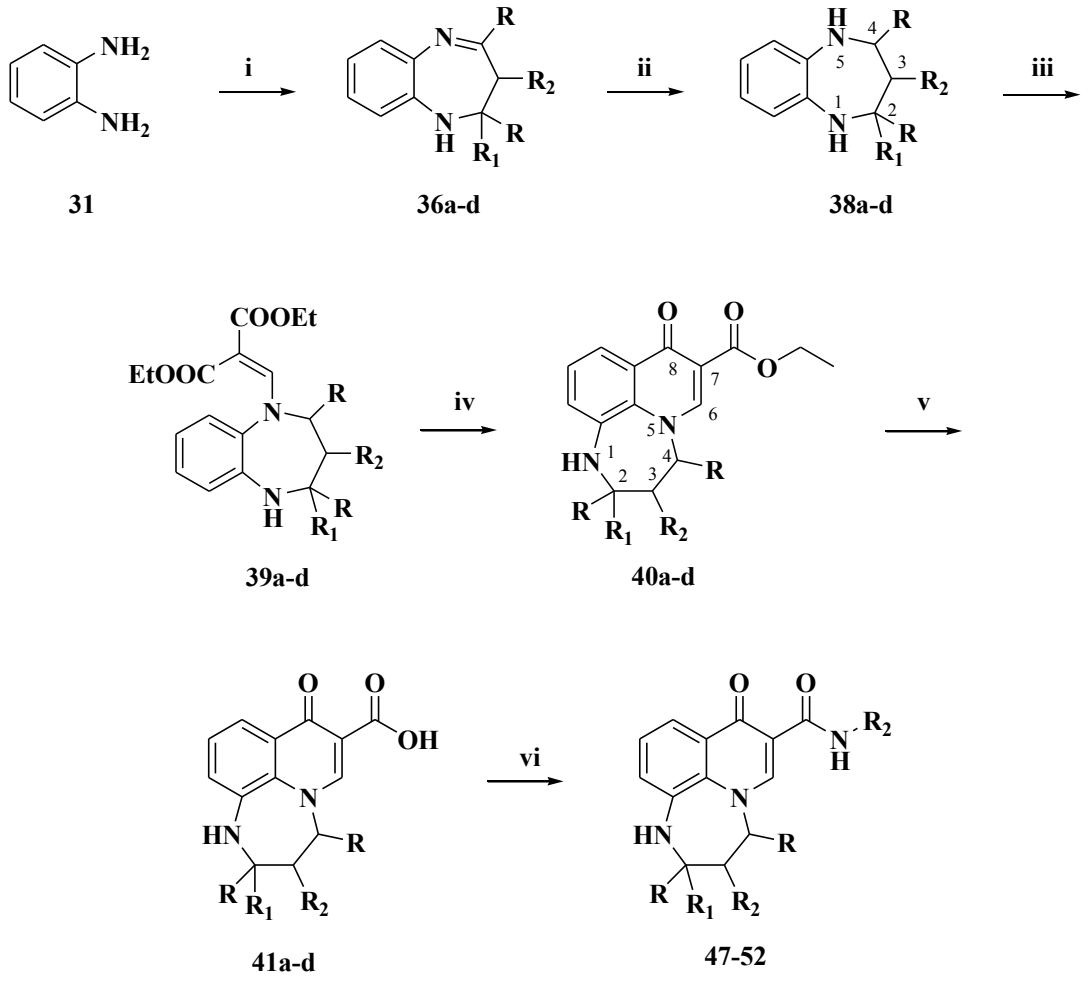
Regarding compound **38d,** surprisingly, hydrogenation with $NaBH_4$ gave only the *syn*-diastereomer. For compound **38d** too, the configuration was assessed through NOEDIFF experiments. The saturation of C-2 methyl (δ = 1.64 ppm) protons resulted in NOE enhancements at H-4 (δ = 4.22 ppm), suggesting that the methyl at C-2 and H-4 should be oriented in the same direction. The ^{1}H and ^{13}C NMR data of this compound are completely consistent with those reported in literature for the *sin*-isomer.[39]

Condensation with DEEM at 140 °C for 2 h yielded the 5-methylenemalonate derivatives **39a-d** (85-90%). The regioselective addition of DEEM at N-5 position was

preferred because of the less steric hindrance present at the 4-position rather than at the 2-position. Subsequently, cyclization reaction with PPA at 130°C in 30 minutes gave compounds **40a-d** in 35-40% yields.[40, 41] The chemical identity of ethyl carboxylate derivatives **40a-d** has been established by ^{1}H NMR and MS spectra, while NMR NOEDIFF studies were conducted in order to analyze the C-4 substituents. Actually, compounds **40a-d** showed NOE enhancement from the position 6-CH to the 4-CH protons on the benzodiazepinoquinoline nucleus, confirming the addition of methylenmalonate chain on the NH close to the less steric hindrance carbon atom.

Eventually, hydrolysis of the ethyl ester derivatives, followed by coupling with appropriate amines, gave the desired compounds **47-52**.

Scheme 3[a]



[a] *Reagents and conditions*:
(i) appropriate ketone, 10mol % NBS, 40 °C, 4h;
(ii) $NaBH_4$, CH_3OH, rt, 2h;
(iii) DEEM, 140 °C, 2 h.
(iv) PPA, 140 °C, 1 h;
(v) HCl 20%, CH_3COOH, 100 °C, 4h;
(vi) amine, DIEA, HBTU, DMF, rt, 16 h, or amine, EDC, HOBt, DMF, rt, 16h.

a: R = R_1 = methyl, R_2 = H
b: R = R_1 = ethyl, R_2 = H
c: RR_1 = cyclopentyl, R_2R = cyclopentyl
d: R =phenyl, R_1 = methyl, R_2 = H

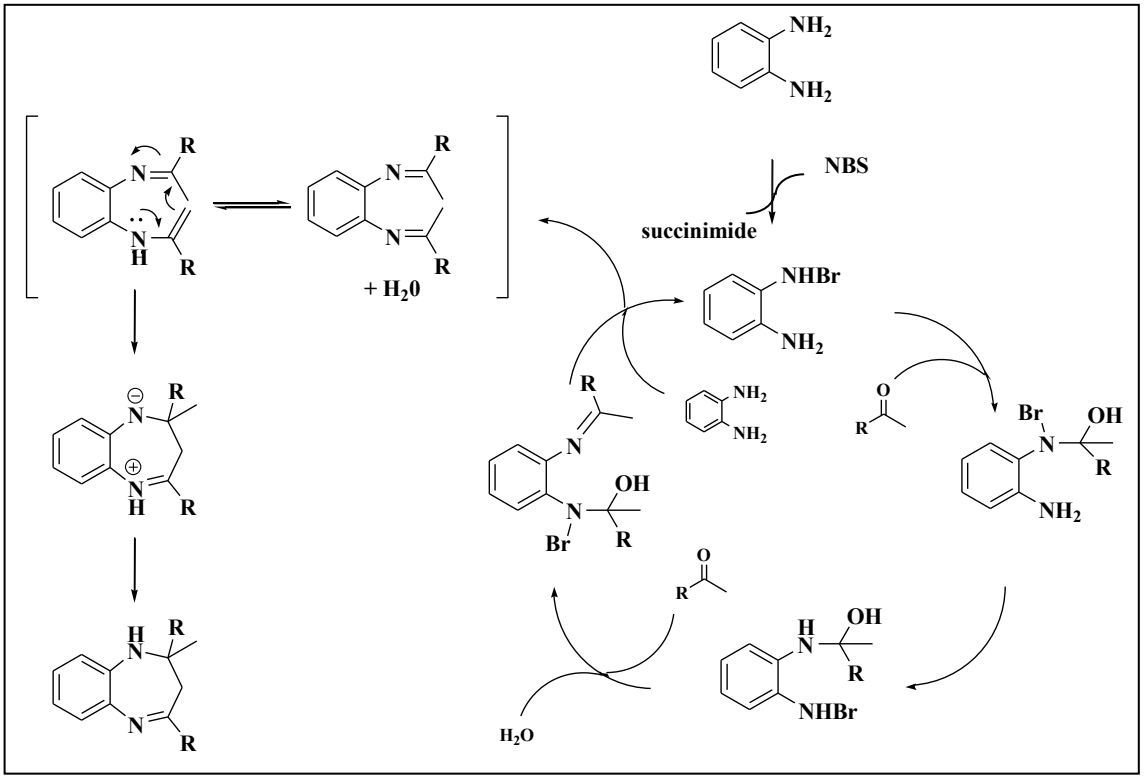


Fig. 16 Mechanism for NBS catalyzed reaction. The amine of *o*-phenylenediamine is activated by NBS in order to attack the carbonyl group of the ketone and providing intermediate diimine. Subsequently, a 1,3-shift of the hydrogen attached to the methyl group occurs to form an isomeric enamine, which cyclizes to afford the diazepine ring.

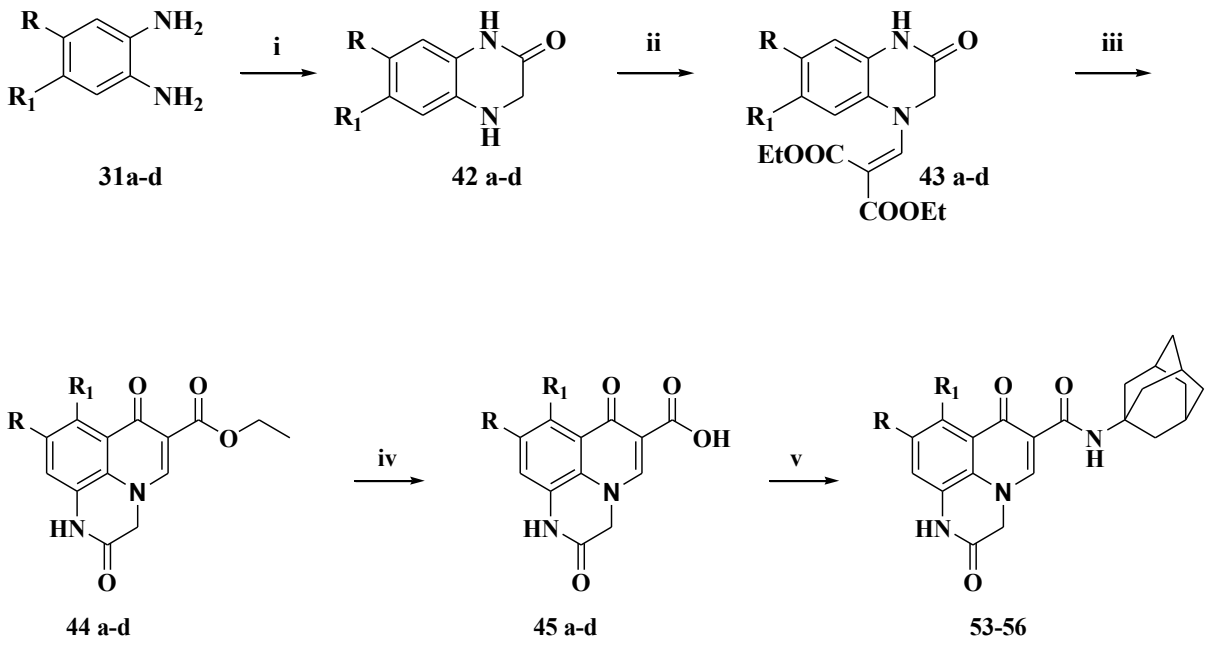
The synthesis of ethyl pyrido[1,2,3-*de*]quinoxaline-6-carboxamide derivatives is depicted in **Scheme 4**. Only few papers reported the preparation of 9-substituted-3,4-dihydroquinoxalin-2(1*H*)-ones and, generally, their synthesis has been developed starting from 2-nitrobenzenamine or 1-fluoro-2-nitrobenzene as starting materials.[42-44]

We optimized a new synthetic approach from *o*-phenylenediamines, that reacted with 2-bromoacetate in presence of triethylamine (TEA) at 80°C to give the 3,4-dihydroquinoxalin-2-(1*H*)-ones **(42a-d)** in 80% yields.[43] Preparation of target compounds **53-56** proceeded analogously to the synthesis of previous tricycles, by addition of DEEM to 3,4-dihydroquinoxalin-2-(1*H*)-one derivatives **43a-d**, followed by cycloacylation in PPA at high temperature to yield compounds **44a-d**. Hydrolysis of ethyl 1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-*de*]quinoxaline-6-carboxylate **44a-d** gave the correspondent carboxylic acids **45a-d** in high yields. Finally, intermediates **45a-d** were converted into the desired compounds **53-56**, as shown previously.

The position of halogens 9-F, 9-Cl on the quinoxaline core in compounds **44b** and **44c** was confirmed by ^{1}H NMR analysis. Moreover, ^{19}F NMR resonance was used to resolve fluorine derivative **44b**: it displays three signals at -114.070, -114.094, -114.119

ppm with scalar couplings of equal intensity (J_{HF} (H-8) = J_{HF} (H-9) = 10 Hz), which confirm the presence of fluorine residue on C-9.

Scheme 4[a]



31a-d **42 a-d** **43 a-d** **44 a-d** **45 a-d** **53-56**

[a] *Reagents and conditions*:
(i) ethyl 2-bromoacetate, TEA, DMF, 80 °C, 3h;
(ii) DEEM, 140 °C, 2 h;
(iii) PPA, 140 °C, 1 h;
(iv) HCl 20%, CH_3COOH, 100 °C, 4h;
(v) adamant-1-ylamine, DIEA, HBTU, DMF, rt, 16 h.

a, R = R_1 = H
b, R = F; R_1 = H
c, R = Cl; R_1 = H
d, R = R_1 = CH_3

Each of the newly synthesized compounds was examined in [^{3}H]CP-55,940 competition binding experiments for their affinity and selectivity towards the rat and human recombinant CB_1 and CB_2 receptors (**Table 1**).

Initially, the choice of the three kind of scaffolds was due to their manageable preparation. Thus, we synthesized the new compounds **46-56**, keeping the lipophilic carboxamide moiety, essential for the receptor binding.[45]

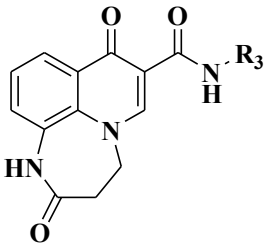
Unfortunately, among the three developed series, only 2,3,4,8-tetrahydro-8-oxo-1*H*-[1,4]diazepino[3,2,1-*ij*]quinoline-7-carboxamide derivatives (**47-52**) showed activity towards CB receptors, while compound **46, 53-56** resulted totally inactive.

In particular, introduction of an alkyl residue on the diazepine ring, such as methyl, ethyl, or cyclopentyl group, provided affinity for CB_2 receptors, with a reasonable selectivity. The best results in this series were obtained with compound **50**, with hK_i CB_2 = 153 nM and SI > 65. On the other hand, replacing the aliphatic moiety with an aromatic residue, like the phenyl ring (**48-49**), gave a total loss of activity.

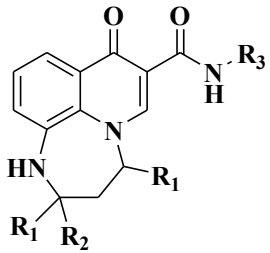
On the basis of these results, we hypothesized that the inactivity was due to the poor lipophilicity of these scaffolds (*c*Log *P* < 2.5). Actually, protein–ligand binding partially depends on lipophilic interactions, and the optimal *c*Log *P* for a class of ligands will depend on the nature of target protein. Since the endogenous ligands for the CB_2 receptor are highly lipophilic fatty acid derivatives, a higher range of *c*Log *P* values is required.[46]

Consequently, we decided to change the diazepine/piperazine cycle of the molecular core, designing a new hybrid chemical structure defined by a oxazinoquinoline scaffold (**Fig.10**).

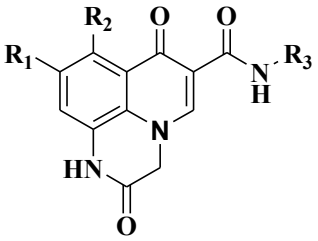
Table 1. Affinity (K_i, nM) and Selectivity Index (SI) of the Novel CB Compounds 46-56 on Rat and Human CB_1 and CB_2 Receptors[a]



46



47-52



53-56

				Ki (nM)				
Cpd	**R_1**	**R_2**	**R_3**	**rat CB_1[b]**	**rat CB_2[c]**	**hCB_1[d]**	**hCB_2[e]**	**SI**
14	(*R*)-(+)-WIN55,212–2			15.6 ± 1.4	7.58 ± 0.72	12.4 ± 1.3	4.53 ± 0.42	2.74
46			adamant-1-yl	>10000 (1%)	>10000 (1%)	>10000 (1%)	>10000 (1%)	
47	CH_3	CH_3	adamant-1-yl	5250 ± 556	213 ± 22	4540 ± 425	165 ± 14	27
48	ethyl	CH_3	adamant-1-yl	>10000 (1%)	194 ± 13	>10000 (1%)	153 ± 12	>65
49	ethyl	H	cyclohexyl	>10000 (1%)	248 ± 16	>10000 (1%)	204 ± 15	>49
50	cyclopentyl	cyclopentyl	adamant-1-yl	>10000 (1%)	3156 ± 282	>10000 (1%)	2852 ± 254	>3.5
51	phenyl	H	adamant-1-yl	>10000 (1%)	>10000 (15%)	>10000 (1%)	>10000 (21%)	
52	phenyl	H	cyclohexyl	>10000 (1%)	>10000 (1%)	>10000 (1%)	>10000 (1%)	
53	H	H	adamant-1-yl	>10000 (1%)	>10000 (1%)	>10000 (7%)	>10000 (1%)	
54	F	H	adamant-1-yl	>10000 (1%)	>10000 (2%)	>10000 (1%)	>10000 (3%)	
55	Cl	H	adamant-1-yl	>10000 (1%)	>10000 (1%)	>10000 (1%)	>10000 (1%)	
56	CH_3	CH_3	adamant-1-yl	>10000 (1%)	>10000 (1%)	>10000 (1%)	>10000 (1%)	

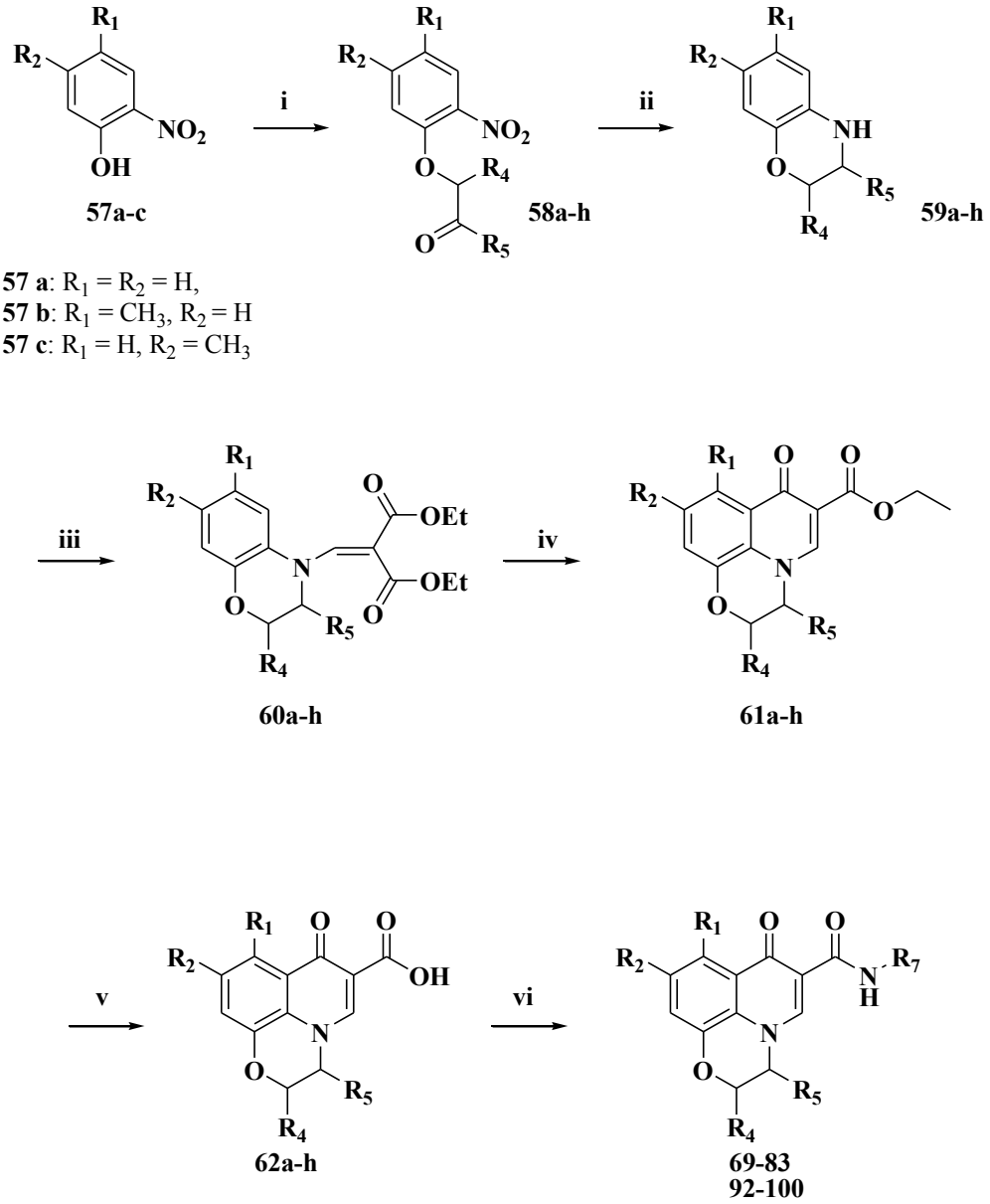
[a]The data are expressed as the mean ± SEM of n = 4 independent experiments. [b]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat brain for CB_1 receptors. [c]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat spleen for CB_2 receptors. [d]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_1 CHO membranes. [e]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_2 CHO membranes.

Synthesis and biological evaluation of oxazinoquinoline derivatives

The desired 7-oxo-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamides **69-109** were developed following two synthetic strategies, based on the availability of the α-halo ketone or amino alcohol necessary to generate the desired substitutions on the oxazine nucleus. Racemic compounds **69-83** and **92-100** were prepared as outlined in **Scheme 5**. Deprotonation of 2-nitrophenols **57a-c**, and subsequent alkylation with the appropriate α-halo ketones led to the formation of nitro ketones **58a-h**. In the second step, reduction of the nitro function of **58a-h** using 10% palladium-on-carbon in methanol (or methanol/THF (85:15) for 3-aryl derivatives) provided the benzoxazines **59a-g**,**i**,**j** by a simultaneous reduction–reductive amination sequence. Apparently, compounds **59i** and **59j** were formed as a consequence of a ring-opening of the cyclopropyl precursors **58g** and **58h**, respectively. Following reaction with DEEM at 140 °C gave the corresponding methylenemalonate derivatives **60a-g**,**i**,**j** in 70–90% yields. Intermediates **60a-g**,**i**,**j** were thermally cyclized in PPA for 1 h, providing the oxazinoquinoline ester derivatives **61a-g**,**i**,**j** in high yields.[47] Saponification of esters **61a**–**g**,**i**,**j** with sodium hydroxide furnished the carboxylic acids **62a-g**,**i**,**j**, which were converted into the target carboxamide derivatives **69-83** and **92-100,** by an amidation reaction with the appropriate amines under peptide coupling conditions.

Alternatively, the synthesis of compounds **84-91**, **101-104**, **108-109** was realized by the procedure utilized for the preparation of optically active ofloxacine (**Scheme 6**).[48] Ethyl 3-oxopropanoate derivatives (**64a-c**) were prepared starting from the corresponding benzoic acids (**63a-c**) by reaction with carbonyldiimidazole (CDI) in THF to give the imidazolide, and consequent condensation with the magnesium salt of monoethyl malonate under neutral conditions (90% yield).[49] Afterwards, intermediates **64 a-c** were converted in ethyl 2-(ethoxymethylene)propionate derivatives **65a-c** by condensation with triethyl orthoformate in refluxing acetic anhydride. Compounds **65a-c** were involved in an addition-elimination reaction with the proper amino alcohols in dry methylene chloride to yield the intermediates **66a**–**i**. The oxazinoquinoline scaffold was obtained by cyclization using potassium carbonate (K_2CO_3) in dimethylformamide (DMF) at 130 °C. After saponification of ethyl ester derivatives **67a-i**, the resulting carboxylic acids were coupled with the appropriate amines as reported in **Scheme 3**, to afford the target carboxamide derivatves. In the same way, reaction of compounds **65a-c** with the proper (*R*)- or (*S*)-amino alcohol gave the corresponding enantiomerically pure derivatives, detailed in **Table 5**.

Scheme 5[a]



57a-c

58a-h

59a-h

57 a: $R_1 = R_2 = H$,
57 b: $R_1 = CH_3$, $R_2 = H$
57 c: $R_1 = H$, $R_2 = CH_3$

60a-h

61a-h

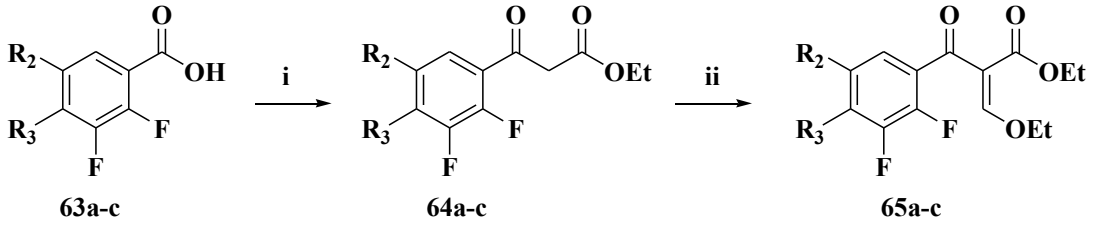
62a-h

69-83
92-100

[a] *Reagents and conditions*:
(i) α-halo ketone, K_2CO_3, acetone (anhydrous), rt, 16 h;
(ii) H_2 (4 atm), 10% Pd/C, MeOH, 4 h;
(iii) DEEM, 140 °C, 2 h;
(iv) PPA, 140 °C, 1 h;
(v) 10% NaOH, CH_3OH, 80 °C, 1 h;
(vi) amine, EDC, HOBt, DMF, rt, 6 h or amine, DIEA, HBTU, DMF, rt, 16 h.

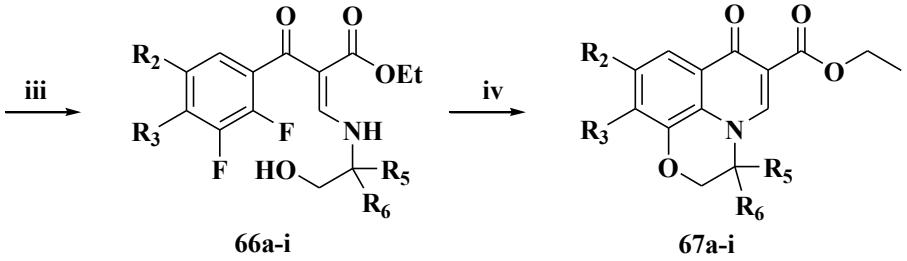
58-62 a: $R_1 = R_2 = R_4 = H$, $R_5 = CH_3$
58-62 b: $R_1 = R_2 = R_4 = H$, R_5 = Phenyl
58-62 c: $R_1 = R_2 = H$, $R_4 = CH_3$, R_5 = Phenyl
58-62 d: $R_1 = R_2 = H$, $R_4 = R_5$ = Phenyl
58-62 e: $R_1 = R_4 = H$, $R_2 = CH_3$, R_5 = Phenyl
58-62 f: $R_1 = R_2 = R_4 = H$, R_5 = 4-Tolyl
58-62 g: $R_1 = R_2 = R_4 = H$, R_5 = Cyclopropyl
58 h: $R_1 = CH_3$, $R_2 = R_4 = H$, R_5 = Cyclopropyl
59-62 i: $R_1 = R_2 = R_4 = H$, R_5 = Propyl
59-62 j: $R_1 = CH_3$, $R_2 = R_4 = H$, R_5 = Propyl

Scheme 6[a]

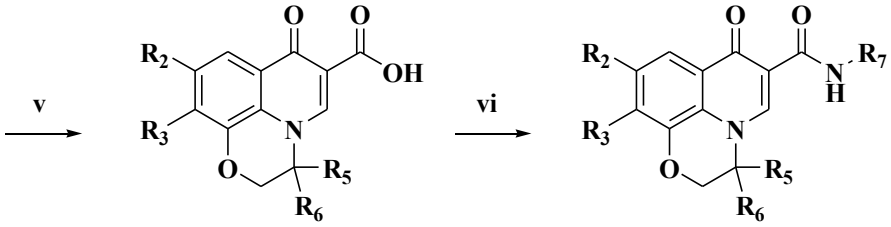


63a-c **64a-c** **65a-c**

63-65 a: $R_1 = R_2 = H$
63-65 b: $R_2 = H$, $R_3 = F$
63-65 c: $R_1 = R_2 = F$



66a-i **67a-i**



68a-i

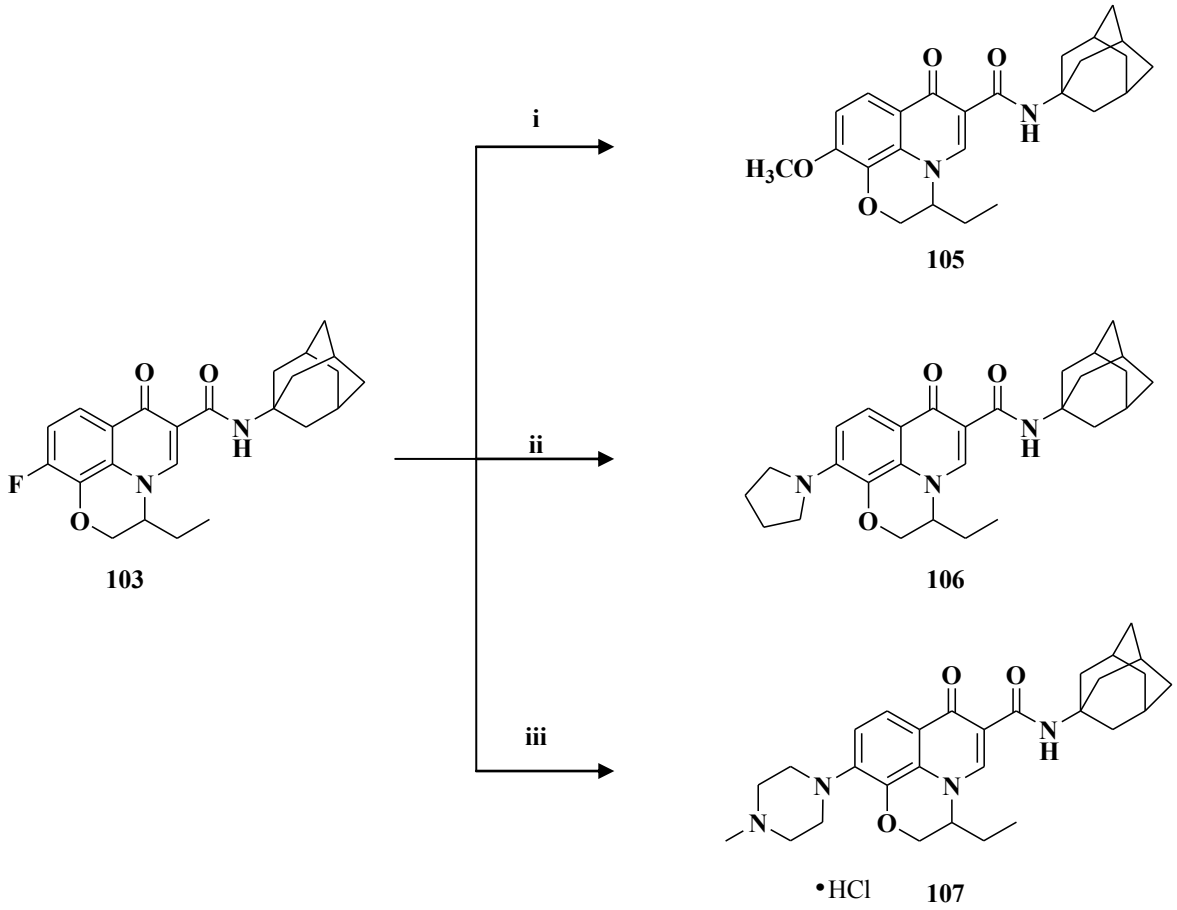
(*R*)-81, (*S*)-81
(*R*)-83, (*S*)-83
84-91
101-104
108-109

[a] *Reagents and conditions*:
(i) CDI, THF, $(C_2H_5O)_2Mg$, monoethyl malonate, rt, 16 h;
(ii) $CH(OEt)_3$, Ac_2O, 110 °C, 3 h;
(iii) appropriate (*R*)-, (*S*)-, or (*R*,*S*)-amino alcohol, CH_2Cl_2, rt, 1 h;
(iv) K_2CO_3, DMF, 130 °C, 7 h;
(v) 10% NaOH, CH_3OH, 80 °C, 1 h;
(vi) amine, EDC, HOBt, DMF, rt, 6 h or amine, DIEA, HBTU, DMF, rt, 16 h.

a: $R_2 = R_3 = H$, R_5, R_6 = H, Methyl
b: $R_2 = R_3 = H$, R_5, R_6 = H, Ethyl
c: $R_2 = R_3 = H$, R_5, R_6 = H, i-Propyl
(*R*)-d: $R_2 = R_3 = R_6 = H$, R_5 = i-Butyl
e: $R_2 = R_3 = H$, R_5, R_6 = H, Phenyl
(*R*)-f: $R_2 = R_3 = H$, R_5, R_6 = H, Benzyl
g: $R_2 = R_3 = H$, $R_5 = R_6 = CH_3$
h: $R_2 = H$, $R_3 = F$, R_5, R_6 = H, Ethyl
i: $R_2 = R_3 = F$, R_5, R_6 = H, Ethyl

Finally, the target compounds **105-107** were synthesized by substitution of the fluorine atom at C-10 by treating the amide **103** with different nucleophilic agents (sodium methoxide, pyrrolidine, *N*-methylpiperazine) in alkaline conditions (**Scheme 7**). Moreover, the 4-methylpiperazine derivative **107** was transformed into the hydrochloride salt by treatment with 1,4-dioxane saturated with gaseous hydrogen chloride.

Scheme 7[a]



103 **105** **106** **107**

[a]Reagents and conditions:
(i) CH_3ONa, THF, 50 °C, 16 h;
(ii) K_2CO_3, DMF, pyrrolidine, 100 °C, 10 h;
(iii) K_2CO_3, DMF, 1-methylpiperazine, 100 °C, 10 h, then 1,4-dioxane saturated with HCl gas, 0 °C, 30 min.

The newly synthesized compounds were tested in [^{3}H]CP-55,940 competition binding experiments in order to value their affinity and selectivity towards the rat and human recombinant CB_1 and CB_2 receptors (**Tables 2-5**).

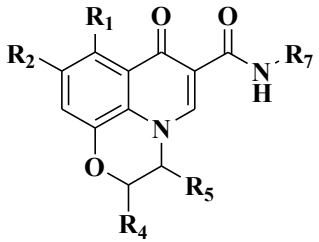
Initially, it has been synthesized a series of 12 compounds (**69**–**80**, **Table 2**) bearing an aromatic moiety (phenyl or 4-tolyl) at C-3 of the oxazinoquinoline tricycle. Substituents on the carboxamide group at C-6 have been selected basing on known cannabinoid pharmacophores, such as those present in the naphthyridine[24, 45, 50] (**21**) and quinolone[51-53] (**22**) derivatives.

As expected, the 3-phenyl analogues bearing a cyclohexyl (**70**), cycloheptyl (**71**), adamant-1-yl (**72**), or 3,5-dimethyladamant-1-yl (**73**) carboxamide showed great affinity at the CB_2 and poor affinity at the CB_1 receptors. Compounds **71** and **73** were the most potent and selective in this group (**71**, h K_i = 0.81 nM, SI = 383; **73**, hK_i = 3.45 nM; SI = 133). Compound **69**, possessing a cyclopentyl carboxamide moiety, showed lower affinity and poor selectivity at the CB receptors, suggesting that the carboxamide group plays an important role, as reported in literature.

Substitution of C-2 position with a methyl group resulted in a dramatic loss of affinity (**74,** hK_i = 536 nM; **75**, hK_i = 332 nM), relative to that of the corresponding compounds lacking the methyl group (compounds **70** and **72**, respectively). Likewise, compound **76**, which bears a phenyl group at C-2, was totally inactive at both CB receptors, confirming that the affinity of this class requires the C-2 position of the oxazinoquinoline nucleus unsubstituted. Intriguingly, the introduction of a methyl group at the *para*-position of the phenyl ring at C-3 (**77**, **78**) provided a remarkable loss of affinity and selectivity for both CB receptors, in comparison with those of the analogous compounds **70** and **72** lacking this substituent. These data suggest that an excessive steric hindrance at this position prevents the receptor binding.

It has been also investigated the substitution of C-9 of oxazinoquinoline scaffold, however the presence of a methyl group resulted in lower affinity (**79**, hK_i =18.4 nM) and selectivity values, compared to those of the analogue without the methyl group (**70**). Derivative **80** was not tested due to its low solubility in DMSO/water.

Table 2. Affinity (K_i, nM) and Selectivity Index (SI) of Novel 3-Aryl CB Compounds on Rat and Human CB_1 and CB_2 Receptors and Potency (IC_{50}, nM) of the Novel CB Compounds in hCB_2 CHO Cells on cAMP Assays[a]



69-80

						K_i (nM)					
Cpd	**R_1**	**R_2**	**R_4**	**R_5**	**R_7**	**rat CB_1**	**rat CB_2**	**hCB_1**	**hCB_2**	**SI**	**hCB_2 IC_{50} (nM)**
14	(*R*)-(+)-WIN 55,212-2					15.6 ± 1.4	7.58 ± 0.72	12.4 ± 1.3	4.53 ± 0.42	2.74	15.7 ± 1.3
69	H	H	H	Phenyl	Cyclopentyl	150 ± 16	110 ± 12	132 ± 12	98 ± 10	1.35	472 ± 43
70	H	H	H	Phenyl	Cyclohexyl	1150 ± 110	19.3 ± 2.3	420 ± 38	2.52 ± 0.21	166	15.2 ± 1.7
71	H	H	H	Phenyl	Cycloheptyl	372 ± 36	14.7 ± 1.5	310 ± 29	0.81 ± 0.07	383	5.23 ± 0.42
72	H	H	H	Phenyl	Adamant-1-yl	670 ± 65	18.3 ± 2.4	265 ± 24	14.2 ± 1.5	19	26 ± 3
73	H	H	H	Phenyl	3,5-Dimethyl-adamant-1-yl	545 ± 53	46 ± 3	460 ± 48	3.45 ± 0.42	133	18.3 ± 1.9
74	H	H	CH_3	Phenyl	Cyclohexyl	3520 ± 320	710 ± 44	3126 ± 288	536 ± 58	5.83	2749 ± 245
75	H	H	CH_3	Phenyl	Adamant-1-yl	2640 ± 225	443 ± 26	2435 ± 231	332 ± 28	7.33	1684 ± 152
76	H	H	Phenyl	Phenyl	Adamant-1-yl	>10000	>10000	>10000	>10000	ND	ND
77	H	H	H	4-Tolyl	Cyclohexyl	4566 ± 425	547 ± 32	4108 ± 380	468 ± 42	8.78	2457 ± 213
78	H	H	H	4-Tolyl	Adamant-1-yl	4154 ± 410	482 ± 43	3877 ± 362	412 ± 40	9.41	2215 ± 211
79	H	CH_3	H	Phenyl	Cyclohexyl	1568 ± 164	25 ± 3	1346 ± 125	18.4 ± 1.9	73	110 ± 9
80	H	CH_3	H	Phenyl	Adamant-1-yl	ND	ND	ND	ND	ND	472 ± 43

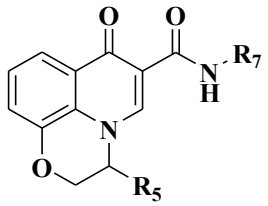
[a]The data are expressed as the mean ± SEM of n = 4 independent experiments. [b]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat brain for CB_1 receptors. [c]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat spleen for CB_2 receptors. [d]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_1 CHO membranes. [e]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_2 CHO membranes. [f]IC_{50} values were calculated on cAMP experiments performed on human CB_2 CHO cells.

The structure–activity relationship (SAR) studies were increased by synthesizing 3-alkyl derivatives, in order to evaluate the effect of the chain length and branching (**Table 3-4**, compounds **81**–**101**). Introduction of a methyl group at this position led to an enhancement in selectivity, although with a modest decrease in affinity. Derivatives **81**–**83** each showed lower affinity for both CB receptors, compared to 3-phenyl analogues **70**–**72**, although the selectivity for the type-2 CB receptor was improved with the cycloheptyl and adamant-1-yl amides (**82** and **83**, respectively). The introduction of two methyl residues at C-3 (compound **102**) led to affinity levels comparable to that of analogue **83**, while the selectivity was drastically reduced (K_i = 56 nM, SI = 4). The most representative compound of this group was **82** (hK_i = 38 nM, SI > 263), bearing a cycloheptyl moiety on the carboxamide chain.

Lengthening the aliphatic chain to an ethyl group (**84**–**86**) enhanced affinity for both receptors, despite an apparent loss in selectivity. Moving to 3-propyl derivatives (**92-95**), further improvements in receptor affinity have been determined, without significantly changing selectivity, relative to those of the corresponding ethyl derivatives. Unfortunately, compound **93**, bearing a cycloheptyl amide, was not tested due to its poor solubility in DMSO/water mixtures. Particularly, compound **92,** with a cyclohexyl carboxamide, showed the greatest affinity for the CB_2 receptor in this series, with an hK_i = 0.32 nM. Substitution of *n*-propyl moiety at C-3 with a cyclopropyl (**98**–**100**) or 2-propyl (**101**) group provided lower affinity compared to that of the analogous compounds with the *n*-propyl side chain.

Basing on these data, we decided to extend the study within the 3-ethyl series, by introducing additional substituents on the heterocyclic nucleus, as a fluorine atom at the C-10 position (**103**): not only this modification did not significantly changed affinity at the CB_2 receptor, relative to that of compound **86**, but also the selectivity decreased about 1.5-fold. An additional fluorine group was introduced at C-9, but derivative **104** turned out to be less active than the analogue **103** (hK_i = 33 nM vs 7.34 nM) without affecting selectivity. In the same way, the introduction of a methyl residue at C-8 (**96**, **97**) in the 3-propyl series caused a remarkable reduction in CB_2 receptor affinity and selectivity, relative to that of the corresponding compounds lacking the methyl moiety (**92** and **94**, respectively). Together, these data point out that modifications in this portion of the molecular core considerably condition the receptor binding. We decided to further explore this sensitivity introducing other substituents at C-10.

Table 3. Affinity (K_i, nM) and Selectivity Index (SI) of the Novel 3-Alkyl CB Compounds on Rat and Human CB_1 and CB_2 Receptors and Potency (IC_{50}, nM) of the Novel CB Compounds in hCB_2 CHO Cells on cAMP Assays[a]



			81-92					
			K_i (nM)					
Cpd	**R_5**	**R_7**	**rat CB_1**[b]	**rat CB_2**[c]	**hCB_1**[d]	**hCB_2**[e]	**SI**	**hCB_2 IC_{50}(nM)**[f]
14	(*R*)-(+)-WIN 55,212-2		15.6 ± 1.4	7.58 ± 0.72	12.4 ± 1.3	4.53 ± 0.42	2.74	15.7 ± 1.3
81	CH_3	cyclohexyl	2630 ± 254	65 ± 6	2150 ± 207	60 ± 8	36	245 ± 28
82	CH_3	cycloheptyl	>10000	52 ± 3	>10000	38 ± 4	>263	210 ± 23
83	CH_3	adamant-1-yl	>10000	55 ± 4	>10000	47 ± 4	>212	230 ± 22
84	ethyl	cyclohexyl	972 ± 83	24 ± 2	821 ± 78	22 ± 2	37	62 ± 7
85	ethyl	cycloheptyl	498 ± 52	11.4 ± 1.3	433 ± 42	9.24 ± 0.92	47	42 ± 3
86	ethyl	adamant-1-yl	725 ± 73	13.5 ± 1.5	689 ± 64	7.83 ± 0.82	88	38 ± 4
87	propyl	adamant-2-yl	425 ± 39	16.1 ± 1.5	389 ± 35	13.2 ± 1.2	30	57 ± 6
88	propyl	5-methylhexan-2-yl	>10000	196 ± 17	>10000	15.8 ± 1.4	>633	62 ± 6
89	propyl	pyridin-4-yl	1375 ± 112	183 ± 16	1150 ± 104	152 ± 14	8	683 ± 66
90	propyl	thiazol-2-yl	1622 ± 157	324 ± 28	1365 ± 115	265 ± 21	5	1127 ± 104
91	propyl	*N*,*N*-diisopropyl	>10000	104 ± 10	>10000	85 ± 9	>118	423 ± 37
92	propyl	cyclohexyl	13.4 ± 1.5	1.84 ± 0.16	10.2 ± 0.9	0.32 ± 0.03	32	1.53 ± 0.16

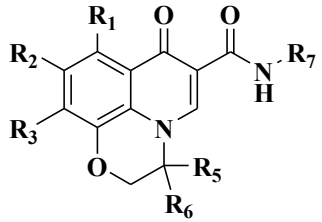
[a]The data are expressed as the mean ± SEM of $n = 4$ independent experiments. [b]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat brain for CB_1 receptors. [c]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat spleen for CB_2 receptors. [d]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_1 CHO membranes. [e]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_2 CHO membranes. [f]IC_{50} values were calculated on cAMP experiments performed on human CB_2 CHO cells.

Compounds **105** and **107,** bearing a methoxy and *N*-methylpiperazine group respectively, showed high affinity at the CB_2 receptor, especially compound **105,** suggesting that the two receptor subtypes present different steric tolerance at this position.

The best results were obtained with compound **106**, in which the 10-fluorine atom was displaced by a pyrrolidine residue, showing high affinity and exceptional selectivity at the CB_2 receptor (hK_i = 8.12, SI > 1231). The choice of these kind of substituents is due to the fact that tertiary amines can be converted into the correspondent hydrochloride salt, making this class of compounds water-soluble. This characteristic is more suitable for in vivo tests, as it will be described later in this work.

Moreover, within the 3-ethyl series, we valued the effect of structural modifications at the 6-carboxamide side chain on receptor affinity and selectivity (compounds **84**–**91**). As shown in the 3-aryl series, the cycloheptyl amide **85** displays higher affinity and selectivity for the CB_2 receptor, rather than the cyclohexyl amide **84**. On the other hand, the adamant-1-yl amide **86** shows affinity for the CB_2 receptor quite similar to that of the cycloheptyl derivative, although with a remarkable increase in selectivity. Moving the point of attachment of the adamantane ring from the bridgehead 1-position (**86**) to the bridging 2-position (**87**) caused a moderate decrease in affinity and selectivity. Substitution of the aliphatic carbocyclic amide moieties with a heteroaromatic 4-pyridyl (**89**) or thiazol-2-yl (**90**) group resulted in a dramatic reduction of affinity and almost complete loss of selectivity for the type-2 receptor. Similarly, significant loss of affinity at the CB_2 receptor was seen with the introduction of a bulky, noncyclic *N*,*N*-diisopropyl amide moiety (**91**), although selectivity was slightly enhanced. In the 3-ethyl series, best values were obtained with the 5-methylhexan-2-yl carboxamide chain (**88**, hK_i = 15.8 nM, SI > 633). These data confirm the importance of 6-carboxamide group in receptor affinity and selectivity.

Table 4. Affinity (K_i, nM) and Selectivity Index (SI) of the Novel 3-Alkyl CB Compounds on Rat and Human CB_1 and CB_2 Receptors and Potency (IC_{50}, nM) of the Novel CB Compounds in hCB_2 CHO Cells on cAMP Assays [a]



93-107

							K_i (nM)				
Cpd	**R_1**	**R_2**	**R_3**	**R_5**	**R_6**	**R_7**	**rat CB_1** [b]	**rat CB_2** [c]	**hCB_1** [d]	**hCB_2** [e]	**SI**	**hCB_2 IC_{50}(nM)** [f]
14	(*R*)-(+)-WIN55,212–2						15.6 ± 1.4	7.58 ± 0.72	12.4 ± 1.3	4.53 ± 0.42	2.74	15.7 ± 1.3
93	H	H	H	Cyclopropyl	H	cycloheptyl	ND	ND	ND	ND	ND	ND
94	H	H	H	Cyclopropyl	H	adamant-1-yl	240 ± 25	2.73 ± 0.25	215 ± 20	2.34 ± 0.21	92	15.3 ± 1.4
95	H	H	H	Cyclopropyl	H	3,5-dimethyladamant-1-yl	476 ± 44	10.7 ± 1.1	200 ± 23	3.62 ± 0.41	55	20 ± 3
96	CH_3	H	H	Isopropyl	H	cyclohexyl	874 ± 78	43 ± 4	756 ± 72	38 ± 4	20	122 ± 11
100	H	H	H	Ethyl	H	adamant-1-yl	482 ± 46	10.3 ± 1.2	389 ± 37	8.92 ± 0.91	44	32 ± 3
101	H	H	H	Ethyl	H	adamant-1-yl	366 ± 31	4.22 ± 0.38	323 ± 28	3.74 ± 0.32	86	12.4 ± 1.7
102	H	H	H	CH_3	CH_3	adamant-1-yl	276 ± 22	68 ± 6	221 ± 18	56 ± 5	4	242 ± 23
103	H	H	F	Ethyl	H	adamant-1-yl	452 ± 44	9.51 ± 0.88	389 ± 34	7.34 ± 0.68	53	27 ± 2
104	H	F	F	Ethyl	H	adamant-1-yl	1824 ± 176	37 ± 4	1752 ± 165	33 ± 3	53	145 ± 12
105	H	H	OCH_3	Ethyl	H	adamant-1-yl	589 ± 51	9.12 ± 0.86	521 ± 48	6.78 ± 0.62	77	24 ± 3
106	H	H	pyrrolidin-1-yl	Ethyl	H	adamant-1-yl	>10000	10.5 ± 1.1	>10000	8.12 ± 0.83	>1231	29 ± 3
107	H	H	4-methylpiperazin-1-yl	Ethyl	H	adamant-1-yl	>10000	61 ± 5	>10000	42 ± 3	>238	196 ± 17

[a]The data are expressed as the mean ± SEM of $n = 4$ independent experiments. [b]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat brain for CB_1 receptors. [c]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat spleen for CB_2 receptors. [d]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_1 CHO membranes. [e]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_2 CHO membranes. [f]IC_{50} values were calculated on cAMP experiments performed on human CB_2 CHO cells.

Finally, given the presence of chiral carbon at C-3, we decided to evaluate the possibility of a difference between the two receptor subtypes in the stereofacial preference for substituents at this position. In order to achieve this goal, we prepared a set of compounds (**81**, **83**, **85**, **86**, and **101**) as single enantiomers in addition to the racemic mixtures (**Table 5**). With the C-3 methyl derivatives (**81**, **83**), a stereochemical preference for the (*R*)-enantiomers over the corresponding (*S*)-enantiomers is observed. More important, the (*R*)-**81** enantiomer (hK_i = 24 nM, SI = 119) shows higher affinity for the CB_2 receptor than the racemic mixture (hK_i = 60 nM, SI = 36), but apparently poorer affinity for the type-1 receptor. As a result, (*R*)-**81** also displays greater selectivity than the racemic mixture. Similarly, the (*R*)-enantiomer of compound **83** displays greater affinity than the (*S*)-enantiomer, although the (*R*)-enantiomer does not significantly alter affinity or selectivity over the racemic compound. As for the 3-ethyl derivatives, the (*R*)-enantiomers of **85** and **86** both provided a 2-fold enhancement in affinity over the racemic compounds. At the same time, they also caused a 2-fold loss in selectivity for the CB_2 receptor, with a consequent 4-fold improvement in affinity for the CB_1 receptor. In contrast, the (*S*)-enantiomers of **85** and **86** caused a notable decrease in affinity for the CB_2 receptor with almost complete loss of selectivity. Analogously, with the C-3 isopropyl derivative **101**, the (*R*)-enantiomer turns out to have greater affinity to both CB receptors rather than the (*S*)-enantiomer or the racemic mixture ((*R*)-**101**, hCB_1K_i = 88 nM; hCB_2K_i = 1.24 nM; (*S*)-**101**, hCB_1K_i = 858 nM; hCB_2K_i = 16.2 nM; (*R*,*S*)-**101**, hCB_1K_i = 323 nM; hCB_2K_i = 3.74 nM). These data confirm a slight decrease in selectivity, relative to those of the racemic mixture.

Basing on the fact that a significant enhancement in affinity and selectivity had been seen only with (*R*)-enantiomers, we continued SAR studies preparing just the enantiopure derivative. Acutally, we extended the side chain length, synthesizing compound (*R*)-**108**, which bears an isobutyl moiety at C-3. Indeed, this compound was found to bind to the CB_2 receptor with high affinity and exceptional selectivity (hK_i = 9.24 nM, SI > 1082). In light of this finding, we also introduced a benzyl moiety at C-3, that led to the formation of compound (*R*)-**109**, in which a methylene spacer between the oxazine ring and the phenyl moiety of compound **72** has been placed. As expected, the compound showed higher affinity at the CB_2 receptor, with a K_i of 3.72 nM, and a modest improvement in selectivity (SI = 28) relative to those of the C-3 phenyl analogue.

The novel 7-oxo-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide derivatives were also examined in functional assays, in order to evaluate their potency. In particular, it has been analyzed the capability of the compounds to inhibit forskolin-induced cAMP production in hCB_2 CHO cells (**Tables 2-5**). In **Fig. 17** are reported competition binding curves (**A**) obtained from receptor binding experiments, and the dose-response curves (**B**) from cAMP assays for select compounds (**92**, **70**, **71**, (*R*)-**86**, and **105**). We can see that high affinity values (K_i) are strictly correlated with high potency values (IC_{50}). All the analysis were performed in comparison to the reference compound WIN55,212-2 (**14)** that is characterized by high affinity and potency but very low selectivity (**Tables 2-5**). These data reveal that modifications on the novel oxazinoquinolin-6-carboxamide scaffold do not affect its functionality, actually all compounds behave as full agonists. Interestingly, the affinity values obtained from rat and human receptors binding were quite similar, confirming a high degree of similarity between the two receptor subtypes across the two species. Consequently, these data support the evaluation of in vivo efficacy in rodent animal models of inflammatory and/or neuropathic pain.[34]

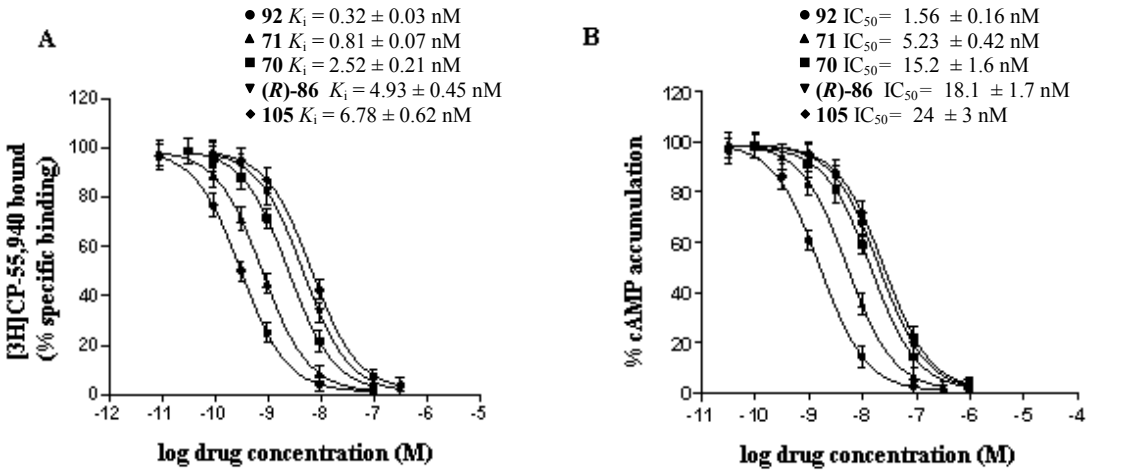
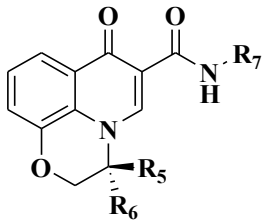


Fig. 3. Affinity (K_i, nM) and potency (IC_{50}, nM) of selected novel CB compounds:(**A**) competition curves on hCB_2 receptors;(**B**) inhibition curves of forskolin-stimulated cAMP accumulation in hCB_2 CHO cells. Results are given as the mean ± SEM (n = 4 independent experiments).

Table 5. Affinity (K_i, nM) and Selectivity Index (SI) of the Novel Chiral CB Compounds on Rat and Human CB_1 and CB_2 Receptors and Potency (IC_{50}, nM) of the Novel CB Compounds in hCB_2 CHO Cells on cAMP Assays[a]



81, 83, 85, 86, 101, 108, 109

				K_i (nM)					
Cpd	**R_5**	**R_6**	**R_7**	**rat CB_1**[b]	**rat CB_2**[c]	**hCB_1**[d]	**hCB_2**[e]	**SI**	**hCB_2 IC_{50}(nM)**[f]
14	(*R*)-(+)-WIN55212-2			15.6 ± 1.4	7.58 ± 0.72	12.4 ± 1.3	4.53 ± 0.42	2.74	15.7 ± 1.3
(*R*)-81	H	CH_3	cyclohexyl	3560 ± 335	30 ± 4	2866 ± 245	24 ± 3	119	90 ± 8
(*S*)-81	CH_3	H	cyclohexyl	5242 ± 534	250 ± 27	4652 ± 425	188 ± 16	25	724 ± 75
(*R*)-83	H	CH_3	adamant-1-yl	>10000	52 ± 5	>10000	47 ± 3	>212	153 ± 14
(*S*)-83	CH_3	H	adamant-1-yl	>10000	86 ± 8	>10000	78 ± 7	>128	250 ± 23
(*R*)-85	H	Ethyl	cycloheptyl	126 ± 11	6.03 ± 0.52	105 ± 9	4.12 ± 0.38	25	17.2 ± 1.6
(*S*)-85	ethyl	H	cycloheptyl	913 ± 87	203 ± 19	843 ± 77	87 ± 16	4.05	682 ± 67
(*R*)-86	H	Ethyl	adamant-1-yl	242 ± 21	6.17 ± 0.53	197 ± 15	4.93 ± 0.45	40	18.1 ± 1.7
(*S*)-86	ethyl	H	adamant-1-yl	653 ± 58	97 ± 8	578 ± 44	88 ± 7	7	413 ± 38
(*R*)-101	H	Isopropyl	adamant-1-yl	95 ± 9	1.75 ± 0.14	88 ± 7	1.24 ± 0.11	71	9.84 ± 0.91
(*S*)-101	isopropyl	H	adamant-1-yl	864 ± 82	19.3 ± 1.9	858 ± 79	16.2 ± 1.8	53	55 ± 6
(*R*)-108	H	Isobutyl	adamant-1-yl	>10000	10.2 ± 1.1	>10000	9.24 ± 0.84	>1082	36 ± 3
(*R*)-109	H	Benzyl	adamant-1-yl	121 ± 10	4.65 ± 0.43	105 ± 9	3.72 ± 0.32	28	17.5 ± 1.8

[a]The data are expressed as the mean ± SEM of $n = 4$ independent experiments. [b]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat brain for CB_1 receptors. [c]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on rat spleen for CB_2 receptors. [d]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_1 CHO membranes. [e]The affinity values were calculated by using [^{3}H]CP-55,940 as a radioligand on human CB_2 CHO membranes. [f]IC_{50} values were calculated on cAMP experiments performed on human CB_2 CHO cells.

Antinociceptive effects of the selective CB_2 agonist MT178

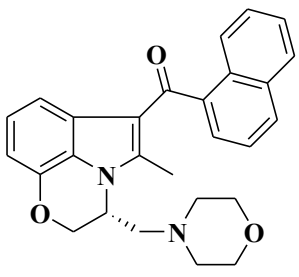
Pain is an unpleasant feeling often caused by intense stimuli, that significantly affects the quality of life. Recent epidemiological studies reported that, in Italy, one out of every four patients suffers from chronic pain and that the available pharmacological treatments are barely effective.

Evidence for the use of Cannabis extracts as analgesic treatment has been known forever. During the last decades, numerous studies have reported marked antinociceptive effects of CB agonists in various models of pain, both inflammatory and neuropathic,[54] that could be very useful as tools to evaluate the therapeutic efficacy of novel potential analgesics.[55]

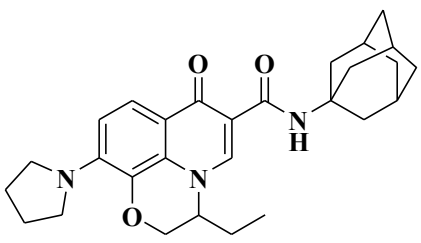
Apparently, the inhibition of inflammatory and neuropathic persistent pain condition involves both CB_1 and CB_2 receptors. However, most drugs interacting with CB_1 receptors displayed marked side central effects that have precluded their application. As a consequence, it became necessary to develop new compounds that are selective for type-2 cannabinoid receptor.[56, 57]

N-adamantyl-3-ethyl-3,7-dihydro-7-oxo-10-(pyrrolidin-1-yl)-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide **106** is one of the most representative compounds in the newly developed series. It showed a high CB_2 selectivity, making it suitable as an antinociceptive drug without CNS side effects. Moreover, the novel CB_2 compound behaves as a potent full agonist, as indicated by cyclic AMP experiments performed in human CB_2 receptors, with an EC_{50} value of 25.31 nM and an efficacy comparable to reference compound WIN 55,212-2 (E_{max} 99 ± 5 and 100 ± 6, respectively).

The presence of a pyrrolidine residue at C-10 allowed the preparation of the correspondent hydrochloric salt, that made the compound water-soluble, and, consequently, suitable for *in vivo* tests, to better investigate the analgesic effect.



WIN 55-212,2
h K_i CB_2 = 4.53 nM
h IC_{50} CB_2 = 15.7 nM
E_{max} = 100%



MT178
h K_i CB_2 = 8.12 nM
h IC_{50} CB_2 = 29 nM
E_{max} = 99%

Actually, labelled as MT178, compound **106** has been tested in two of the most used inflammatory pain models, such as formalin and writhing tests, where it produced a dose-dependent antihyperalgesic effect in a similar way to WIN55,212-2. Moreover, the effect of MT178 was reversed by the selective CB_2 antagonist AM630, but not by the selective CB_1 antagonist AM251, supporting a CB_2-mediated mechanism of action, and the high selectivity.

The effect of MT178 was also analyzed in different chronic pain models, that is to say streptozotocin (STZ)-induced neuropathy, bone cancer pain and acid-induced muscle pain (AIMP).[58] In a very encouraging way, compound **106** significantly reduced mechanical allodinya in all the three models, with an efficacy comparable to that obtained with WIN55,212-2.

Moreover, rotarod and catalepsy assays were performed to evaluate CNS adverse effects, and, as expected, WIN55,212-2 showed marked central side effects. On the other hand, the treatment with MT178 did not cause any locomotor disturbance or catalepsy, even at doses up to 100-fold the doses that caused antinociception.

These results confirm that systemic administration of MT178 produces strong analgesia in different pain models through selective activation of CB_2 receptors, providing an interesting approach to analgesic therapy in inflammatory and chronic pain without CB_1-mediated central side effects.

Conclusions

This project focused on the design and synthesis of novel cannabinoid CB_2 receptor ligands. Initially, the synthetic routes of three new scaffolds were optimized: 2,8- dioxo -1,2,3,4-tetrahydro-8*H*[1,4]diazepino[3,2,1-*ij*]quinoline-7-carboxamides, 8-oxo-1,2,3,4-tetrahydro-8*H*[1,4]diazepino[3,2,1-*ij*]quinoline-7-carboxamides and 2,7-dioxo-2,3-dihydro-1*H*,7*H*-pyrido[1,2,3-*de*]quinoxaline-6-carboxamides. These classes of compounds did not show encouraging results (K_iCB_2 > 150 nM).

Consequent modification of the scaffold led to the discovery of a novel 7-oxo-[1,4]oxazino[2,3,4-*ij*]quinoline carboxamide series. In particular, we obtained excellent CB_2 receptor affinity, with K_i values less than 100 nM.

Furthermore, considering the presence of a chiral center in position C-3, we evaluated the stereoselectivity of CB_2 receptor, and data showed that the (*R*)-isomer provided higher affinity compared to the corresponding (*S*)-enantiomer. In particular, best results were obtained with compound (*R*)-**108** (K_iCB_2 = 9.24 nM; SI > 1082).

Moreover, the cAMP functional assays showed that the novel oxazinoquinoline series behaves as full agonist.

Finally, one of the most selective compound, **106** (MT178), was analyzed *in vivo* both in inflammatory and neuropatic pain animal models and it revealed great efficacy as an antinociceptive agent.

In conclusion, this work represents an interesting starting point for further optimization of CB_2-receptor agonists as tools to study their therapeutic potential in various disease settings, especially pain.

Experimental Section

Pharmacology

Competition binding experiments were performed by using [^{3}H]CP-55,940 (specific activity 180 Ci/mmol) that was obtained from Perkin-Elmer Life and Analytical Sciences (Waltham, MA). Human CB_1 and CB_2 receptors expressed in CHO cells were purchased from Perkin-Elmer Life and Analytical Sciences. All other reagents were of analytical grade and were obtained from commercial sources.

Competition Binding Experiments on CB_1 and CB_2 Receptors

To study CB_1 receptors, rat brain (male Sprague-Dawley rats, Charles River, Wilmington, MA) was removed, frozen in liquid nitrogen, and stored at -80 °C. The rat brain tissue was suspended in 50 mM Tris-HCl buffer, pH 7.4, at 4 °C. The suspension was homogenized with a Polytron and centrifuged for 10 min at 2000*g*, and the supernatant was centrifuged again for 20 min at 40000*g*. The pellet was resuspended in a buffer containing 50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, and 0.5% fatty acid free bovine serum albumin (BSA), pH 7.4, at 30 °C. Competition binding experiments to rat CB_1 receptors were carried out using [^{3}H]CP-55,940 (1.0 nM), a membrane suspension containing 40 μg of protein/100 μL and different concentrations (1 nM to 10 μM) of the examined compounds.

To investigate CB_2 receptors, a [^{3}H]CP-55,940 binding assay was performed by using rat spleen (male Sprague–Dawley rats, Charles River) that was homogenized in 50 mM Tris-HCl buffer, pH 7.4, at 4 °C with a Polytron and centrifuged for 10 min at 2000*g*, and the supernatant was centrifuged for 20 min at 40000*g*. The pellet was resuspended in a buffer containing 50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, and 0.5% fatty acid free BSA, pH 7.4, at 30 °C. Competition binding experiments to rat CB_2 receptors were performed using [^{3}H]CP-55,940 (0.5 nM), a membrane suspension containing 80 μg of protein/100 μL and different concentrations (1 nM to 10 μM) of the examined compounds.[59]

Human CB_1 and CB_2 receptors expressed in CHO cells were grown adherently and maintained in Ham's F12 containing 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and Geneticin (G418; 0.4 mg/mL) at 37 °C in 5% CO_2/95% air.[60-62] For membrane preparation the culture medium was removed, and the cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris-HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with a Polytron and centrifuged for 10 min at 1000*g*, and the supernatant was then centrifuged for 30 min at

100000*g*. The membrane pellet was suspended in 50 mM Tris-HCl buffer, 0.5% BSA (pH 7.4) containing 5 mM $MgCl_2$, and 2.5 mM EDTA or 1 mM EDTA for hCB_1 or hCB_2 receptors, respectively. Competition binding experiments were performed using 0.5 nM [^{3}H]CP-55,940 and different concentrations (1 nM to 10 μM) of the examined compounds or the reference agonist WIN55,212-2 for an incubation time of 90 or 60 min at 30 °C for CB_1 or CB_2 receptors, respectively.

Bound and free radioactivities were separated by filtering the assay mixture through Whatman GF/C glass fiber filters using a Brandel cell harvester (Brandel Instruments, Unterföhring, Germany). The filter-bound radioactivity was counted using a Packard Tri Carb 2810 TR scintillation counter from Perkin-Elmer Life and Analytical Sciences.

cAMP Assay to Human CB_2 Receptors

CHO cells transfected with human CB_2 receptors were washed with phosphate-buffered saline and diluted trypsin and centrifuged for 10 min at 200*g*. The pellet containing CHO cells (1×10^6 cells/assay) was suspended in 0.5 mL of an incubation mixture, NaCl (150 mM), KCl (2.7 mM), NaH_2PO_4 (0.37 mM), $MgSO_4$ (1 mM), $CaCl_2$ (1 mM), Hepes (5 mM), $MgCl_2$ (10 mM), glucose (5 mM), pH 7.4, at 37 °C. Then 0.5 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as a phosphodiesterase inhibitor was added and preincubated for 10 min in a shaking bath at 37 °C.[63] The potency of the novel CB compounds in comparison with a well-known CB agonist (**14**) was studied in the presence of forskolin (1 μM). The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C, and the supernatant was extracted four times with water-saturated diethyl ether. The final aqueous solution was tested for cAMP levels by a competition protein binding assay. Samples of cAMP standard (0-10 pmol) were added to each test tube containing the incubation buffer (Trizma base (0.1 M), aminophylline (8.0 mM), 2-mercaptoethanol (6.0 mM), pH 7.4) and [^{3}H]cAMP in a total volume of 0.5 mL. The binding protein previously prepared from beef adrenals was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal, the samples were centrifuged at 2000*g* for 10 min. The clear supernatant was counted by using a Perkin-Elmer 2810 TR scintillation counter (Perkin-Elmer Life and Analytical Sciences). The protein concentration was determined according to a Bio-Rad method (Bradford, 1976) with bovine albumin as a standard reference. Inhibitory binding constant values, K_i, were calculated from the IC_{50} according to the Cheng and Prusoff

equation.[64] A weighted nonlinear least-squares curve fitting program, LIGAND,[65] was used for computer analysis of inhibition experiments. All the data are expressed as the mean ± SEM of n = 4 independent experiments for the in vitro assays. Statistical analysis of the data was performed using unpaired two-sided Student's t test.

Chemistry

Reagent grade solvents were dried according to standard techniques. Sodium sulphate was used as a drying agent for water containing organic phases. All reported yields are of isolated products and are not optimized. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (F245 Merck plates). Chromatographic spots were visualized by UV light. Purification of crude compounds and separation of reaction mixtures were carried out by column chromatography on silica gel 60 (230–400 mesh from Merck). Melting points (Mp) (uncorrected) were determined in a 240 Buchi-Tottoli melting point apparatus. Chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent central peak. ^{1}H NMR spectra were recorded at 200 MHz or 400 MHz on a Bruker AC 200 spectrometer. Electron spray ionization mass spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD model in positive scan mode. The molecular weights from the MS spectra were in full agreement with the proposed chemical structures of target compounds. Elemental analysis data for final compounds were obtained from the micro-analytical laboratory of Department of Chemistry, Ferrara University and were within ±0.40 of theoretical values for formulas given. Reagents and solvents were provided by Fluka-Aldrich, Sigma and Alfa-Aesar.

General Procedures

Synthesis of 4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (32)[35]

A mixture of 1,2-phenyldiamine (**31**, 0.018 mol) and 60% aqueous acrylic acid (0.027 mol) dissolved in water (3.5 mL) and conc. hydrochloric acid (3.5 mL) was stirred at 70°C for 3 h and monitored through TLC. The solvent was evaporated by distillation, basified with conc. ammonium hydroxide. The reaction mixture was partitioned between H_2O and ethyl acetate (EtOAc). The organic layer was washed with brine, and dried over sodium sulfate (Na_2SO_4). The solvent was evaporated under reduced pressure. The desired product was crystallized from EtOAc.

32 *4,5-Dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one*
Pale white solid. Mp: 138 °C. Yield: 68%. MS *m*/*z* 163 (MH^+). ^{1}H NMR (200 MHz, DMSO): δ 7.99 (br s, 1 H); 7.02-6.70 (m, 4 H); 4.10 (br s, 1 H); 3.65 (m, 2 H); 2.72 (t, *J* = 5.4 Hz, 2 H).

Synthesis of diethyl 2-[(1,2,3,4-tetrahydro-2-oxobenzo[*b*][1,4]diazepin-5-yl) methylene]malonate (33)

A mixture of 4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**32**, 0.005 mol**)** and Diethyl ethoxymetylenmalonate (DEEM) (0.005 mol) was heated at 140 °C for 1 h. The reaction mixture was poured into petroleum ether and the precipitate was collected by filtration to give the desired compound as a pale white solid (yield: 90%) that was filtered and used without any further purification in the next step.

Synthesis of ethyl 2,3,4,8-tetrahydro-2,8-dioxo-1*H*-[1,4]diazepino[3,2,1-*ij*] quinoline-7-carboxylate (34)

A mixture of diethyl 2-((1,2,3,4-tetrahydro-2-oxobenzo[*b*][1,4]diazepin-5-yl)methylene) malonate (**33,** 0.003 mol) and polyphosphoric acid (5 g) was heated at 130 °C for 1 h. The mixture was poured into ice and water to form a white precipitate that was filtered and washed with cold water to afford:

34 *Ethyl 2,3,4,8-tetrahydro-2,8-dioxo-1H-[1,4]diazepino[3,2,1-ij]quinoline-7-carboxylate*

White solid. Mp: 295 °C. Yield: 95%. MS *m*/*z* 287 (MH^+), ^{1}H NMR (200 MHz, DMSO): δ 10.11 (br s, 1 H); 8.53 (s, 1 H); 8.10-8.00 (m, 1 H); 7.50-7.25 (m, 2 H); 4.51 (t, *J* = 4.6 Hz, 2 H); 4.23 (q, *J* = 7.2 Hz, 2 H); 2.96 (t, *J* = 4.8 Hz, 2 H); 1.28 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (100 MHz, DMSO): δ 173.7, 172.2, 164.3, 150.2, 130.6, 130.1, 129.0, 125.7, 124. 5, 121.5, 108.5, 59.7, 52.2, 36.4, 14.3.

Synthesis of 2,3-dihydro-1*H*-benzo[*b*][1,4]diazepines (36)[36]

A mixture of 1,2-phenylendiamine (**31**, 18.5 mmol), appropriate ketone (74 mmol) and *N*-Bromosuccinimide (1.85 mmol) was heated at 40 °C for 4 h and monitored by TLC (for compound **36a**, the reaction mixture was stirred at 80 °C for 0.5 h, using p-Toluensulfonic Acid as catalyst[66] (1.85 mmol)). Reaction mixture was basified with NH_3 to pH = 9 and then extracted with EtOAc. Organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under vacuum. The desired products were purified on silica gel column, using a mixture of petroleum ether and EtOAc as eluent.[35]

36a *2,3-Dihydro-2,2,4-trimethyl-1H-benzo[b][1,4]diazepine*

Pale yellow solid. Mp: 146-147 °C. Yield: 80%. MS *m*/*z* 189 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.10 (m, 1 H); 7.00-6.69 (m, 2 H); 6.75 (m, 1 H); 2.98 (br s, 1 H); 2.6 (s, 3 H); 2.22 (s, 2 H); 1.34 (s, 6 H).

36b *2,4-Diethyl-2,3-dihydro-2-methyl-1H-benzo[b][1,4]diazepine*

Yellow oil. Yield: 67%. MS *m*/*z* 217 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.15-7.10 (m, 1 H); 6.97-6.92 (m, 2 H); 6.72-6.68 (m, 1 H); 3.01 (br s, 1 H); 2.63- 2.52 (q, *J* = 7.4 Hz, 2 H); 2.25-2.19 (d, *J* = 12.6 Hz, 1 H); 2.15-2.08 (d, *J* = 12.6 Hz, 1 H); 1.65-1.61 (m, 2 H); 1.27-1.19 (m, 6 H); 0.96-0.89 (t, *J* = 7.6 Hz, 3 H).

36c *2,3,9,10a-Tetrahydro-1H-spiro[benzo[b]cyclopenta[e][1,4]diazepine-10,1'-cyclopentane]*

Brown solid. Mp: 134-136 °C. Yield: 67%. MS *m*/*z* 241 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.34-7.29 (m, 1 H); 6.98-6.93 (m, 1 H); 6.82-6.78 (m, 1 H); 6.59-6.54 (m, 1 H); 3.99 (br s, 1 H); 2.77 (t, *J* = 6.8 Hz, 1, H); 2.16-1.54 (m, 14 H).

36d *2,3-Dihydro-2-methyl-2,4-diphenyl-1H-benzo[b][1,4]diazepine*

Yellow solid. Mp: 107-109 °C. Yield: 92%. MS *m*/*z* 313 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 7.63-7.57 (m, 4 H); 7.31-7.20 (m, 7 H); 7.08-6.87 (m, 3 H); 3.52 (br s, 1 H); 3.12-3.11 (d, *J* = 13 Hz, 1 H); 3.01-2.94 (d, *J* = 13 Hz, 1 H); 1.76 (s, 3 H).

General synthetic procedure for of 2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine (38).

A mixture of benzodiazepine (**36**, 0.02 mol), methanol (50 mL) and sodium borohydride (0.02 mol) was stirred at room temperature for 2 h and monitored by TLC (for compound **38c**, the reaction mixture was stirred at 40 °C for 8 h). Methanol was evaporated under reduced pressure and the reaction mixture was partitioned between H_2O and ethyl acetate (EtOAc). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The resulting product was purified on silica gel column, using a mixture of petroleum ether and EtOAc as eluent.

38a *2,3,4,5-Tetrahydro-2,2,4-trimethyl-1H-benzo[b][1,4]diazepine*

Pale white solid. Mp: 60-61 °C. Yield: 80%. , MS *m*/*z* 191 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 6.79-6.61 (m, 4 H); 3.26 (br s, 2 H); 3.22 (m, 1 H); 1.66-1.53 (m, 2 H); 1.32 (s, 3 H); 1.22 (d, *J* = 6.4 Hz, 3 H); 1.08 (s, 3 H).

38b *2,4-Diethyl-2,3,4,5-tetrahydro-2-methyl-1H-benzo[b][1,4]diazepine*

Yellow oil. Yield: 72 %. MS *m*/*z* 219 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 6.68-6.59 (m, 4 H); 3.29 (br s, 2 H); 2.93 (m, 1 H); 1.66-1.52 (m, 6 H); 1.05-0.95 (m, 9 H).

38c *2,3,3a,4,9,10a-Hexahydro-1H-spiro[benzo[b]cyclopenta[e][1,4]diazepine-10,1'-cyclopentane]*

Pale yellow solid. Mp: 70 °C. Yield: 75%. MS *m*/*z* 243 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 6.70- 6.61 (m, 4 H); 4.00 (br s, 2 H); 3.5 (m, 1 H); 2.7-1.2 (m, 15 H).

38d *2,3,4,5-Tetrahydro-2-methyl-2,4-diphenyl-1H-benzo[b][1,4]diazepine*

Pale white solid. Mp: 132-133 °C. Yield: 80%. MS *m*/*z* 315 (MH^+). ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (m, 2 H); 7.46-7.25 (m, 8 H); 6.83 (m, 4 H); 4.22 (d d, *J* = 13 Hz, *J* = 12 Hz, 1 H); 3.83 (br s, 2 H); 2.44 (m, 1 H); 1.88 (d d, *J* = 13.6 Hz, *J* = 2 Hz, 1 H); 1.64 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.3, 145.2, 139.7, 137.2, 128.8, 128.5, 127.7, 127.0, 126.7, 125.3, 122.3, 120.6, 57.7, 57.2, 55.4, 23.7.

General synthetic procedure for diethyl 2-[(1,2,3,4-tetrahydro-2,2,4-trimethylbenzo[*b*][1,4]diazepin-5-yl)methylene]malonate (39)

Starting with 3,4,5-tetrahydro-2,2,4-trimethyl-1H-benzo[*b*][1,4]diazepines **38**, the title compounds were prepared in a manner analogous to that described for compound **33** in 85-90% yields. Compounds 3**9a-d** were used in the next step without purification.

General synthetic procedure for ethyl 2,3,4,8-tetrahydro-8-oxo-1*H*-[1,4]diazepino [3,2,1-*ij*]quinoline-7-carboxylate (40)

Starting with diethyl 2-((1,2,3,4-tetrahydro-2,2,4-trimethylbenzo[b][1,4]diazepin-5-yl)methylene)malonates **39 a-d**, the title compounds were prepared in a manner analogous to that described for compound **34**.

40a *Ethyl 2,3,4,8-tetrahydro-2,2,4-trimethyl-8-oxo-1H-[1,4]diazepino[3,2,1-ij] quinoline-7-carboxylate*

Purification on silical gel column: EtOAc:petroleum ether 7/3. Yellow solid. Mp: 126 °C. Yield: 40%. MS *m*/*z* 315 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1 H); 8.07 (m, 1 H); 7.18 (m, 1 H); 6.91 (m, 1 H); 4.93 (m, 1 H); 4.39 (q, 2 H, *J* = 7.6 Hz); 3.46 (br s, 1H); 2.07 (d d, *J* = 14 Hz, *J* = 3.2 Hz, 1 H); 1.95 (m, 1 H); 1.41 (t, *J* = 7.2 Hz, 3 H); 1.28 (s, 3 H); 1.16 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 166.7,. 146.2, 138.0, 125.8, 123.76, 120.1, 109.5, 60.8, 60.0, 54.7, 46.7, 32.0, 30.2, 21.5, 14.5.

40b *Ethyl 2,4-diethyl-2,3,4,8-tetrahydro-2-methyl-8-oxo-1H-[1,4]diazepino[3,2,1-ij] quinoline-7-carboxylate*

Purification on silical gel column: EtOAc:petroleum ether 6/4. Yellow solid. Mp: 135 °C. Yield: 38%. MS *m*/*z* 343 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (s, 1 H); 8.05-8.02 (d, *J* = 8 Hz, 1 H); 7.22-7.18 (t, *J* = 8 Hz, 1 H); 6.89-6.87 (d, *J* = 7.6 Hz, 1 H); 4.57-4.48 (m, 1 H); 4.40-4.38 (q, *J* = 7.2 Hz, 2 H); 3.63 (br s, 1 H); 2.32-2.21 (m, 1 H); 2.17-2.07 (m, 1 H); 1.97-1.94 (m, 2 H); 1.48-1.46 (q, *J* = 7.2 Hz, 2 H); 1.43-1.39 (t, *J* = 7.2 Hz, 3 H); 1.16 (s, 3 H); 0.90-0.86 (t, *J* = 7.6 Hz, 3 H); 0.82-0.79 (t, *J* = 7.6 Hz, 3 H).

40c *Ethyl 3-oxo-9,10,11,11a-tetrahydro-3H,7H,8aH-spiro [cyclopenta[6,7] [1,4] diazepino[3,2,1-ij]quinoline-8,1'-cyclopentane]-2-carboxylate*

Purification on silica gel column: EtOAc:petroleum ether 1/1. Pale yellow solid. Mp: 109 °C. Yield: 35%. MS *m*/*z* 367 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1 H);

7.96 (m, 1 H); 7.19 (m, 1 H); 6.80 (m, 1 H); 4.43 (m, 1 H); 4.39 (q, *J* = 7.2 Hz, 2 H); 3.72 (br s, 1 H); 2.39 (m, 1 H); 2.04-1.58 (m, 14 H); 1.43 (t, *J* = 7.2 Hz, 3 H).

40d *Ethyl 2,3,4,8-tetrahydro-2-methyl-8-oxo-2,4-diphenyl-1H-[1,4]diazepino[3,2,1-ij] quinoline-7-carboxylate*

Purification on silica gel column: EtOAc:petroleum ether 8/2. Pale white solid. Mp: 150 °C. Yield: 40%. MS *m*/*z* 439 (MH^+). ^1H NMR (400 MHz, $CDCl_3$): δ 8.16 (m, 1 H); 8.11 (s, 1 H); 7.29-7.11 (m, 11 H); 6.98 (m, 1 H); 5.99 (d d, *J* = 8.4 Hz, *J* = 1.6 Hz, 1 H); 4.25 (q, *J* = 7.2 Hz, 2 H); 3.77 (br s, 1 H); 3.37 (d d, *J* = 15.2 Hz, *J* = 8.4 Hz, 1 H); 2.72 (d d, *J* = 15.2 Hz, *J* = 2 Hz, 1 H); 1.64 (s, 3 H); 1.28 (t, *J* = 7.6 Hz).

General synthetic procedure for 3,4-dihydroquinoxalin-2(1*H*)-one (32)[43]

To a solution of appropriate 1,2-phenylendiamine (**31**, 27.8 mmol) in dimethylformamide (DMF) (30 mL) were added triethylamine (55.6 mmol) and ethyl 2-bromoacetate (30.5 mmol). The reaction was stirred at room temperature for 16 h, and then heated at 80 °C for 3h. Solvent was removed under reduced pressure and the residue was poured into water and extracted with EtOAc. Organic layer was washed with aqueous saturated sodium bicarbonate solution and brine, dried over sodium sulfate, filtered and concentrated under vacuum. The desired products were purified on silica gel column.

42a *3,4-Dihydroquinoxalin-2(1H)-one*

Purified on silica gel column (EtOAc:petroleum ether 1/1). Tan solid. Mp: 127-129 °C. Yield: 55%. MS *m*/*z* 149 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 8.92 (br s, 1 H); 7.20-6.93 (m, 4 H); 3.99 (s, 2 H); 3.40 (br s, 1 H).

42b *6-Fluoro-3,4-dihydroquinoxalin-2(1H)-one*

Purified on silica gel column (EtOAc:petroleum ether 8/2). Tan solid. Mp: 245-246 °C. Yield: 60%. MS *m*/*z* 167 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 10.32 (br s, 1 H); 7.84 (m, 1 H); 7.21-7.05 (m, 2 H); 5.88 (br s, 1 H); 3.68 (s, 2 H).

42c *6-Chloro-3,4-dihydroquinoxalin-2(1H)-one*

Purified on silica gel column (EtOAc-:petroleum ether 1/1). Tan solid. Mp: 214-215 °C. Yield: 55%. MS *m*/*z* 183 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 10.00 (br s, 1 H); 7.20 (m, 1 H); 7.05- 6.99 (m, 2 H); 5.80 (br s, 1 H); 3.65 (s, 2 H).

42d *3,4-Dihydro-6,7-dimethylquinoxalin-2(1H)-one*

Purified on silica gel column (EtOAc-:petroleum ether 1/1). Tan solid. Mp: 134-136 °C. Yield: 48%. MS *m*/*z* 177 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.87 (br s, 1 H); 7.10 (m, 1 H); 6.09 (m, 1 H); 5.72 (br s, 1 H); 3.72 (s, 2 H); 2.17 (m, 6 H).

General synthetic procedure for diethyl 2-[(2,3-dihydro-2-oxoquinoxalin-4(1*H*)-yl) methylene]malonate (43)

Starting with 3,4-dihydroquinoxalin-2(1H)-one **42a-d**, the title compounds were prepared (70-75%) in a manner analogous to that described for compound **33**. Compounds **43a-d** were used in the next step without purification.

General synthetic procedure for ethyl 1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-*de*]quinoxaline-6-carboxylate (44)

Starting with diethyl 2-((2,3-dihydro-2-oxoquinoxalin-4(1H)-yl)methylene) malonates **43a-d**, the title compounds were prepared in a manner analogous to that described for compound **34**.

44a *Ethyl 1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylate*

Purified on silica gel column (EtOAc:methanol 9/1). White solid. Mp >300 °C. Yield: 55%. MS *m*/*z* 273 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 11.16 (br s, 1 H); 8.51 (s, 1 H); 7.71 (m, 1 H); 7.37-7.15 (m, 2 H); 5.01 (s, 2 H); 4.17 (q, *J* =7 Hz, 2 H); 1.28 (t, *J* = 7 Hz, 3 H). ^{13}C NMR (100 MHz, DMSO): δ 172.2, 164.3,. 162.2, 147.2, 129.3, 127.6, 126.0, 125.2, 118.9, 116.6, 111.0, 59.8, 52.0, 14.3.

44b *Ethyl 9-fluoro-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylate*

Purified on silica gel column (EtOAc). White solid. Mp >300 °C. Yield: 50%. MS *m*/*z* 291 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 11.18 (br s, 1 H); 8.55 (s, 1 H); 7.56-7.51 (m, 1 H); 7.17-7.11 (m, 1 H); 5.02 (s, 2 H); 4.18 (q, *J* =7.22 Hz, 2 H); 1.27 (t, *J* = 7.4 Hz, 3 H). ^{16}F-NMR (397 MHz, DMSO): δ = -114.094 (t, 1 F, *J* = 10 Hz, F-9).

44c *Ethyl 9-chloro-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylate*

Purified on silica gel column (EtOAc). White solid. Mp: 292 °C.Yield: 50%. MS *m*/*z* 307 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 11.16 (br s, 1 H); 8.51 (s, 1 H); 7.71 (m, 1 H); 7.13 (s, 1 H); 5.01 (s, 2 H); 4.18 (q, *J* =7.2 Hz, 2 H); 1.25 (t, *J* = 7.4 Hz, 3 H).

44d *Ethyl 1,2,3,7-tetrahydro-8,9-dimethyl-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylate*

Purified on silica gel column (EtOAc). White solid. Mp: 271 °C.Yield: 50%. MS *m*/*z* 301 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 10.98 (br s, 1 H); 8.34 (s, 1 H); 6.98 (s, 1 H); 4.92 s, 2 H); 4.22-4.18 (q, *J* = 7.2 Hz, 2 H); 2.64 (s, 3 H); 2.29-2.26 (m, 3 H); 1.30-1.21 (t, *J* = 7 Hz, 3 H).

Synthesic genereal procedures for Carboxylic Acid Derivatives (35, 41, 44)

Starting products **34, 40a-d, 44a-d** (0.5 mmol) were dissolved in acetic acid (15 mL) and aqueous solution of HCl 20% (6.5 mL) was added. The reaction was heated at 100 °C for 4h and monitored by TLC. Solvent was evaporated under reduced pressure, and the residue was basified with an aqueous solution of NaOH 5% to pH = 6, to give a white precipitate that was filtered and washed with cold water.

35 *2,3,4,8-Tetrahydro-2,8-dioxo-1H-[1,4]diazepino[3,2,1-ij]quinoline-7-carboxylic acid*

White solid. Mp: 265-267 °C.Yield: 99%. MS *m*/*z* 255 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 15.12 (br s, 1 H); 10.32 (br s, 1 H); 8.93 (s, 1 H); 8.15-8.10 (d, *J* = 7.6 Hz, 1 H); 7.71-7.54 (m, 2 H); 4.75-4.71 (m, 2 H); 3.08-3.04 (m, 2 H).

41a *2,3,4,8-Tetrahydro-2,2,4-trimethyl-8-oxo-1H-[1,4]diazepino[3,2,1-ij]quinoline-7-carboxylic acid*

White solid. Mp: 228 °C.Yield: 85%. MS *m*/*z* 287 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 15.2 (br s, 1 H); 8.77 (s, 1 H); 7.81-7.76 (m, 1 H);7.41-7.38 (m, 2 H); 5.73 (br s, 1 H); 5.06 (m, 1 H); 2.11-2.04 (m, 2 H); 1.68-1.65 (m, 3 H); 1.19 (s, 3 H); 1.10 (s, 3 H).

41b *2,4-Diethyl-2,3,4,8-tetrahydro-2-methyl-8-oxo-1H-[1,4]diazepino[3,2,1-ij] quinoline-7-carboxylic acid*

Pale yellow solid. Mp: 194 °C.Yield: 90%. MS *m*/*z* 315 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 15.35 (br s, 1 H); 8.86-8.82 (d, *J* = 7.4 Hz, 1 H); 7.79-7.74 (m, 1 H); 7.42-

7.39 (m, 2 H); 5.86-5.79 (d, *J* = 15.2 Hz, 1 H); 4.79-4.61 (m, 1 H); 2.24-2.05 (m, 2 H); 2.05-2.01 (m, 2 H); 1.42-1.36 (m, 2 H); 1.07 (s, 3 H); 0.78-0.66 (m, 6 H).

41c *9,10,11,11a-Tetrahydro-3H,7H,8aH-spiro[cyclopenta[6,7][1,4]diazepino[3,2,1-ij] quinoline-8,1'-cyclopentane]-2-carboxylic acid*

Yellow solid. Mp:>300 °C.Yield: 72%. MS *m*/*z* 339 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 15.01 (br s, 1 H); 8.76 (s, 1 H); 7.98-7.93 (d, *J* = 7.8 Hz, 1 H); 7.37-7.29 (t, *J* = 7.8 Hz, 1 H); 6.97-6.93 (d, J = 7.6 Hz, 1 H); 4.61-4.42 (m, 1 H); 3.95 (br s, 1 H); 2.57-2.41 (m, 1 H); 2.09-1.25 (m, 14 H).

41d *2,3,4,8-Tetrahydro-2-methyl-8-oxo-2,4-diphenyl-1H-[1,4]diazepino[3,2,1-ij] quinoline-7-carboxylic acid*

Yellow solid. Mp: 204 °C.Yield: 75%. MS *m*/*z* 402 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 14.9 (br s, 1 H); 8.10 (s, 1 H); 7.72-7.69 (m, 1 H); 7.58-7.35 (m, 10 H); 7.13-7.05 (m, 3 H); 6.50 (m, 1 H); 3.71-3.57 (m, 1 H); 3.78-3.62 (m, 1 H); 1.56 (s, 3 H).

45a *1,2,3,7-Tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylic acid*

White solid. Mp:>300 °C.Yield: 70%. MS *m*/*z* 245 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 15.18 (br s, 1 H); 11.35 (br s, 1 H); 8.90 (s, 1 H); 7.88-7.84 (d, *J* = 8 Hz, 1 H); 7.52 (t, *J*= 8 Hz, 1 H); 7.34-7.30 (d, *J* = 7.4 Hz, 1 H); 5.18 (s, 2 H).

45b *9-Fluoro-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylic acid*

White solid. Mp:>300 °C.Yield: 99%. MS *m*/*z* 263 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 15.05 (br s, 1 H); 11.51 (br s, 1 H); 8.92 (s, 1 H); 7.56-7.51 (d d, *J* = 8.6 Hz, 1 H); 7.17-7.11 (d d, *J* = 9.2 Hz, 1 H); 5.20 (s, 2 H).

45c *9-Chloro-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylic acid*

Brown solid. Mp:>300 °C.Yield: 68%. MS *m*/*z* 279 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 14.85 (br s, 1 H); 11.48 (br s, 1 H); 8.93 (s, 1 H); 7.79-7.78 (m, 1 H); 7.27-7.26 (m, 1 H); 5.18 (s, 2 H).

45d *1,2,3,7-Tetrahydro-8,9-dimethyl-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxylic acid*

White solid. Mp:>300 °C.Yield: 85%. MS *m*/*z* 273 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 15.65 (br s, 1 H); 11.18 (br s, 1 H); 8.80 (s, 1 H); 7.14 (s, 1 H); 5.12 (s, 2 H); 2.76 (s, 3 H); 2.33 (s, 3 H).

Synthesis of *N*-adamantanylcarboxamide derivatives (46-48, 50-51, 53-56)

To a stirred solution of the respective carboxylic acid (**35, 41, 45,** 0.2 mmol) in dry DMF (3 mL) was added diisopropylethylamine (DIPEA, 0.8 mmol). The resulting solution was stirred at room temperature for 10 min before addition of *o*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 0.3 mmol) and then stirred for another 3 h. 1-Aminoadamantane (0.3 mmol) was then added, and the solution was stirred for 16 h. DMF was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate and successively washed with water-saturated sodium bicarbonate, water, and brine. The organic phase was dried over anhydrous sodium sulfate, evaporated, and finally purified by flash chromatography.

46 *N-Adamant-1-yl-2,3,4,8-tetrahydro-2,8-dioxo-1H-[1,4]diazepino[3,2,1-ij]quinoline-7-carboxamide*

White solid. Mp: >300 °C. Yield: 45%. MS *m*/*z* 392 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.20 (br s, 1 H); 9.85 (br s, 1 H); 8.66 (s, 1 H); 8.11-8.07 (d d, *J* = 7.8 Hz, 1 H); 7.58-7.53 (d d, *J* = 7.8 Hz, 1 H), 7.48-7.40 (t, *J* = 7.8 Hz, 1 H); 4.63-4.58 (m, 2 H); 3.03-2.98 (m, 2 H); 1.98 (s, 9H); 1.67 (s, 6 H).

47 *N-Adamant-1-yl-2,3,4,8-tetrahydro-2,2,4-trimethyl-8-oxo-1H-[1,4]diazepino[3,2,1-ij]quinoline-7-carboxamide*

White solid. Mp: 222 °C.Yield: 79%. MS *m*/*z* 420 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.98 (br s, 1 H); 8.67 (s, 1 H); 7.75-7.72 (m, 1 H); 7.26-7.24 (m, 2 H); 5.48 (br s, 1 H); 4.99-4.85 (m, 1 H), 2.05-2.01 (m, 11 H); 1.66-1.61 (m, 9 H); 1.19 (s, 3 H); 1.08 (s, 3 H).

48 *N-Adamant-1-yl-2,4-diethyl-2,3,4,8-tetrahydro-2-methyl-8-oxo-1H-[1,4]diazepino [3,2,1-ij]quinoline-7-carboxamide*

White solid. Mp: 215 °C.Yield: 50%. MS *m*/*z* 448 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.95 (br s, 1 H); 8.67 (s, 1 H); 7.74-7.65 (m, 1 H); 7.27-7.26 (m, 2 H); 5.62 (br s, 1 H); 4.57-4.43 (m, 1H); 2.35-2.18 (m, 2 H); 2.05 (s, 9 H); 2.04-1.87 (m, 2 H); 1.66 (s, 6 H); 1.40 (m, 2 H); 1.12 (s, 3 H); 0.73-0.65 (m, 6 H).

50 *N-Adamant-1-yl-9,10,11,11a-tetrahydro-3H,7H,8aH-spiro[cyclopenta[6,7][1,4] diazepino[3,2,1-ij]quinoline-8,1'-cyclopentane]-2-carboxamide*

Pale yellow solid. Mp:>300 °C.Yield: 45%. MS *m*/*z* 472 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.91 (br s, 1 H); 8.74 (s, 1 H); 7.63-7.58 (d d, *J* = 7.6 Hz, 1 H); 7.23-7.16

(m, 2 H); 5.96 (br s, 1 H); 4.73-4.67 (m, 1 H); 2.46-2.38 (m, 1 H); 1.98 (m, 9 H); 1.96-1.38 (m, 20 H).

51 *N-Adamant-1-yl-2,3,4,8-tetrahydro-2-methyl-8-oxo-2,4-diphenyl-1H-[1,4]diazepino [3,2,1-ij]quinoline-7-carboxylic acid*

White solid. Mp: 293 °C.Yield: 45%. MS *m*/*z* 402 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.81 (br s, 1 H); 8.12 (s, 1 H); 7.74-7.68 (m, 1 H); 7.46-7.25 (m, 9 H); 7.12-7.08 (m, 3 H); 6.41-6.37 (m, 1 H); 6.18 (br s, 1H); 3.61-3.42 (m, 1 H); 2.67-2.58 (m, 1 H); 1.93 (s, 9 H); 1.61 (s, 6 H); 1.53 (s, 3 H).

53 *N-Adamant-1-yl-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxamide*

White solid. Mp:>300 °C.Yield: 50%. MS *m*/*z* 378 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.22 (br s, 1 H); 9.91 (br s, 1 H); 8.63 (s 1 H); 7.83-7.78 (d d, J = 8.4 Hz, 1 H); 7.41-7.34 (t, J = 7.6 Hz, 1 H); 7.23-7.18 (d d, J = 7.6 Hz, 1 H); 5.10 (br s, 2 H); 2.06 (s, 9 H); 1.67 (s, 6 H).

54 *N-Adamant-1-yl-9-fluoro-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxamide*

White solid. Mp:>300 °C.Yield: 45%. MS *m*/*z* 397 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.34 (br s, 1 H); 9.83 (br s, 1H); 8.64 (s, 1 H); 7.48-7.42 (d d, J = 9.2 Hz, 1 H); 7.05-6.99 (d d, J = 9 Hz, 1 H); 5.12 (br s, 2 H); 1.99 (s, 9 H); 1.67 (s, 6 H).

55 *N-Adamant-1-yl-9-chloro-1,2,3,7-tetrahydro-2,7-dioxopyrido[1,2,3-de]quinoxaline-6-carboxamide*

Pale orange solid. Mp:>300 °C.Yield: 48%. MS *m*/*z* 412 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.17 (br s, 1 H); 9.78 (br s, 1 H); 8.65 (s, 1 H); 7.73-7.70 (m, 1 H); 7.17-7.16 (m, 1 H); 5.11 (s, 2 H); 2.06 (s, 9 H); 1.67 (s, 6 H).

56 *N-Adamant-1-yl-1,2,3,7-tetrahydro-8,9-dimethyl-2,7-dioxopyrido[1,2,3-de] quinoxaline-6-carboxamide*

White solid. Mp:>300 °C.Yield: 55%. MS *m*/*z* 407 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.02 (br s, 1 H); 9.97 (br s, 1 H); 8.51 (s, 1 H); 7.02 (s, 1 H); 5.02 (s, 2 H); 2.71 (s, 3 H); 2.29 (s, 3 H); 2.05 (s, 9 H); 1.67 (s, 6 H).

Synthesis of *N*-cyclohexylcarboxamide derivatives (49, 52)

To a solution of the appropriate carboxylic acid (**41b**, **41d**, 0.6 mmol) in DMF (5 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.6 mmol), 1-hydroxybenzotriazole (HOBt, 0.6 mmol), and finally cyclohexylamine (0.9 mmol). The

reaction mixture was stirred at room temperature for 6 h. The solvent was evaporated, and the residue was purified by column chromatography to yield a white solid.

49 *N-Cyclohexyl-2,4-diethyl-2,3,4,8-tetrahydro-2-methyl-8-oxo-1H-[1,4] diazepino [3,2,1-ij]quinoline-7-carboxamide*

White solid. Mp: 191 ° C. Yield: 48%. MS *m*/*z* 273 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.01 (br d, *J* = 7.8 Hz, 1 H); 8.71 (s, 1 H); 7.77-7.68 (m, 1 H); 7.28-7.26 (m, 2 H); 5.62 (s, 1 H); 4.55-4.45 (m, 1 H); 3.82-3.74 (m, 1 H); 2.35-1.31 (m, 10 H); 1.30-1.05 (m, 10 H); 0.71-0.68 (m, 5 H).

52 *N-Cyclohexyl-2,3,4,8-tetrahydro-2-methyl-8-oxo-2,4-diphenyl-1H-[1,4]diazepino [3,2,1-ij]quinoline-7-carboxamide*

White solid. Mp: 278 ° C. Yield: 45%. MS *m*/*z* 492 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.90-9.86 (d, *J* = 8 Hz, 1 H); 8.13 (s, 1 H); 7.73-7.69 (d d, *J* = 7.8 Hz, 1 H); 7.44-7.01 (m, 12 H); 6.37-6.33 (d, *J* = 8 Hz, 1 H); 6.19 (br s, 1 H); 3.63-3.51 (m, 1 H); 2.62-2.58 (m, 1 H); 1.78-1.18 (m, 14 H).

Synthesis of 2-Nitrophenoxy Ketones (58a-h)

To a solution of 2-nitrophenol (**57a-c**, 14 mmol) in anhydrous acetone (10 mL) were added K_2CO_3 (15 mmol) and the appropriate halo ketone (1.1 eq). After being stirred vigorously at room temperature until the starting material disappeared as determined by TLC, the reaction mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in ethyl acetate (150 mL) and washed with water (30 mL). The organic layer was dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography using ethyl acetate/petroleum ether as the eluent or by crystallization to yield the desired compound.

58a *1-(2-Nitrophenoxy)propan-2-one*

White solid. Mp: 72 °C. Yield: 72%. MS: *m*/*z* 196.0 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.91–7.86 (m, 1H), 7.61–7.57 (m, 1H), 7.24–7.13 (m, 2H), 5.05 (s, 2H), 2.17 (s, 3H).

58b *2-(2-Nitrophenoxy)-1-phenylethanone*

Yellow solid. Mp: 116–117 °C. Yield: 71%. MS: *m*/*z* 258.2 (M + H). ^{1}H NMR ($CDCl_3$): δ 8.03–7.98 (m, 2H), 7.90–7.85 (m, 1H), 7.61–7.45 (m, 4H), 7.11–6.95 (m, 2H), 5.45 (s, 2H).

58c *2-(2-Nitrophenoxy)-1-phenylpropan-1-one*

Yellow oil. Yield: 69%. MS: *m*/*z* 272.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.08–8.04 (m, 2H), 7.89–7.85 (m, 1H), 7.72–7.59 (m, 3H), 7.15–7.11 (m, 2H), 6.29 (q, *J* = 6.8 Hz, 1H), 1.57 (d, *J* = 6.6 Hz, 3H).

58d *2-(2-Nitrophenoxy)-1,2-diphenylethanone*

Yellow solid. Mp: 58 °C. Yield: 15%. MS: *m*/*z* 334.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.08–7.86 (m, 2H), 7.30–7.54 (m, 5H), 7.03–7.19 (m, 7H), 6.48 (s, 1H).

58e *2-(5-Methyl-2-nitrophenoxy)-1-phenylethanone*

Yellow solid. Mp: 132–134 °C. Yield: 75%. MS: *m*/*z* 272.1 (M + H). ^{1}H NMR ($CDCl_3$): δ 8.03–7.99 (m, 2H), 7.83–7.54 (m, 4H), 7.16 (s, 1H), 6.96–6.91 (m, 1H), 5.83 (s, 2H), 2.32 (s, 3H).

58f *1-(4-Methylphenyl)-2-(2-nitrophenoxy)ethanone*

White solid. Mp: 125 °C. Yield: 73%. MS: *m*/*z* 272.8 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.92–7.86 (m, 3H), 7.58–7.53 (m, 1H), 7.40–7.36 (m, 2H), 7.28–7.23 (m, 1H), 7.16–7.11 (m, 1H), 5.81 (s, 2H), 2.40 (s, 3H).

58g *1-Cyclopropyl-2-(2-nitrophenoxy)ethanone*

White solid. Mp: 58 °C. Yield: 75%. MS: *m*/*z* 222.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.91–7.86 (m, 1H), 7.61–7.57 (m, 1H), 7.20–7.13 (m, 2H), 5.21 (s, 2H), 2.22 (m, 1H), 1.01–0.90 (m, 4H).

58h *1-Cyclopropyl-2-(4-methyl-2-nitrophenoxy)ethanone*

Brown oil. Yield: 69%. MS: *m*/*z* 236.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.89 (m, 1H), 7.19–7.12 (m, 2H), 5.20 (s, 2H), 2.32 (s, 3H), 2.21 (m, 1H), 1.01–0.90 (m, 4H).

Synthesis of Benzo[1,4]oxazines (59a–g,i,j)

To a solution of the nitro ketone **58a**–**h** (7 mmol) in methanol (100 mL) (in the case of 3-aryl in methanol/THF, 85:15) was added 10% palladium on carbon (0.3 mmol), and the mixture was shaken under 4 atm of hydrogen at room temperature for 6 h. The reaction was monitored by TLC, and when complete, the suspension was filtered through a pad of Celite to remove the catalyst. The filtrate was concentrated under vacuum to give a yellow residue that was purified by column chromatography using ethyl acetate/petroleum ether (0.5/9.5) to yield the desired compounds. The cyclopropyl derivative **59g** and propyl derivative **59i** were separated by column chromatography using ethyl acetate/petroleum ether (0.2/9.8) as the eluent.

59a *3,4-Dihydro-3-methyl-2H-benzo[b][1,4]oxazine*

Colorless oil. Yield: 80%. MS: *m*/*z* 150.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 6.68–6.40 (m, 4H), 5.72 (br s, 1H), 4.15–4.07 (m, 1H), 3.65–3.56 (m, 1H), 3.37–3.36 (m, 1H), 1.07 (d, *J* = 6.4 Hz, 3H).

59b *3,4-Dihydro-3-phenyl-2H-benzo[b][1,4]oxazine*

White solid. Mp: 40 °C. Yield: 76%. MS: *m*/*z* 212.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.43–7.37 (m, 5H), 6.85–6.67 (m, 4H), 5.99 (br s, 1H); 4.53–4.49 (m, 1H),4.34–4.27 (m, 1H), 4.06–3.95 (m, 1H).

59c *3,4-Dihydro-2-methyl-3-phenyl-2H-benzo[b][1,4]oxazine*

Pale yellow oil. Yield: 83%. MS: *m*/*z* 226.0 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.34–7.30 (m, 5H), 6.74–6.69 (m, 3H), 6.58–6.43 (m, 1H), 6.38 (br s, 1H), 4.47-.4.44 (m, 2H), 0.98 (d, *J* = 6.4 Hz, 3H).

59d *3,4-Dihydro-2,3-diphenyl-2H-benzo[b][1,4]oxazine*

Orange solid. Mp: 161–162 °C dec. Yield: 71%. MS: *m*/*z* 288.8 (M + H). ^{1}H NMR ($CDCl_3$): δ 7.18–7.13 (m, 7H), 6.99–6.75 (m, 8H), 5.44 (d, *J* = 3.2 Hz, 1H), 4.70 (d, *J* = 3 Hz, 1H).

59e *3,4-Dihydro-7-methyl-3-phenyl-2H-benzo[b][1,4]oxazine*

White solid. Mp: 88 °C. Yield: 77%. MS: *m*/*z* 226.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.41–7.33 (m, 5H), 6.62–6.50 (m, 3H), 6.03 (br s, 1H), 4.48 (m, 1H), 4.17 (m, 1H), 3.91–3.82 (m, 1H), 2.13 (s, 3H).

59f *3,4-Dihydro-3-(4-methylphenyl)-2H-benzo[b][1,4]oxazine*

Colorless oil. Yield: 83%. MS: *m*/*z* 226.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.29 (dd, *J* = 8 Hz, 2H), 7.17 (dd, *J* = 8 Hz, 2H), 6.71–6.66 (m, 3H), 6.54–6.48 (m, 1H), 6.20 (br s, 1H), 4.39 (m, 1H), 4.21–4.14 (m, 2H), 3.89–3.80 (m, 1H), 2.30 (s, 3H).

59g *3,4-Dihydro-3-cyclopropyl-2H-benzo[b][1,4]oxazine*

Yellow oil. Yield: 38%. MS: *m*/*z* 176.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 6.65–6.59 (m, 3H), 6.45–6.41 (m, 1H), 5.80 (br s, 1H), 4.19–4.15 (m, 1H), 3.56–3.81 (m, 1H), 2.58–2.57 (m, 1H), 0.78–0.76 (m, 1H) 0.48–0.32 (m, 4H).

59i *3,4-Dihydro-3-propyl-2H-benzo[b][1,4]oxazine*

Yellow oil. Yield: 54%. MS: *m*/*z* 178.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 6.66–6.58 (m, 3H), 6.46–6.42 (m, 1H), 5.71 (br s, 1H), 4.14–4.10 (m, 1H), 3.72–3.67 (m, 1H), 3.25–3.24 (m, 1H), 1.41–1.37 (m, 4H), 0.91 (t, *J* = 6.8 Hz, 3H).

59j *3,4-Dihydro-6-methyl-3-propyl-2H-benzo[b][1,4]oxazine*

Yellow oil. Yield: 81%. MS: *m*/*z* 192.3 (M + H). ^{1}H NMR ($CDCl_3$): δ 6.68 (m, 1H), 6.48–6.42 (m, 2H), 4.22–4.16 (m, 1H), 3.86–3.77 (m, 1H), 3.67 (br s, 1H), 3.40–3.35 (m, 1H), 2.21 (s, 3H), 1.49–1.41 (m, 4H), 1.01–0.94 (m, 3H).

Synthesis of Diethyl 2-[(2,3-Dihydro-2/3-substituted-benzo[b][1,4]oxazin-4-yl) methylene]malonate derivatives (60a–g,i,j)

A mixture of benzo[1,4]oxazine **59a**–**g**,**i**,**j** (4.7 mmol) and diethyl (ethoxymethylene) malonate (4.7 mmol) was heated with stirring at 140 °C for 2 h. The solvent was removed under reduced pressure, and cold diethyl ether was added to form a precipitate that was filtered and subsequently purified by column chromatography using ethyl acetate/petroleum ether (0.5/9.5).

60a *Diethyl 2-((2,3-Dihydro-3-methylbenzo[b][1,4]oxazin-4-yl)methylene)malonate*

Pale yellow oil. Yield: 73%. MS: *m*/*z* 320.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.71 (s, 1H), 7.21–6.95 (m, 4H), 4.21–3.87 (m, 7H), 1.26–1.16 (m, 9H).

60b *Diethyl 2-((2,3-Dihydro-3-phenylbenzo[b][1,4]oxazin-4-yl)methylene)malonate*

White solid. Mp: 103–104 °C. Yield: 81%. MS: *m*/*z* 382.2 (M + H). ^{1}H NMR ($CDCl_3$): δ 8.12 (s, 1H) 7.38–6.87 (m, 9H), 4.55–3.93 (m, 6H) 3.42–3.21 (m, 1H), 1.26 (t, *J* = 7 Hz, 3H), 0.90 (t, *J* = 7.2 Hz, 3H).

60c *Diethyl 2-((2,3-Dihydro-2-methyl-3-phenylbenzo[b][1,4]oxazin-4-yl)methylene) malonate*

White solid. Mp: 130 °C. Yield: 89%. MS: *m*/*z* 396.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.85 (s, 1H), 7.33–7.27 (m, 4H), 7.10–6.93 (m, 5H), 5.02 (d, *J* = 2.6 Hz, 1H), 4.60–4.43 (m, 1H), 4.11–3.95 (m, 3H), 3.64–3.61 (m, 1H), 1.20–1.13 (m, 6H), 0.93 (t, *J* = 7.2 Hz, 3H).

60d *Diethyl 2-((2,3-Dihydro-2,3-diphenylbenzo[b][1,4]oxazin-4-yl)methylene)malonate*

White solid. Mp: 114 °C. Yield: 96%. MS: *m*/*z* 458.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.96 (s, 1H), 7.42–7.30 (m, 4H), 7.18–7.04 (m, 8H), 6.43–6.40 (m, 2H), 5.69 (d, *J* = 2.6 Hz, 1H), 5.34 (d, *J* = 2.8 Hz, 1H), 4.14–4.10 (m, 2H), 3.98–3.81 (m, 1H), 3.56–3.41 (m, 1H), 1.18 (t, *J* = 7.2 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H).

60e *Diethyl 2-((2,3-Dihydro-7-methyl-3-phenylbenzo[b][1,4]oxazin-4-yl)methylene) malonate*

White solid. Mp: 105 °C. Yield: 92%. MS: *m*/*z* 396.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.93 (s, 1H), 7.30–7.15 (m, 6H), 6.87–6.82 (m, 1H), 6.64–6.63 (m, 1H), 5.14 (m, 1H), 4.72–4.65 (m, 1H), 4.36–4.29 (m, 1H), 4.11 (q, *J* = 6.2 Hz, 2H), 3.88–3.79 (m, 1H), 3.32–3.23 (m, 1H), 2.20 (s, 3H), 1.17 (t, *J* = 7 Hz, 3H), 0.77 (t, *J* = 7 Hz, 3H).

60f *Diethyl 2-((2,3-Dihydro-3-(4-methylphenyl)benzo[b][1,4]oxazin-4-yl)methylene) malonate*

White solid. Mp: 94 °C. Yield: 87%. MS: *m*/*z* 396.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.94 (s, 1H), 7.33 (m, 1H), 7.08–6.99 (m, 6H), 6.83–6.78 (m, 1H), 5.13 (m, 1H), 4.78–4.62 (m, 1H), 4.38–4.31 (m, 1H), 4.11 (q, *J* = 6.2 Hz, 2H), 3.97–3.78 (m, 1H), 3.42–3.21 (m, 1H), 2.23 (s, 3H), 1.82 (t, *J* = 7.4 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H).

60g *Diethyl 2-((3-Cyclopropyl-2,3-dihydrobenzo[b][1,4]oxazin-4-yl)methylene) malonate*

Yellow oil. Yield: 92%. MS: *m*/*z* 346.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.72 (s, 1H), 7.12–6.90 (m, 4H), 4.36–4.35 (m, 1H), 4.20–4.05 (m, 3H), 3.97–3.65 (m, 2H), 3.51–3.47 (m, 1H), 1.19 (t, *J* = 7 Hz, 3H), 1.06 (t, *J* = 7 Hz, 3H), 0.98–0.83 (m, 2H), 0.53–0.43 (m, 3H).

60i *Diethyl 2-((2,3-Dihydro-3-propylbenzo[b][1,4]oxazin-4-yl)methylene)malonate*

Yellow oil. Yield: 84%. MS: *m*/*z* 348.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.70 (s, 1H), 7.14–6.89 (m, 4H), 4.35 (m, 1H), 4.17–3.78 (m, 6H), 1.48–1.05 (m, 10H), 0.85 (t, *J* = 7.4 Hz, 3H).

60j *Diethyl 2-(2,3-Dihydro-6-methyl-3-propylbenzo[b][1,4]oxazin-4-yl)methylene) malonate*

Yellow oil. Yield: 78%. MS: *m*/*z* 362.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 7.84 (s, 1H), 6.88–6.78 (m, 3H), 4.30–4.07 (m, 6H), 3.82 (m, 1H), 2.27 (s, 3H), 1.63–1.59 (m, 4H), 1.32–1.22 (m, 6H), 0.92 (t, *J* = 7 Hz, 3H).

Synthesis of Ethyl 3,7-Dihydro-2/3-substituted-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*] quinoline-6-carboxylates (61a–g,i,j)

A mixture of diethyl ester **60a–g**,**i**,**j** (1.5 mmol) and poly(phosphoric acid) (3 g) was heated at 140 °C for 1 h. The mixture was poured into ice and water to form a precipitate that was filtered and washed with cold water.

61a *Ethyl 3,7-Dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 189 °C dec. Yield: 95%. MS: *m*/*z* 274.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.65 (s, 1H), 7.80–7.75 (m, 1H), 7.40–7.31 (m, 2H), 4.79–4.63 (m, 1H), 4.42–4.34 (m, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 1.41 (d, *J* = 6.6 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 3H).

61b *Ethyl 3,7-Dihydro-3-phenyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 239 °C. Yield: 87%. MS: *m*/*z* 336.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.55 (s, 1H), 7.87–7.82 (m, 1H), 7.43–7.28 (m, 5H), 7.13–7.08 (m, 2H), 5.91 (m, 1H), 4.76–4.64 (m, 2H), 4.19 (q, *J* = 6.2 Hz, 2H), 1.24 (t, *J* = 7 Hz, 3H).

61c *Ethyl 3,7-Dihydro-2-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 243–244 °C. Yield: 81%. MS: *m*/*z* 350.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.53 (s, 1H), 7.84–7.83 (m, 1H), 7.43–7.34 (m, 5H), 7.11–7.07 (m, 2H), 5.73 (d, *J* = 3 Hz, 1H), 4.82–4.78 (m, 1H), 4.21–4.16 (m, 2H), 1.27–1.18 (m, 6H).

61d *Ethyl 3,7-Dihydro-7-oxo-2,3-diphenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 289–290 °C dec. Yield: 75%. MS: *m*/*z* 412.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.63 (s, 1H), 7.93–7.90 (m, 1H), 7.53–7.49 (m, 2H), 7.32–7.13 (m, 8H), 6.66 (d, *J* = 6.4 Hz, 2H), 6.15–5.95 (m, 2H), 4.22–4.17 (m 2H), 1.25 (t, *J* = 7.2 Hz, 3H).

61e *Ethyl 3,7-Dihydro-9-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 199 °C dec. Yield: 79%. MS: *m*/*z* 350.0 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.49 (s, 1H), 7.63–7.62 (m, 1H), 7.36–7.32 (m, 3H), 7.13–7.04 (m, 3H), 5.87 (m, 1H), 4.72–4.59 (m 2H), 4.16 (q, *J* = 6.4 Hz, 2H), 2.38 (s, 3H), 1.21 (t, *J* = 7 Hz, 3H).

61f *Ethyl 3,7-Dihydro-7-oxo-3-(4-methylphenyl)-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 183 °C. Yield: 83%. MS: *m*/*z* 350.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.50 (s, 1H), 7.86–7.81 (m, 1H), 7.43–7.27 (m, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 7.00 (d, *J* = 8 Hz, 2H), 5.84 (m, 1H), 4.72–4.61 (m, 2H), 4.18 (q, *J* = 7 Hz, 2H), 2.27 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H).

61g *Ethyl 3-Cyclopropyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 190 °C. Yield: 77%. MS: *m*/*z* 300.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 7.82–7.76 (m, 1H), 7.37–7.32 (m, 2H), 4.58–4.31 (m, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 3.97 (m, 1H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.18 (m, 1H), 0.59 (m, 4H).

61i *Ethyl 3,7-Dihydro-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 133 °C. Yield: 80%. MS: *m*/*z* 302.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H); 7.79–7.74 (m, 1H), 7.36–7.30 (m, 2H), 4.60–4.54 (m, 2H), 4.28–4.21 (m, 3H), 1.82–1.63 (m, 2H), 1.48–1.33 (m, 2H), 1.29 (d, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H).

61j *Ethyl 3,7-Dihydro-8-methyl-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

Pale white solid. Mp: 125–127 °C. Yield: 95%. MS: *m*/*z* 316.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.47 (s, 1H), 7.16–7.07 (m, 2H), 4.79 (m, 1H), 4.53–4.47 (m, 2H), 4.24–4.16 (m, 3H), 2.71 (s, 3H), 1.81–1.68 (m, 4H), 1.31–1.24 (m, 6H), 0.88 (t, *J* = 7.4 Hz, 3H).

Synthesis of ethyl 3-oxopropanoate derivatives (64a–c)

Carbonyldiimidazole (11 mmol) was added to a solution of the requisite benzoic acid (**63a–c**, 10 mmol) in anhydrous THF (50 mL). After the mixture was stirred at room temperature for 6 h, the magnesium salt of ethyl malonic acid half-ester (prepared by stirring monoethyl malonate (10 mmol) and magnesium ethoxide (5 mmol) in anhydrous THF (25 mL) for 1 h at room temperature) was added, and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was partitioned between 5% hydrochloric acid (20 mL) and ether (50 mL). The aqueous layer was further extracted with ether, and the combined extracts were washed with aqueous saturated sodium bicarbonate solution (10 mL) and dried. Evaporation of the solvent in vacuo led to the desired compounds as clear oils (79%), which were used without further purification in the next step.

64a *Ethyl 3-(2,3-Difluorophenyl)-3-oxopropanoate*

64b *Ethyl 3-(2,3,4-Trifluorophenyl)-3-oxopropanoate*

64c *Ethyl 3-(2,3,4,5-Tetrafluorophenyl)-3-oxopropanoate*

Synthesis of 2-(ethoxymethylene)-3-oxo-3-phenyl-propionate derivatives (65a–i)

A mixture of the appropriate ethyl 3-(2,3-difluorophenyl)-, 3-(2,3,4-trifluorophenyl)-, or 3-(2,3,4,5-tetrafluorophenyl)-3-oxopropanoate (**64a–c**, 7.7 mmol), triethyl orthoformate (11.5 mmol), and acetic anhydride (45 mmol) was stirred at 110–120 °C for 3 h. The reaction was concentrated under reduced pressure to leave an oily residue, which was diluted with toluene and concentrated. This process was repeated two more times to afford the desired ethyl 2-(ethoxymethylene)-3-oxo-3-(2,3-difluorophenyl)-, 3-(2,3,4-trifluorophenyl)-, or 3-(2,3,4,5-tetrafluorophenyl)propionate (**65a**–**c**) as an oil, which was used directly without further purification.

Synthesis of Ethyl 2-benzoyl-3-((2-hydroxy-1-alkylethyl)amino)acrylates (66a–i)

To a solution of ethyl 2-(ethoxymethylene)-3-oxo-3-(2,3-difluorophenyl)propionate (**65a**; 7.7 mmol) in dry methylene chloride (20 mL) was added a solution of the desired (*R*,*S*)-, (*R*)-, or (*S*)-2-amino-1-hydroxyalkane (11 mmol) in methylene chloride (5 mL) dropwise under cooling with an ice bath. The whole mixture was stirred at room temperature for 1 h and then concentrated in vacuo to yield the requisite (*R*,*S*)-, (*R*)-, or (*S*)-ethyl 2-(2,3-difluorobenzoyl)-3-((2-hydroxy-1-alkylethyl)amino)acrylates **66a**–**g** as oils, which were used in the next step without further purification.
In identical fashion, ethyl 2-(ethoxymethylene)-3-oxo-3-(2,3,4-trifluorophenyl) propionate (**65b**) or ethyl 2-(ethoxymethylene)-3-oxo-3-(2,3,4,5-tetrafluorophenyl) propionate (**65c**) was reacted with 2-amino-1-hydroxybutane to afford the desired ethyl 2-(2,3,4-trifluorobenzoyl)-3-((2-hydroxy-1-ethylethyl)amino) acrylate (**66h**) and ethyl 2-(2,3,4,5-tetrafluorobenzoyl)-3-((2-hydroxy-1-ethylethyl)amino) acrylate (**66i**) as oils, which were used in the next step without further purification.

Synthesis of Ethyl 3,7-Dihydro-7-oxo-3-substituted-2*H*-[1,4]oxazino[2,3,4-*ij*] quinoline-6-carboxylates (67a–i)

A mixture of the requisite ethyl 2-(2,3-difluorobenzoyl)-, 2-(2,3,4-trifluorobenzoyl)-, or 2-(2,3,4,5-tetrafluorobenzoyl)-3-((2-hydroxy-1-substituted-ethyl)amino)acrylate (**66a**–**i**, 5.5 mmol), K_2CO_3 (16 mmol), and anhydrous DMF (30 mL) was stirred at 130–140 °C for 7 h. After cooling, ethyl acetate (50 mL) was added to the reaction mixture. The organic phase was washed with water, dried, filtered, and concentrated. The residue was

purified by flash chromatography, eluting with ethyl acetate/petroleum ether (7/3) to afford the desired product as a white solid.

(*R*)-67a *(R)-Ethyl 3,7-Dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

(*S*)-67a *(S)-Ethyl 3,7-Dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

The NMR data and melting points are identical to those reported for compound **61a**.

67b *(R,S)-Ethyl 3,7-Dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate,*

(*R*)-67b *(R)-Ethyl 3,7-Dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

(*S*)-67b *(S)-Ethyl 3,7-Dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 157 °C dec. Yield: 86%. MS: *m*/*z* 288.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.63 (s, 1H), 7.79–7.44 (m, 1H), 7.35–7.30 (m, 2H), 4.61–4.49 (m, 2H), 4.32–4.18 (m, 3H), 1.75–1.72 (m, 2H), 1.28 (t, *J* = 7 Hz, 3H), 0.93 (t, *J* = 7.6 Hz, 3H).

67c *(R,S)-Ethyl 3,7-Dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*,

(*R*)-67c *(R)-Ethyl 3,7-Dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

(*S*)-67c *(S)-(-)-Ethyl 3,7-Dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxylate*

White solid. Mp: 142 °C. Yield: 71%. MS: *m*/*z* 302.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H), 7.79–7.74 (m, 1H), 7.39–7.29 (m, 2H), 4.75 (d, *J* = 10.8 Hz, 1H), 4.32–4.18 (m, 4H), 2.07–1.91 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 6.8 Hz, 3H).

(*R*)-67d *(R)-(+)-Ethyl 3,7-Dihydro-3-isobutyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxyla*te

Colorless oil. Yield: 54%. MS: *m*/*z* 316.3 (M + H). ^{1}H NMR ($CDCl_3$): δ 8.33 (s, 1H), 8.08–8.04 (m, 1H), 7.32–7.21 (m, 2H), 4.45–4.17 (m, 4H), 1.95–1.51 (m, 4H), 1.22 (m, 3H), 1.05 (t, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6 Hz, 3H).

(*R*)-67e *(R)-Ethyl 3,7-Dihydro-3-phenyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

(*S*)-67e *(S)-Ethyl 3,7-Dihydro-3-phenyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

The NMR data and melting points are identical to those reported for compound **11b**.

(*R*)-67f *(R)-Ethyl 3,7-Dihydro-3-benzyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 179–180 °C. Yield: 57%. MS: *m*/*z* 350.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.74 (s, 1H), 8.07–8.03 (m, 1H), 7.74–7.39 (m, 2H), 7.23–7.16 (m, 5H), 5.33–5.29 (m, 2H), 4.25 (q, *J* = 6.8 Hz, 2H), 3.83 (m, 2H), 3.26 (m, 1H), 1.30 (t, *J* = 7.2 Hz, 3H).

67g *Ethyl 3,7-Dihydro-3,3-dimethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

Yellow solid. Mp: 216–217 °C. Yield: 28%. MS: *m*/*z* 289.3 (M + H). ^{1}H NMR ($CDCl_3$): δ 8.61 (s, 1H), 8.11–8.07 (m, 1H), 7.36–7.23 (m, 2H), 4.40 (q, *J* = 7.2 Hz, 2H), 4.12 (s, 2H), 1.62 (s, 6H), 1.42 (t, *J* = 7.2 Hz, 3H).

67h *Ethyl 3,7-Dihydro-3-ethyl-10-fluoro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 190–191 °C dec. Yield: 40%. MS: *m*/*z* 306.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.66 (s, 1H), 7.82–7.70 (m, 1H), 7.45–7.31 (m, 1H), 4.79–4.54 (m 2H), 4.40–4.31 (m, 1H), 4.23 (q, *J* = 7 Hz, 2H), 1.91–1.83 (m, 2H), 1.28 (t, *J* = 7 Hz, 3H), 0.94 (t, *J* = 7.4 Hz, 3H).

67i *Ethyl 3,7-Dihydro-3-ethyl-9,10-difluoro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate*

White solid. Mp: 190–191 °C dec. Yield: 40%. MS: *m*/*z* 324.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.68 (s, 1H), 7.71–7.57 (m, 1H), 4.79–4.54 (m 2H), 4.40–4.33 (m, 1H), 4.23 (q, *J* = 7 Hz, 2H), 1.82–1.63 (m, 2H), 1.28 (t, *J* = 7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).

Synthesis of 3,7-Dihydro-2/3-substituted-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic Acids (62a–g,i,j; 68a–i)

A solution of the requisite ethyl 3,7-dihydro-2-substituted-3-substituted-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylate (**61a–g**,**i**,**j, 67a–i**, 7.4 mmol), NaOH 10% (10 mL), and methanol (90 mL) was heated at 80 °C for 1 h. The solvent was evaporated under reduced pressure, and the residue was acidified with 10% HCl at 0 °C. The precipitate was collected by filtration to give the desired product as a white solid.

62a *(R,S)-3,7-Dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*R*)-68a *(R)-3,7-Dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*S*)-18a *(S)-3,7-Dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 240 °C dec. Yield: 76%. MS: *m*/*z* 246.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.23 (br s, 1H), 9.05 (s, 1H), 7.95–7.91 (m, 1H), 7.60–7.47 (m, 2H), 4.99–4.84 (m, 1H), 4.50–4.42 (m, 2H), 1.46 (d, J = 7 Hz, 3H).

62b *(R,S)-3,7-Dihydro-3-phenyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*R*)-68e *(R)-3-Phenyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*S*)-68e *(S)-3-Phenyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 284 °C. Yield: 90%. MS: *m*/*z* 308.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.10 (br s, 1H), 8.90 (s, 1H), 8.02–7.98 (m, 1H),7.64–7.37 (m, 5H), 7.17–7.12 (m, 2H), 6.10 (m, 1H), 4.85–4.83 (m, 1H), 4.73–4.70 (m, 1H).

62c *3,7-Dihydro-2-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 291–292 °C dec. Yield: 82%. MS: *m*/*z* 322.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.02 (br s, 1H), 8.93 (s, 1H), 8.02–7.98 (m, 1H), 7.61–7.56 (m, 2H), 7.38–7.34 (m, 3H), 7.11–7.09 (m, 2H), 5.93–5.91 (m, 1H), 4.96–4.84 (m, 1H), 2.03 (m, 3H).

62d *3,7-Dihydro-7-oxo-2,3-diphenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: >300 °C. Yield: 86%. MS: *m*/*z* 384.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.03 (br s, 1H), 8.73 (s, 1H), 8.02–7.99 (m, 1H), 7.55–7.51 (m, 2H), 7.29–7.12 (m, 8H), 6.71–6.67 (m, 2H), 6.20 (m, 1H), 5.95 (m, 1H).

62e *3,7-Dihydro-9-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: >300 °C. Yield: 86%. MS: *m*/*z* 322.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.21 (br s, 1H), 8.85 (s, 1H), 7.81–7.79 (m, 1H), 7.39–7.35 (m, 4H), 7.15–7.10 (m, 2H), 6.08 (m, 1H), 4.83–4.69 (m, 2H), 2.48 (s, 3H).

62f *3,7-Dihydro-7-oxo-3-(4-methylphenyl)-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 294 °C dec. Yield: 90%. MS: *m*/*z* 322.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.08 (br s, 1H), 8.82 (s, 1H), 8.01–7.96 (m, 1H), 7.63–7.47 (m, 2H), 7.20 (d, *J* = 8 Hz, 2H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.02 (m, 1H), 4.80–4.69 (m, 2H), 2.28 (s, 3H).

62g *3-Cyclopropyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 227–228 °C. Yield: 76%. MS: *m*/*z* 272.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.17 (br s, 1H), 9.06 (s, 1H), 7.95–7.90 (m, 1H), 7.59–7.46 (m, 2H), 4.64–4.46 (m, 2H), 4.14–4.07 (m, 1H), 1.38–1.11 (m, 1H), 0.79–0.52 (m, 4H).

62i *3,7-Dihydro-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 213 °C. Yield: 82%. MS: *m*/*z* 274.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.21 (br s, 1H), 9.00 (s, 1H), 7.95–7.90 (m, 1H), 7.60–7.46 (m, 2H), 4.85 (m, 1H), 4.69–4.63 (m, 1H), 4.42–4.41 (m, 1H), 1.98–1.11 (m, 4H), 0.88 (t, *J* = 7 Hz, 3H).

62j *3,7-Dihydro-8-methyl-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 205 °C dec. Yield: 78%. MS: *m*/*z* 289.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.53 (br s, 1H), 8.91 (s, 1H), 7.36–7.24 (m, 2H), 4.84–4.77 (m, 1H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.29 (d, *J* = 10 Hz, 1H), 2.80 (s, 3H), 1.95–1.20 (m, 4H), 0.87 (t, *J* = 7.4 Hz, 3H).

68b *(R,S)-3,7-Dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*R*)-68b *(R)-3,7-Dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*S*)-18b *(S)-3,7-Dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 190 °C. Yield: 78%. MS: *m*/*z* 260.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.21 (br s, 1H), 9.03 (s, 1H), 7.94–7.90 (m, 1H), 7.59–7.45 (m, 2H), 4.81–4.65 (m, 2H), 4.42–4.35 (m, 1H), 1.80–1.75 (m, 2H), 0.93 (t, *J* = 6.6 Hz, 3H).

68c *(R,S)-3,7-Dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*R*)-68c *(R)-3,7-Dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

(*S*)-68c *(S)-3,7-Dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 210 °C. Yield: 86%. MS: *m*/*z* 274.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 14.99 (br s, 1H), 8.99 (s, 1H), 8.31–7.44 (m, 3H), 5.28 (m, 1H), 4.99–3.91 (m, 3H), 2.01 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.09 (d, *J* = 6.6 Hz, 3H).

(*R*)-68d *(R)-3,7-Dihydro-3-isobutyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 131–132 °C. Yield: 80%. MS: *m*/*z* 288.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 14.99 (br s, 1H), 8.62 (s, 1H), 8.11–8.06 (m, 1H), 7.52–7.26 (m, 2H), 4.82–4.21 (m, 3H), 1.99–1.38 (m, 3H), 1.07–0.95 (m, 6H).

(*R*)-68f *(R)-3-Benzyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 239–240 °C. Yield: 82%. MS: *m*/*z* 323.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.08 (br s, 1H), 8.58 (s, 1H), 7.97–7.92 (m, 1H), 7.63–7.54 (m, 2H), 7.31–7.14 (m, 5H), 5.08 (m, 1H), 4.59–4.15 (m, 2H), 3.13–3.05 (m, 2H).

68g *3,7-Dihydro-3,3-dimethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: >300 °C. Yield: 68%. MS: *m*/*z* 260.1 (M + H). ^{1}H NMR ($CDCl_3$): δ 15.00 (br s, 1H), 8.88 (s, 1H), 8.15–8.10 (m, 1H), 7.53–7.36 (m, 2H), 4.18 (s, 2H), 1.67 (s, 6H).

68h *3-Ethyl-10-fluoro-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 230 °C. Yield: 65%. MS: *m*/*z* 278.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.00 (br s, 1H), 9.01 (s, 1H), 7.97–7.90 (m, 1H), 7.64–7.54 (m, 1H), 4.84–4.75 (m, 2H), 4.64–4.39 (m, 1H), 1.85–1.73 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H).

68i *9,10-Difluoro-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic Acid*

White solid. Mp: 246–248 °C. Yield: 50%. MS: *m*/*z* 296.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 15.00 (br s, 1H), 9.01 (s, 1H), 7.71–7.65 (m, 1H), 4.84–4.76 (m, 2H), 4.44–4.38 (m, 1H), 1.84–1.76 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).

Synthesis of *N*-Cycloalkyl- or *N*,*N*-Diisopropyl-3,7-dihydro-2/3-substituted-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamides

To a solution of the appropriate 3,7-dihydro-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid (**62**, **(*R*)-68a**, **(*S*)-68a**, **68b**, 0.6 mmol) in DMF (5 mL) were added EDC (0.6 mmol), HOBt (0.6 mmol), and finally the desired amine (0.9 mmol). The reaction mixture was stirred at room temperature for 6 h. The solvent was evaporated, and the residue was purified by column chromatography to yield a white solid.

69 *N-Cyclopentyl-3,7-dihydro-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 237 °C. Yield: 64%. MS: *m*/*z* 375.2 (M + H). ^{1}H NMR (DMSO-d_6): 10.00 (br d, *J* = 7.2 Hz, 1H), 8.67 (s, 1H), 7.96–7.91 (m, 1H), 7.46–7.36 (m, 5H), 7.14–7.09 (m, 2H), 6.01 (m, 1H), 4.82–4.59 (m, 2H), 4.25–4.16 (m, 1H), 1.98–1.30 (m, 8H). Anal. ($C_{23}H_{22}N_2O_3$) C, H, N.

70 *N-Cyclohexyl-3,7-dihydro-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 207 °C. Yield: 58%. MS: *m*/*z* 389.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.01 (br d, *J* = 7.6 Hz, 1H), 8.69(s, 1H), 7.96–7.91 (m, 1H), 7.49–7.08 (m, 7H), 6.01 (m, 1H), 4.76 (m, 1H), 4.65 (m, 1H), 3.95 (m, 1H), 1.88–1.30 (m, 10H). Anal. ($C_{24}H_{24}N_2O_3$) C, H, N.

71 *N-Cycloheptyl-3,7-dihydro-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 169 °C. Yield: 55%. MS: *m*/*z* 403.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.03 (br d, *J* = 7.8 Hz, 1H), 8.66 (s, 1H), 7.96–7.92 (m, 1H), 7.46–7.34 (m, 5H), 7.14–7.09 (m, 2H), 6.01 (m, 1H), 4.83–4.58 (m, 2H), 3.95 (m, 1H), 1.95–1.35 (m, 12H). Anal. ($C_{25}H_{26}N_2O_3$) C, H, N.

74 *N-Cyclohexyl-3,7-dihydro-2-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 254 °C. Yield: 61%. MS: *m*/*z* 403.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.97 (br d, *J* = 7.4 Hz, 1H), 8.68 (s, 1H), 7.97–7.93 (m, 1H), 7.50–7.35 (m, 5H), 7.10 (m, 2H), 5.88 (m, 1H), 4.89–4.84 (m, 1H), 3.91 (m, 1H), 1.97–1.40 (m, 10H), 1.33 (d, *J* = 6.2 Hz, 3H). Anal. ($C_{25}H_{26}N_2O_3$) C, H, N.

77 *N-Cyclohexyl-3,7-dihydro-7-oxo-3-p-tolyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 237 °C. Yield: 64%. MS: *m*/*z* 403.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.99 (br d, *J* = 7.4 Hz, 1H), 8.62 (s, 1H), 7.95–7.90 (m, 1H), 7.49–7.34 (m, 2H), 7.19 (d, *J* = 8.00 Hz, 2H), 7.02 (d, *J* = 8.00 Hz, 2H), 5.94 (m, 1H), 4.72–4.63 (m, 2H), 3.89 (m, 1H), 2.28 (s, 3H), 1.92–1.26 (m, 10H). Anal. ($C_{25}H_{26}N_2O_3$) C, H, N.

79 *N-Cyclohexyl-3,7-dihydro-9-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 286 °C. Yield: 66%. MS: *m*/*z* 403.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.04 (br d, *J* = 7.8 Hz, 1H), 8.63 (s, 1H), 7.74 (s, 1H), 7.38–7.35 (m, 3H), 7.21 (s, 1H), 7.011–7.07 (m, 2H), 5.99 (m, 1H), 4.75–4.59 (m, 2H), 3.89 (m, 1H), 2.43 (s, 3H), 1.92–1.24 (m, 10H). Anal. ($C_{25}H_{26}N_2O_3$) C, H, N.

81 *N-Cyclohexyl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 245 °C. Yield: 58%. MS: *m*/*z* 327.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (br d, *J* = 7.8 Hz, 1H), 8.82 (s, 1H), 7.89–7.85 (m, 1H), 7.46–7.33 (m, 2H), 4.82 (m, 1H), 4.45–4.36 (m, 2H), 3.81 (m, 1H), 1.88–1.28 (m, 13H). Anal. ($C_{19}H_{22}N_2O_3$) C, H, N.

82 *N-Cycloheptyl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 208 °C. Yield: 47%. MS: *m*/*z* 403.3 (M + H). ^{1}H NMR ($CDCl_3$): δ 10.05 (br d, *J* = 7.4 Hz, 1H), 8.71 (s, 1H), 8.09–8.04 (m, 1H), 7.41–7.24 (m, 2H), 4.82 (m, 1H), 4.35–4.26 (m, 2H), 4.21–4.08 (m, 1H), 2.04–1.97 (m, 2H), 1.71–1.57 (m, 13H). Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

84 *N-Cyclohexyl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 175 °C. Yield: 69%. MS: *m*/*z* 341.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (br d, *J* = 7.8 Hz, 1H), 8.80 (s, 1H), 7.89–7.84 (m, 1H), 7.46–7.32 (m, 2H), 4.67–4.60 (m, 2H), 4.36–4.30 (m, 1H), 3.99 (m, 1H), 1.87–1.28 (m, 12H), 0.92 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

85 *N-Cycloheptyl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 165 °C. Yield: 51%. MS: *m*/*z* 355.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.09 (br d, *J* = 7.8 Hz, 1H), 8.79 (s, 1H), 7.89–7.84 (m, 1H), 7.41–7.35 (m, 2H), 4.70–

4.59 (m, 2H), 4.36–4.29 (m, 1H), 4.07–4.01 (m, 1H), 1.87–1.56 (m, 14H), 0.92 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N.

91 *3,7-Dihydro-N,N-diisopropyl-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 187 °C. Yield: 36%. MS: *m*/*z* 336.2 (M + H). ^{1}H NMR (DMSO-d_6):8.06 (s, 1H), 7.74–7.69 (m, 1H), 7.27–7.23 (m, 2H), 4.63–4.21 (m, 3H), 3.75 (m, 1H), 3.50 (m, 1H), 1.80–1.42 (m, 2H), 1.42 (d, *J* = 6.4 Hz, 6H), 1.08 (m, 6H), 0.90 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{20}H_{26}N_3O_3$) C, H, N.

92 *N-Cyclohexyl-3,7-dihydro-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 191 °C. Yield: 68%. MS: *m*/*z* 355.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.04 (br d, *J* = 8 Hz, 1H), 8.77 (s, 1H), 7.87–7.85 (m, 1H), 7.43–7.33 (m, 2H), 4.75–4.59 (m, 2H), 4.34–4.31 (m, 1H), 3.85 (m, 1H), 1.91–1.29 (m, 14H), 0.87 (t, *J* = 7.6 Hz, 3H). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N.

93 *N-Cycloheptyl-3,7-dihydro-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: >300 °C. Yield: 46%. MS: *m*/*z* 369.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.08 (br d, *J* = 8 Hz, 1H), 8.75 (s, 1H), 7.89–7.84 (m, 1H), 7.45–7.31 (m, 2H), 4.74–4.57 (m, 2H), 4.34–4.29 (m, 1H), 4.04 (m, 1H), 3.98 (m, 1H), 1.89–1.29 (m, 15H), 0.88 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{22}H_{28}N_2O_3$) C, H, N.

96 *N-Cyclohexyl-3,7-dihydro-8-methyl-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 213 °C. Yield: 55%. MS: *m*/*z* 369.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (br d, *J* = 7.8 Hz, 1H); 8.67 (s, 1H), 7.22–7.08 (m, 2H); 4.73–4.65 (m, 1H); 4.56–4.50 (m, 1H); 4.26–4.19 (m, 1H); 3.82 (m, 1H); 2.78 (s, 3H); 1.88–1.19 (m, 14H); 0.87 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{22}H_{28}N_2O_3$) C, H, N.

98 *N-Cyclohexyl-3-cyclopropyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 212 °C. Yield: 63%. MS: *m*/*z* 353.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (br d, *J* = 8 Hz, 1H), 8.90 (s, 1H), 7.90–7.86 (m, 1H), 7.43–7.38 (m, 2H), 4.61–4.46 (m, 2H), 4.10–4.05 (m, 1H), 3.85 (m, 1H), 1.99–1.32 (m, 11H), 0.70.65 (m, 4H). Anal. ($C_{21}H_{24}N_2O_3$) C, H, N.

99 *N-Cycloheptyl-3-cyclopropyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 185 °C. Yield: 52%. MS: *m*/*z* 367.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.09 (br d, *J* = 8.4 Hz, 1H), 8.90 (s, 1H), 7.89–7.87 (m, 1H), 7.45–7.35 (m, 2H), 4.55–4.46 (m, 2H), 4.05–4.02 (m, 2H), 1.99–1.90 (m, 2H),1.69–1.41 (m, 10H), 1.30–1.19 (m, 1H), 0.75–0.54 (m, 4H). Anal. ($C_{22}H_{26}N_2O_3$) C, H, N.

(*R*)-91 *(R)-N-Cyclohexyl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 245 °C. Yield: 62%. MS: *m*/*z* 327.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (br d, *J* = 7.8 Hz, 1H), 8.82 (s, 1H), 7.89–7.85 (m, 1H), 7.46–7.33 (m, 2H), 4.82 (m, 1H), 4.45–4.36 (m, 2H), 3.81 (m, 1H), 1.88–1.28 (m, 13H). Anal. ($C_{19}H_{22}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = +81 (*c* = 0.01, CH_3OH).

(*S*)-91 *(S)-N-Cyclohexyl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 245 °C. Yield: 69%. MS: *m*/*z* 327.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (br d, *J* = 7.8 Hz, 1H), 8.82 (s, 1H), 7.89–7.85 (m, 1H), 7.42–7.37 (m, 2H), 4.82 (m, 1H), 4.49–4.37 (m, 2H), 3.81 (m, 1H), 1.88–1.28 (m, 13H). Anal. ($C_{19}H_{22}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = −81 (*c* = 0.01, CH_3OH).

(*R*)-95 *(R)-(+)-N-Cycloheptyl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 165 °C. Yield: 72%. MS: *m*/*z* 355.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.09 (br d, *J* = 7.8 Hz, 1H), 8.79 (s, 1H), 7.89–7.84 (m, 1H), 7.45–7.35 (m, 2H), 4.71–4.60 (m, 2H), 4.36–4.29 (m, 1H), 4.07–4.01 (m, 1H), 1.87–1.56 (m, 14H), 0.92 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = +100 (*c* = 0.01, $CHCl_3$).

(*S*)-95 *(S)-(-)-N-Cycloheptyl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 165 °C. Yield: 54%. MS: *m*/*z* 355.0 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.09 (br d, *J* = 7.8 Hz, 1H), 8.79 (s, 1H), 7.89–7.84 (m, 1H), 7.45–7.32 (m, 2H), 4.70–4.60 (m, 2H), 4.36–4.29 (m, 1H), 4.07–4.03 (m, 1H), 1.87–1.57 (m, 14H), 0.92 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = −100 (*c* = 0.01, $CHCl_3$).

Synthesis of *N*-Adamantyl-, *N*-Alkyl-, and *N*-Heteroaryl-3,7-dihydro-2/3-substituted-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamides

To a stirred solution of the respective carboxylic acid (**62a**–**g**,**i**,**j**, **68a**–**i**, 0.2 mmol) in dry DMF (3 mL) was added diisopropylethylamine (0.8 mmol). The resulting solution was stirred at room temperature for 10 min before addition of HBTU (0.3 mmol) and then stirred for another 3 h. 1-Aminoadamantane, 2-aminoadamantane (for **87**), or 1-amino-3,5-dimethyladamantane (for **95**) (0.3 mmol) was then added, and the solution was stirred for 16 h. DMF was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate and successively washed with water-saturated sodium bicarbonate, water, and brine. The organic phase was dried over anhydrous sodium sulfate, evaporated, and finally purified by flash chromatography using ethyl acetate/petroleum ether (8/2) as the eluent.

72 *N-Adamant-1-yl-3,7-dihydro-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: >300 °C. Yield: 71%. MS: *m*/*z* 441.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.89 (br s, 1H), 8.59 (s, 1H), 7.95–7.90 (m, 1H), 7.45–7.34 (m, 5H), 7.16–7.11 (m, 2H), 5.96 (m, 1H); 4.92–4.58 (m, 2H), 2.03 (s, 9H), 1.65 (s, 6H). Anal. ($C_{28}H_{28}N_2O_3$) C, H, N.

73 *3,7-Dihydro-N-(3,5-dimethyladamant-1-yl)-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 264 °C. Yield: 63%. MS: *m*/*z* 469.5 (M + H). ^{1}H NMR (DMSO-d_6): 9.92 (br s, 1H), 8.56 (s, 1H), 7.95–7.90 (m, 1H), 7.45–7.37 (m, 5H), 7.17–7.12 (m, 2H), 5.95 (m, 1H), 4.82–4.59 (m, 2H), 2.10–1.59 (m, 7H), 1.39–1.14 (m, 6H), 0.83 (s, 6H). Anal. ($C_{30}H_{32}N_2O_3$) C, H, N.

75 *N-Adamant-1-yl-3,7-dihydro-2-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: >300 °C. Yield: 71%. MS: *m*/*z* 455.6 (M + H). ^{1}H NMR (DMSO-d_6): 9.85 (br s, 1H), 8.62 (s, 1H), 7.95–7.91 (m, 1H), 7.52–7.33 (m, 5H), 7.11–7.06 (m, 2H), 5.82 (d, J = 2.8 Hz, 1H), 4.83–4.78 (m, 1H), 2.02 (s, 9H), 1.64 (s, 6H), 1.20 (d, J = 6.2 Hz, 3H). Anal. ($C_{29}H_{30}N_2O_3$) C, H, N.

76 *N-Adamant-1-yl-3,7-dihydro-7-oxo-2,3-diphenyl-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 260 °C. Yield: 45%. MS: *m*/*z* 517.3 (M + H). ^{1}H NMR (DMSO-d_6): 9.86 (br s, 1H), 8.73 (s, 1H), 8.02–7.98 (m, 1H), 7.58–7.54 (m, 2H), 7.33–7.13(m, 8H), 6.71–6.66 (m, 2H), 6.25 (d, *J* = 2.8 Hz, 1H), 5.96 (d, *J* = 2.8 Hz, 1H), 2.04 (s, 9H), 1.65 (s, 6H). Anal. ($C_{34}H_{32}N_2O_3$) C, H, N.

78 *N-Adamant-1-yl-3,7-dihydro-7-oxo-3-(4-methylphenyl)-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 171 °C. Yield: 59%. MS: *m*/*z* 455.8 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.89 (br s, 1H), 8.53 (s, 1H), 7.94–7.89 (m, 1H), 7.45–7.34 (m, 2H), 7.21 (d, *J* = 8.00 Hz, 2H), 7.06 (d, *J* = 8.00 Hz, 2H), 5.88 (m, 1H), 4.79–4.51 (m, 2H), 2.29 (s, 3H), 2.03 (s, 9H), 1.65 (s, 6H). Anal. ($C_{29}H_{30}N_2O_3$) C, H, N.

80 *N-Adamant-1-yl-3,7-dihydro-9-methyl-7-oxo-3-phenyl-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: >300 °C. Yield: 48%. MS: *m*/*z* 455.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.93 (br s, 1H), 8.55 (s, 1H), 7.73 (s, 1H), 7.39–7.36 (m, 3H), 7.20 (s, 1H), 7.14–7.10 (m, 2H), 5.94 (m, 1H), 4.82–4.53 (m, 2H), 2.43 (s, 3H), 2.03 (s, 9H), 1.65 (s, 6H). Anal. ($C_{29}H_{30}N_2O_3$) C, H, N.

83 *N-Adamant-1-yl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 282 °C. Yield: 55%. MS: *m*/*z* 379.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.95 (br s, 1H), 8.77 (s, 1H), 7.88–7.84 (m, 1H), 7.45–7.33 (m, 2H), 4.82 (m, 1H), 4.52–4.23 (m, 2H), 2.07 (s, 9H), 1.67 (s, 6H), 1.42 (d, *J* = 6.8 Hz, 3H). Anal. ($C_{23}H_{26}N_2O_3$) C, H, N.

86 *N-Adamant-1-yl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 219 °C. Yield: 66%. MS: *m*/*z* 393.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.93 (br s, 1H), 8.74 (s, 1H), 7.88–7.83 (m, 1H), 7.41–7.34 (m, 2H), 4.65–4.59 (m, 2H), 4.39–4.12 (m, 1H), 2.07 (s, 9H), 1.72 (m, 2H), 1.67 (s, 6H), 0.92 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{24}H_{28}N_2O_3$) C, H, N.

87 *N-Adamant-2-yl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 240 °C. Yield: 45%. MS: *m*/*z* 393.1 (M + H). ^{1}H NMR (DMSO-d_6): 10.61 (d, *J* = 8.4 Hz, 1H), 8.81 (s, 1H), 7.92–7.87 (m, 1H), 7.46–7.32 (m, 2H), 4.70–

4.60 (m, 2H), 4.36–4.30 (m, 1H), 4.13 (d, *J* = 8.4 Hz, 1H), 1.98–1.60 (m, 16H), 0.96 (t, *J* = 7.2 Hz 3H). Anal. ($C_{24}H_{28}N_2O_3$) C, H, N.

88 *3,7-Dihydro-3-ethyl-N-(5-methylhexan-2-yl)-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 240 °C. Yield: 24%. MS: *m*/*z* 357.1 (M + H). ^{1}H NMR (DMSO-d_6): 10.61 (d, *J* = 8.4 Hz, 1H), 8.80 (s, 1H), 7.88–7.80 (m, 1H), 7.48–7.34 (m, 2H), 4.78–4.66 (m, 2H), 4.40–4.28 (m, 1H), 4.12–3.88 (m, 1H), 1.83–1.40 (m, 5H), 1.18–1.11 (m, 5H), 0.88–0.83 (m, 9H). Anal. ($C_{21}H_{28}N_2O_3$) C, H, N.

89 *3,7-Dihydro-3-ethyl-7-oxo-N-pyridin-4-yl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 280 °C. Yield: 44%. MS: *m*/*z* 336.2 (M + H). ^{1}H NMR (DMSO-d_6): 12.68 (br s, 1H), 9.00 (s, 1H), 8.50–8.47 (m, 2H), 7.97–7.93 (m, 1H), 7.74–7.70 (m, 2H), 7.54–7.39 (m, 2H), 4.83–4.64 (m, 2H), 4.42–4.31 (m, 1H), 1.96–1.65 (m, 2H), 0.95 (t, *J* = 7.6 Hz, 3H). Anal. ($C_{19}H_{17}N_3O_3$) C, H, N.

90 *3,7-Dihydro-3-ethyl-7-oxo-N-thiazol-2-yl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 221 °C. Yield: 58%. MS: *m*/*z* 342.2 (M + H). ^{1}H NMR (DMSO-d_6): 13.62 (s, 1H), 9.05 (s, 1H), 7.96–7.94 (m, 1H), 7.54–7.28 (m, 4H), 4.78–4.65 (m, 2H), 4.43–4.3 (m, 1H), 1.92–1.61 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{17}H_{15}N_3O_3S$) C, H, N.

94 *N-Adamant-1-yl-3,7-dihydro-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 218 °C. Yield: 43%. MS: *m*/*z* 407.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.93 (br s, 1H), 8.71 (s, 1H), 7.86–7.84 (m, 1H), 7.42–7.32 (m, 2H), 4.71 (m, 1H), 4.61–4.58 (m, 1H), 4.33–4.30 (m, 1H), 2.06 (s, 9H), 1.78 (m, 2H), 1.67 (s, 6H), 1.43–1.21 (m, 2H), 0.89 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{25}H_{30}N_2O_3$) C, H, N.

95 *3,7-Dihydro-N-(3,5-dimethyladamant-1-yl)-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 219 °C. Yield: 48%. MS: *m*/*z* 435.7 (M + H). ^{1}H NMR (DMSO-d_6): 9.96 (br s, 1H), 8.68 (s, 1H), 7.87–7.83 (m, 1H), 7.45–7.31 (m, 2H), 4.79–4.57 (m, 2H), 4.33 (m, 1H), 2.12–1.15 (m, 17H), 0.92–0.85 (m, 9H). Anal. ($C_{27}H_{34}N_2O_3$) C, H, N.

97 *N-Adamant-1-yl-3,7-dihydro-8-methyl-7-oxo-3-propyl-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 237 °C. Yield: 53%. MS: *m*/*z* 421.3 (M + H). ^{1}H NMR (DMSO-d_6): 9.94 (br s, 1H), 8.62 (s, 1H), 7.17–7.05 (m, 2H), 4.85–4.60 (m, 2H), 4.22 (m, 1H), 3.43–

3.36 (m, 4H), 2.77 (s, 3H), 2.06 (s, 9H), 1.67 (s, 6H), 0.88 (m, 3H). Anal. ($C_{26}H_{32}N_2O_3$) C, H, N.

100 *N-Adamant-1-yl-3-cyclopropyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 245 °C. Yield: 55%. MS: *m*/*z* 405.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.94 (br s, 1H), 8.88 (s, 1H), 7.89–7.85 (m, 1H), 7.42–7.36 (m, 2H), 4.63–4.18 (m, 2H), 4.04–3.97 (m, 2H), 2.07 (s, 9H), 1.68 (s, 6H), 1.33–1.15 (m, 1H), 0.66–0.51 (m, 4H). Anal. ($C_{25}H_{28}N_2O_3$) C, H, N.

101 *N-Adamant-1-yl-3,7-dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 220 °C. Yield: 62%. MS: *m*/*z* 407.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.90 (br s, 1H), 8.68 (s, 1H), 7.85–7.81 (m, 1H), 7.42–7.28 (m, 2H), 4.79–4.73 (m, 1H), 4.42–4.24 (m, 2H), 2.04 (s, 10H), 1.92 (m, 1H), 1.65 (s, 6H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.75 (d, *J* = 6.6 Hz, 3H). Anal. ($C_{25}H_{30}N_2O_3$) C, H, N.

102 *N-Adamant-1-yl-3,7-dihydro-3,3-dimethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

Pale white solid. Mp: 223 °C. Yield: 26%. MS: *m*/*z* 393.1 (M + H). ^{1}H NMR ($CDCl_3$): 9.98 (br s, 1H), 8.95 (s, 1H), 8.10–8.05 (m, 1H), 7.41–7.23 (m, 2H), 4.12 (s, 2H), 2.19–2.11 (m, 9H), 1.73 (s, 6H), 1.62 (s, 6H). Anal. ($C_{24}H_{28}N_2O_3$) C, H, N.

103 *N-Adamant-1-yl-3-ethyl-10-fluoro-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 232 °C. Yield: 65%. MS: *m*/*z* 411.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.84 (s, 1H), 8.77 (s, 1H), 7.90–7.82 (m, 1H), 7.48–7.38 (m, 1H), 4.75–4.64 (m, 2H), 4.39–4.32 (m, 1H), 2.06 (s, 9H), 1.82–1.74 (m, 2H), 1.67 (s, 6H), 0.93 (t, *J* = 7.6 Hz, 3H). Anal. ($C_{24}H_{27}FN_2O_3$) C, H, N.

104 *N-Adamant-1-yl-9,10-difluoro-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 262 °C. Yield: 50%. MS: *m*/*z* 429.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.78 (s, 1H), 8.79 (s, 1H), 7.77–7.68 (m, 1H), 4.82–4.68 (m, 2H), 4.45–4.38 (m, 1H), 2.06 (s, 9H), 1.82–1.78 (m, 2H), 1.67 (s, 6H), 0.92 (t, *J* = 7.6 Hz, 3H). Anal. ($C_{24}H_{26}FN_2O_3$) C, H, N.

(*R*)-83 *(R)-(+)-N-Adamant-1-yl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 282 °C. Yield: 56%. MS: *m*/*z* 379.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.95 (br s, 1H), 8.77 (s, 1H), 7.88–7.84 (m, 1H), 7.45–7.33 (m, 2H), 4.82 (m, 1H), 4.52–

4.23 (m, 2H), 2.07 (s, 9H), 1.67 (s, 6H), 1.42 (d, J = 6.8 Hz, 3H). Anal. ($C_{23}H_{26}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = +69 (c = 0.01, $CDCl_3$).

(*S*)-83 *(S)-(-)-N-Adamant-1-yl-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 282 °C. Yield: 50%. MS: *m*/*z* 379.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.95 (br s, 1H), 8.77 (s, 1H), 7.88–7.84 (m, 1H), 7.45–7.33 (m, 2H), 4.82 (m, 1H), 4.52–4.23 (m, 2H), 2.07 (s, 9H), 1.67 (s, 6H), 1.42 (d, J = 6.8 Hz, 3H). Anal. ($C_{23}H_{26}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = −69 (c = 0.01, $CDCl_3$).

(*R*)-86 *(R)-(+)-N-Adamant-1-yl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 219 °C. Yield: 69%. MS: *m*/*z* 393.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.94 (br s, 1H), 8.75 (s, 1H), 7.88–7.83 (m, 1H), 7.41–7.34 (m, 2H), 4.65–4.59 (m, 2H), 4.39–4.12 (m, 1H), 2.07 (s, 9H), 1.72 (m, 2H), 1.67 (s, 6H), 0.92 (t, J = 7.4 Hz, 3H). Anal. ($C_{24}H_{28}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = +142 (c = 0.01, $CHCl_3$).

(*S*)-86 *(S)-(-)-N-Adamant-1-yl-3,7-dihydro-3-ethyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxamide*

White solid. Mp: 219 °C. Yield: 53%. MS: *m*/*z* 393.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.94 (br s, 1H), 8.75 (s, 1H), 7.88–7.83 (m, 1H), 7.45–7.31 (m, 2H), 4.65–4.59 (m, 2H), 4.39–4.12 (m, 1H), 2.07 (s, 9H), 1.77 (m, 2H), 1.68 (s, 6H), 0.92 (t, J = 7.4 Hz, 3H). Anal. ($C_{24}H_{28}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = −142 (c = 0.01, $CHCl_3$).

(*R*)-101 *(R)-(+)-N-Adamant-1-yl-3,7-dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino [2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 212 °C. Yield: 47%. MS: *m*/*z* 407.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.90 (br s, 1H), 8.68 (s, 1H), 7.86–7.81 (m, 1H), 7.42–7.28 (m, 2H), 4.79–4.73 (m, 1H), 4.42–4.24 (m, 2H), 2.04 (s, 10H), 1.92 (m, 1H), 1.65 (s, 6H), 0.98 (d, J = 6.6 Hz, 3H), 0.76 (d, J = 6.6 Hz, 3H). Anal. ($C_{25}H_{30}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = +42 (c = 0.01, CH_3OH).

(*S*)-101 *(S)-(-)-N-Adamant-1-yl-3,7-dihydro-3-isopropyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 220 °C. Yield: 53%. MS: *m*/*z* 407.5 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.90 (br s, 1H), 8.68 (s, 1H), 7.85–7.81 (m, 1H), 7.42–7.28 (m, 2H), 4.79–4.73 (m, 1H), 4.42–4.24 (m, 2H), 2.04 (s, 10H), 1.92 (m, 1H), 1.65 (s, 6H), 0.99 (d, J = 6.6 Hz, 3H), 0.75 (d, J = 6.6 Hz, 3H). Anal. ($C_{25}H_{30}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = −42 (c = 0.01, CH_3OH).

(*R*)-108 *(R)-(+)-N-Adamant-1-yl-3,7-dihydro-3-isobutyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 130 °C. Yield: 28%. MS: *m*/*z* 421.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.92 (br s, 1H), 8.67 (s, 1H), 7.88–7.83 (m, 1H), 7.45–7.32 (m, 2H), 4.82–4.75 (m, 1H), 4.63–4.57 (m, 1H), 4.35–4.24 (m, 1H), 2.07 (s, 10H), 1.68 (s, 6H),1.44 (m, 1H), 0.99 (d, *J* = 5.8 Hz, 3H), 0.90 (d, *J* = 5.8 Hz, 3H). Anal. ($C_{26}H_{32}N_2O_3$) C, H, N.

(*R*)-109 *(R)-(+)-N-Adamant-1-yl-3-benzyl-3,7-dihydro-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 153 °C. Yield: 55%. MS: *m*/*z* 455.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.85 (br s, 1H), 8.50 (s, 1H), 7.89–7.85 (m, 1H), 7.43–7.19 (m, 7H), 4.99 (m, 1H), 4.41–4.23 (m, 2H), 3.04 (d, *J* = 7.4 Hz, 2H), 2.03 (s, 9H), 1.66 (s, 6H). Anal. ($C_{29}H_{30}N_2O_3$) C, H, N. $[\alpha]_D^{25}$ = +190 (*c* = 0.01, CH_3OH).

Synthesis of *N*-Adamant-1-yl-3,7-dihydro-3-ethyl-10-methoxy-7-oxo-2*H*-[1,4] oxazino[2,3,4-*ij*]quinoline-6-carboxamide (105)

A solution of *N*-adamant-1-yl-3,7-dihydro-3-ethyl-10-fluoro-7-oxo-2*H*-[1,4]oxazino [2,3,4-*ij*]quinoline-6-carboxamide (**103**; 0.14 mmol) in dry THF (3 mL) was added to a solution of freshly prepared CH_3ONa (0.28 mmol) under a nitrogen atmosphere. The reaction mixture was heated at 50 °C for 16 h and neutralized using 1 N HCl. The mixture was concentrated in vacuo and the residue extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The crude residue was purified by chromatography using petroleum ether/ethyl acetate (1/1) as the eluent.

105 *N-adamant-1-yl-3,7-dihydro-3-ethyl-10-methoxy-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide*

White solid. Mp: 279 °C. Yield: 33%. MS: *m*/*z* 423.1 (M + H). ^{1}H NMR ($CDCl_3$): δ 9.98 (br s, 1H), 8.59 (s, 1H), 8.07 (d, *J* = 9 Hz, 1H), 7.12 (d, *J* = 9 Hz, 1H), 4.65–4.59 (m, 1H), 4.30–4.21 (m, 1H), 4.19 (m, 1H), 4.02 (s, 3H), 2.16–2.10 (m, 9H), 1.91–1.88 (m, 2H), 1.73 (m, 6H), 1.05 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{25}H_{30}N_2O_3$) C, H, N.

Synthesis of *N*-Adamant-1-yl-3,7-dihydro-3-ethyl-7-oxo-10-pyrrolidin-1-yl-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide (106)

K_2CO_3 (1 mmol) was added to a mixture of *N*-adamant-1-yl-3,7-dihydro-3-ethyl-10-fluoro-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide (**103**; 0.36 mmol), pyrrolidine (2.5 mmol), and anhydrous DMF (5 mL). The resulting mixture was stirred at 100 °C for 10 h. The solvent was removed under vacuum, the residue dissolved in ethyl acetate (30 mL), and the organic layer washed with water (5 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, evaporated, and purified by flash chromatography using ethyl acetate/petroleum ether (3/7) as the eluent.
Pale white solid. Mp: 282 °C. Yield: 50%. MS: *m*/*z* 462.4 (M + H). ^{1}H NMR ($CDCl_3$): δ 10.01 (br s, 1H), 8.50 (s, 1H), 7.71 (d, *J* = 9 Hz, 1H), 6.80 (d, *J* = 9.2 Hz, 1H), 4.532–4.38 (m, 2H), 4.14–4.07 (m, 1H), 3.51 (m, 4H), 2.06 (s, 9H), 1.96–1.73 (m, 6H), 1.66 (s, 6H), 0.96 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{28}H_{35}N_3O_3$) C, H, N.

Synthesis of *N*-Adamant-1-yl-3,7-dihydro-3-ethyl-7-oxo-10-(4-methylpiperazin-1-yl)-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide (107)

K_2CO_3 (1 mmol) was added to a mixture of *N*-adamant-1-yl-3,7-dihydro-3-ethyl-10-fluoro-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide (**103**; 0.36 mmol), 1-methylpiperazine (2.5 mmol), and anhydrous DMF (5 mL). The resulting mixture was stirred at 100 °C for 10 h. The solvent was removed in vacuo, the residue dissolved in ethyl acetate (30 mL), and the organic layer washed with water (5 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, evaporated, and purified by flash chromatography using ethyl acetate/petroleum ether (3/7) as the eluent.
White solid. Mp: 294 °C dec. Yield: 40%. MS: *m*/*z* 491.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.95 (s, 1H), 8.61 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 4.75–4.67 (m, 2H), 4.45–4.38 (m, 1H), 3.25–3.11 (m, 4H), 2.49–2.46 (m, 4H), 2.21 (s, 3H), 2.03 (s, 9H), 1.84–1.69 (m, 2H), 1.65 (s, 6H), 0.93 (t, *J* = 7.4 Hz, 3H).

Synthesis of *N*-Adamant-1-yl-3-ethyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]-oxazino[2,3,4-*ij*]quinoline-6-carboxamide Hydrochloride (107·HCl)

A solution of *N*-adamant-1-yl-3-ethyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-7*H*-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (**107**, 0.04 mmol) in 1,4-dioxane saturated with gaseous hydrogen chloride (4 mL) was stirred at 0 °C for 30 min. The solvent was concentrated in vacuo, and the residue was suspended in diethyl ether (10 mL), filtered, and washed with cold diethyl ether. Pale yellow solid. Mp: 240 °C dec. Yield: 69%. MS: *m*/*z* 527.8 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.48 (br s, 1H), 9.94 (s, 1H), 8.67 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 4.68–4.57 (m, 2H), 4.30–4.25 (m, 1H), 3.70–2.88 (m, 11H), 2.05 (m, 9H), 1.67 (m, 8H), 0.92 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{29}H_{39}ClN_4O_3$) C, H, N.

Refereneces

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