Preparation of bicyclic nitrogen intermediates as useful scaffolds for the synthesis of new biologically active compounds

PhD Thesis of
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Chapter 1. The orexin system, general overview and aim of the project
1.1 The Orexin System Pharmacology

1.1.1 Introduction

The orexins (also called hypocretins) are neuropeptides that were first discovered in 1998 by two independent research groups, utilizing different methodologies. Sakurai et al. named these peptides orexin-A and -B because they were originally thought to promote feeding (the term orexin comes from the Greek word orexis that means appetite).\(^1\) The team led by de Lecea et al. named the peptides hypocretin-1 and hypocretin-2 because they are produced in the hypothalamus and have some similarities to the incretin family of peptides.\(^2\)

The Sakurai et al. research team reported the identification of orexins by “reverse pharmacological” approach. Most neuropeptides work through G Protein-Coupled Receptors (GPCRs) and there are many “orphan” GPCR genes in the human genome (the related ligands for these receptors molecules have not been identified yet). Sakurai et al. team transfected cells with orphan GPCR genes in order to obtain their expression. These transfected cells were subsequently used as reporter systems in order to detect endogenous ligands in tissue extracts. In this process orexin A and orexin B were identified as endogenous peptidic ligands for two orphan GPCRs: orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R).\(^1\)

The group of de Lecea et al. utilized a technique based on subtractive-PCR to identify transcripts that are specifically expressed in the hypothalamus.\(^2\) Previously, they had obtained a library of cDNAs corresponding to a number of mRNA sequences selectively expressed in this region of the brain.\(^3\) From this library, they subsequently identified one cDNA sequence that contains the entire coding region of a putative secretory protein of 131 amino acids. They predicted that this protein gives rise to two novel peptide products that are structurally related to each other (hypocretin-1 and -2).

These findings led to establish that the orexin system is composed of two endogenous peptides (Orexin A and Orexin B), that mediate their actions via interactions with two closely related GPCRs (OXR1 and OXR2). The orexins are produced by specialized neurons in the hypothalamus, which project to many different regions of the brain.\(^4\)

Physiological studies over the last decade showed that orexin signalling plays a key role in a variety of important biological processes, including feeding, sleep-wake cycle, energy homeostasis,

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addiction and reward seeking, amongst others. In fact, there is clear evidence for defects in orexin signalling being involved in diet-induced obesity and diabetes, narcolepsy, panic anxiety disorder, drug addiction and Alzheimer’s Disease. These evidences justify the considerable efforts of pharmaceutical industries to develop new potent compounds active on the orexinergic system.

In particular, it has become clear that the effects of orexin peptides on arousal and sleep are profound. In fact, narcolepsy, one of the most common causes of sleepiness, is caused by a loss of the orexin-producing neurons. This consideration has fueled a strong interest in developing orexin antagonists as a novel approach for promoting sleep and treating insomnia.

Almost all hypnotics used so far in the clinic enhance γ-aminobutyric acid (GABA) signaling or alter monoamine signaling. Unfortunately these neurotransmitters affect numerous brain functions, and therefore these drugs can cause several side effects, such as unsteady gait and confusion. In contrast, orexin antagonists are expected to promote sleep with fewer side effects, and recent, large clinical studies look so promising that the first OX1R/OX2R antagonist Suvorexant has been recently approved for insomnia in USA (Aug 2014).

Another important evidence is the role of the orexins in mediating the effects of several drugs of abuse, such as cocaine, morphine and alcohol via projections to key brain regions. So at present, there are several orexin-based pharmacotherapies under development for the treatment of addiction.

1.1.2 Overview of orexin signaling

**Orexin Peptides**

As previously stated, molecular cloning studies showed that orexin A and orexin B are derived from a common precursor peptide, prepro-orexin encoded by the hypocretin gene (Human: HCRT; Rat/Mouse: Hcrt) located on chromosome 17q21, Figure 1.

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Figure 1. Orexins and orexin receptors. (a) Sequences of orexin A (identical in all studied mammalian species) and of orexin B in various species. The topology of the two intrachain disulfide bonds of orexin A is indicated above the sequence. Shadows indicate amino acid identity. (The picture is taken from TRENDS in Pharmacological Sciences 2011, note the pyroglutamic N-terminal, whereas usually the structure is reported with the unmodified N-terminal Gln residue, see for instance panel b and Figure 2). (b) OX-A and OX-B are encoded by the HCRT gene. Structures of the human gene [from UCSB genome browser (http://genome.ucsc.edu); intronic sequence is shown at 1/10th scale of exon sequence], mRNA, and protein gene products shown (picture elaboration from Pharmacol Rev 64:389–420, 2012).

The structure and organization of the hypocretin gene has been largely conserved through evolution. In all vertebrates examined, the gene is composed of two exons with the intron splice falling within the early portion of the open reading frame encoding the secretory signal sequence. The HCRT gene is expressed in the hypothalamus, where the mRNA is translated to the prepro-orexin precursor peptide (131-amino acid for human prepro-orexin peptide) that is proteolytically processed to give rise to orexin A (OX-A; 33 amino acids; 3562 Da) and Orexin B (OX-B; 28 amino acids; 2937 Da).

Structures of orexins were chemically determined by biochemical purification and sequence analysis by Edman sequencing and mass spectrometry. In mammals, the sequence of the mature OX-A ligand is entirely conserved among all species examined and contains two disulphide bonds, formed by [Cys\(^6\)-Cys\(^{12}\), Cys\(^7\)-Cys\(^{14}\)]. Mature OX-A is further post-translationally modified with an N-terminal pyroglutamic acid.

Mammalian OX-B sequences, on the other hand, are also very well conserved but are not identical across the species. In fact, in dogs, cows, sheeps, and pigs a proline residue substitutes for the serine residue present in humans in the second amino acid position. Moreover, in rodents besides this variation, another residue differs from the human sequence (asparagine 18, for serine 18).

It is interesting to notice that there is some sequence similarity between OX-A and OX-B, this homology could explain the relative overlapping of their pharmacological functions, i.e. their ability to serve as ligands for both OX1 and OX2 receptors, albeit with differing affinities.

Indeed, it should be emphasized that mammalian OX-A and OX-B sequences are identical in the C-terminal portion of the mature peptides, including the peptidic sequence Gly-Asn-His-Ala-Ala-Gly-
Ile-Leu-Thr. They also share the sequence Arg-Leu-X-X-Leu-Leu (X = non-conserved amino acids) spaced three amino acids N-terminal of the conserved peptidic sequence mentioned above. This evidence suggests that these residues may exist at one surface of an α-helical secondary structure.\textsuperscript{1,11} Because both peptides have measurable affinities for each of the OX1 and OX2 receptors, these observations indicate that these residues are essential for orexin receptor interaction. This feature is shown in Figure 2 (http://www.rcsb.org/pdb/home/home.do).

![Figure 2](http://www.rcsb.org/pdb/home/home.do). Representation of OXA and OxB peptidic sequences (From Protein Data Bank: [http://www.rcsb.org/pdb/home/home.do](http://www.rcsb.org/pdb/home/home.do); PDB ID: 1r02 and 1cq0).

The x-ray structures of both orexin A and orexin B peptides were resolved ([Protein Data Bank](http://www.rcsb.org/pdb/home/home.do)). A graphic representation of the PDB files of orexin peptides as cartoon was performed using [PyMol\textsuperscript{™}] program, Figure 3.

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Figure 3. (a) OXA (blue) and OXB (orange) peptides. (b) OxA/OXB Alignment. The peptidic sequences are shown as cartoon and disulphide bonds in Orexin A as spheres. PDB files (1r02 and 1cq0) elaboration with PyMol™ 1.5.0.3 program.

Orexin Receptors

The orexin peptides bind selectively to the OX1 and OX2 receptors (OX1R and OX2R, also known as HCRTR1 and HCRTR2).\textsuperscript{1} As already mentioned, these are GPCRs having, as usual for this class of receptors, 7-transmembrane domains and some similarity to other neuropeptide receptors (see below). All pharmacological actions of orexins seem to be mediated by these two receptors. Among various classes of GPCRs, OX1R is structurally more similar to certain neuropeptide receptors, in particular to the Y2 Neuropeptide Y (NPY) receptor (26% similarity), followed by the thyrotropin-releasing hormone (TRH) receptor, choleystokinin type-A receptor and NK2 neurokinin receptor.

In spite of these structural similarities to other neuropeptide receptors, neither OX1R nor OX2R has any significant affinity for neuropeptide Y, secretin, or similar peptides.\textsuperscript{1,12} The amino acid identity between the deduced full-length human OX1R and OX2R sequences is 64%. Thus, the similarity between the two orexin receptors is considerably higher than that with the other GPCRs listed above.\textsuperscript{1} OX1R and OX2R are strongly conserved across mammals: amino acid identities between the human and rat homologues are 94% for OX1R and 95% for OX2R, Table 1 and Table 2.\textsuperscript{1}

Human OXA has nearly equal activity on both orexin receptors, with ligand binding affinities (IC50) of 20 and 38 nM for OX1R and OX2R, respectively, and EC50 values of 30 and 34 nM in [Ca2+]i mobilization assays of CHO cells transfected to express human OX1R and OX2R, respectively, Figure 4.11 OX-B, however, has markedly less activity toward OX1R with an IC50 of 420 nM and an EC50 of 2500 nM. It is, therefore, somehow selective for OX2R, exhibiting an IC50 of 36 nM and EC50 of 60 nM.1 This selectivity of OXB for OX2R has been used to interpret the relative roles of OX2R and OX1R in biological functions. Definitive receptor selective function, however, is demonstrated only with genetic and/or highly selective orexin receptor antagonist reagents.

Further, recent studies showed molecular interactions and cross-talks between orexin receptors and other receptors. Hilairet et al. showed that when the cannabinoid receptor (CB1) and OX1R are co-expressed, there is a CB1-dependent enhancement of the orexin A potency to activate precise intracellular signal-transduction pathways. It was also shown that CB1 and OX1R are closely apposed at the plasma membrane to form heterodimers.13 It was also showed that OX1R and OX2R are capable of forming a homo- or heterodimer.14 These observations suggest complex signaling cascade might exist in the downstream of orexin receptors.

**Signal Transduction Systems of Orexin Receptors**

Numerous studies have shown that orexins depolarize neurons and increase excitability and ignition rate for many minutes.\(^{15}\) In general it is thought that OX1R couples to Gq, and OX2R can signal through Gq or Gi/Go, but coupling mechanisms seem to be different by cell type and have not been thoroughly examined in neurons, Figure 5.\(^{16}\)

![Figure 5](image)

**Figure 5.** Orexin signaling mechanisms. Orexin-A signals through both OX1R and OX2R, whereas orexin-B signals mainly through OX2R. Intracellular cascades mediated by G proteins increase intracellular calcium and activate the Na+/Ca2+ exchanger, which depolarizes target neurons. These cascades also inactivate G protein–regulated inward rectifier (GIRK) channels. Increased expression of NMDA receptors on the cell surface produces long-lasting increases in neuronal excitability (picture from *Annu. Rev. Pharmacol. Toxicol.* 2011 February 10; 51: 243–266).

Several ionic mechanisms mediate the acute effects of orexins. An inhibition of K-channels (including G protein-regulated inward rectifier -GIRK- channels) may make more excitable some neurons.\(^{17}\) Further, the orexin receptor signalling can induce a sustained and fast intracellular Ca\(^{2+}\) gain by voltage-gated Ca-channels, by transient receptor potential channels or from intracellular stores.\(^{1,18}\) Lastly, the Na\(^{+}/Ca^{2+}\) exchanger activation can contribute to the target neurons excitation.\(^{19}\) Further to these postsynaptic events, orexin can work at presinaptic level on nerve terminals to induce GABA or glutamate release. This generates more complex effects on downstream neurons.\(^{16,18a}\)

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Orexin-mediated signalling can also give enduring increasing in neuronal excitability: in the ventral tegmental area (VTA), orexin increases the NMDA receptors numbers on the cell surface (neurons more reactive to the glutamate excitatory effect for hours). Through this pathway, the orexins may generally excite neurons that promote arousal. The neurons that release the orexin neurotransmitters also produce glutamate, dynorphin and other dynorphin-related neuropeptides. These co-neurotransmitters may be physiologically remarkable, but less is known about their roles or conditions under which they are released.

1.2 Medicinal Chemistry Approach

The great importance of the orexinergic system in the research projects aimed to find new drugs active on CNS is witnessed by the large number of medicinal chemistry projects in this field. For instance, the database Integrity (Thompson Reuters), consulted in November 2014, reports more than 200 leads.

The R&D activity on orexin antagonists involves either the big pharma companies and smaller biotech group. The following chart represents the major player found in an Integrity query on “Orexin”.

![Pie Chart illustrating the competition in R&D development in orexin antagonists (Source: Thomson Reuters Integrity, Nov 2014).](image1)

**Figure 1.** Pie Chart illustrating the competition in R&D development in orexin antagonists (Source: Thomson Reuters Integrity, Nov 2014).

As previously illustrated, while sleep disorders are the principal clinical field of investigation, the role of orexinergic system in controlling other aspects of behavior is mirrored by the following scheme, produced again by Integrity, illustrating the conditions targeted by the leads.

![Pie Chart illustrating the major clinical conditions targeted by the orexin antagonists in development (Source: Thomson Reuters Integrity, Nov 2014).](image2)

**Figure 2.** Pie Chart illustrating the major clinical conditions targeted by the orexin antagonists in development (Source: Thomson Reuters Integrity, Nov 2014).
Since the pharmacology of OX1R is not fully superimposable to that of OX2R (and the same is for OXA and OXB) - for instance, OX1Rs seem to have a predominant role in the control of anxiety, panic and addiction, while OX2Rs seem predominantly involved in sleep control – the R&D projects on these targets are almost evenly divided into three main classes, the dual orexin receptor antagonists (DORAs) representing approx. the 44% of the lead projects listed in Integrity, and the selective orexin receptor antagonists (SORAs) towards OX1R or OX2R, 26% and 30%, respectively. The scope of this thesis is on DORAs, since they are the most promising agents for treating insomnia. Indeed, Suvorexant, a DORA compound, is the first orexin antagonist approved for the market for treating sleep disorders (August 2014), and, therefore we tried to ameliorate some pharmacological features of Suvorexant that emerged during its clinical development and that induced the FDA to lower its posology. Nevertheless, in a brief summary of the state of the art of orexinoergic medicinal chemistry, it is worth to mention also the efforts made to obtain SORAs (mainly targeting OX2R) as hypnotic compounds devoid of the putative side effects of non-selective antagonists.

The following table, Table 1, taken from a recent paper by Khoo et al., summarizes very well the evolution of the research efforts in the orexinoergic field.

Table 1 taken from Khoo et al., CNS Drugs (2014) 28, 713–730

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Company</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA (17-33)</td>
<td>SmithKline Beecham</td>
<td>Truncated orexin-A peptide with reduced potency but enhanced selectivity for the OX₁ receptor. Partial agonist</td>
</tr>
<tr>
<td>[Ala¹¹,D-Leu¹⁵] Orexin B (SB-69875)</td>
<td>Banyu</td>
<td>Substituted orexin-B peptide with enhanced selectivity for the OX₂ receptor. Agonist</td>
</tr>
<tr>
<td>SB-334867</td>
<td>GlaxoSmithKline</td>
<td>SORA. MW 319.11, Research only. First selective OX₂ receptor antagonist</td>
</tr>
<tr>
<td>SB-408124</td>
<td>GlaxoSmithKline</td>
<td>SORA. MW 356.14, Research only. Selective OX₁ receptor antagonist</td>
</tr>
<tr>
<td>SB-674042</td>
<td>GlaxoSmithKline</td>
<td>SORA. MW 448.51, Research only. Selective OX₂ receptor antagonist</td>
</tr>
<tr>
<td>ACT-335827</td>
<td>Actelion</td>
<td>SORA. MW 518.64, Research only. Selective OX₁ receptor antagonist</td>
</tr>
<tr>
<td>TCS OX2 29</td>
<td>Banyu</td>
<td>SORA. MW 397.24, Research only. First small molecule selective OX₂ receptor antagonist</td>
</tr>
<tr>
<td>JNJ-10397049</td>
<td>Johnson and Johnson</td>
<td>SORA. MW 481.98, Research only. Selective OX₁ receptor antagonist</td>
</tr>
<tr>
<td>EMPA</td>
<td>Roche</td>
<td>SORA. MW 510.23, Research only. Selective OX₁ receptor antagonist</td>
</tr>
<tr>
<td>LSN2424100</td>
<td>Eli Lilly</td>
<td>SORA. MW 407.42, Research only. Selective OX₂ receptor antagonist</td>
</tr>
<tr>
<td>MK-1064</td>
<td>Merck</td>
<td>SORA. MW 461.83, Clinical candidate. Selective OX₂ receptor antagonist</td>
</tr>
<tr>
<td>Almorexant (ACT-07573)</td>
<td>Actelion and GlaxoSmithKline</td>
<td>DORA. MW 512.23, Treatment for insomnia. Reached Phase III trials before development was discontinued</td>
</tr>
<tr>
<td>SB-649868</td>
<td>GlaxoSmithKline</td>
<td>DORA. MW 477.15, Treatment for insomnia. Reached Phase II trials before development was discontinued</td>
</tr>
<tr>
<td>Filorexant (MK-6098)</td>
<td>Merck</td>
<td>DORA. MW 430.2, Treatment for insomnia. Currently under development (Phase II). No active clinical trials</td>
</tr>
<tr>
<td>Suvorexant (MK-4305)</td>
<td>Merck</td>
<td>DORA. MW 450.16, Treatment for insomnia. Phase III trials complete, awaiting FDA and PDEA approval. Proposed addition to Schedule IV by the United States Drug Enforcement Agency</td>
</tr>
</tbody>
</table>

MW molecular weight

Being OXA and OXB neuropeptides, the first logical approaches towards new ligands acting at OX1R and/or OX2R as agonists or antagonists were with truncated and/or modified orexin peptides. These attempts gave origin to promising tools, such as OXA (17–33), which is a partial agonist at the OX1 receptor and [Ala^{11}, D-Leu^{15}]-Orexin B (SB-668875), in which the substitution of two residues in the orexin-B peptide produced, a potent agonist with increased selectivity for the OX2 receptor.

However, the most important expansion of our knowledge of orexinergic pharmacology has been produced with small molecule antagonists. The prototype was SB-334867 (developed by GlaxoSmithKline), a naphthyridine-substituted diarylurea with high affinity for the OX1 receptor (Selective Orexin Receptor 1 Antagonist; SORA 1) without relevant cross-activity towards 50 other targets (GPCRs and ion channels). However, its selectivity for OX1R is only approximately 50-fold higher than that for OX2R (Haynes et al., 2000; Porter et al., 2001), at higher doses, SB-334867 is likely to block both orexin receptors, moreover it has demonstrated binding activity with a number of other receptors and transporters, complicating its use as an orexin receptor probe. This OX1R/OX2R selectivity is therefore, relatively modest compared to more recently developed antagonists. GlaxoSmithKline has developed further several commercially available small molecule OX1 receptor antagonists: in particular SB-408124 and SB-674042. SB-408124 is urea-based small molecule, like SB-334867, that has a similar dissociation constant (Kb) for the OX1 receptor, 21.7 nM and 27.8 nM, respectively. SB-408124 Kb on OX2R is, on the other hand, 1410 nM. SB-674042, in contrast to the two ureas, has a proline-like core with two 5-membered heteroaromatic rings and two phenyl groups. It is significantly more potent than both SB-334867 and SB-408124, with a Kb of 1.1 nM for the OX1 receptor and 129 nM for the OX2 receptor: it is also a more selective OX1 receptor antagonist than either of its predecessors. More recently, Actelion Pharmaceuticals has reported a tetrahydropapaverine derivative OX1 receptor antagonist designated ACT-335827. It is about 10 times more selective towards OX1R respect the OX2R, with Kb values of 41 and 560 nM respectively. It is orally bioavailable, able to cross the blood brain barrier (BBB).

The structure of the selective orexin receptor 1 antagonists (SORAs) described above are reported in Figure 3.

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The small molecules selective OX2 receptor antagonists (SORA-2), in contrast, were developed by a different group of companies and have different structures. The first, TCS-OX2-29, is a tetrahydroisoquinoline reported in 2003 and developed by Banyu Pharmaceutical Co., a branch of Merck Sharpe & Dohme. It is highly selective for the OX2 receptor (IC₅₀ of 40 nM), while having no effect at the OX1 receptor (above µM range). Subsequently was discovered JNJ-10397049 (Johnson&Johnson), a phenyl-dioxanyl urea compound with 600-fold selectivity for the OX2 receptor.

In 2013 it was reported by literature EMPA (Hoffman-La Roche): it contain an acetamidic and sulfonamidic groups in a branched structure. It inhibits OX2 receptor in the nanomolar range, while inhibit OX1 receptor only over µM concentrations. Furthermore, Eli Lilly developed LSN2424100, that present a sulfonamide as structural motif with aromatic/heteroaromatic substitutions. LSN2424100 has highly activity towards the OX2R with a Kb of 0.44 nM, while show less activity for the OX1R with a Kb value of 90.3 nM (about 200-fold selective at OX2R). LSN2424100 had also shown antidepressant activity in mice and rats.

Merck has reported developing a SORA 2 (MK- 1064) for insomnia, this product is reported in phase I clinical studies.

The structure of the SORAs described were reported in Figure 4.

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Several Dual Orexin Receptor Antagonist (DORAs, Figure 5), that bind both the orexin receptors, have entered in the clinical trials in the last years, mainly for the treatment of insomnia. The most representative are Almorexant, SB-649868 (GlaxoSmithKline), Filorexant and Suvorexant (Merk). Almorexant is a tetrahydroisoquinoline, but presents additional substitutions respect TCS-OX2-29 (SORA-2) and it was developed for the treatment of insomnia.\textsuperscript{15}

After in depth studies emerged that Almorexant may interact with the transmembrane domines 3 and 5-7 of both OXRs (is the same domain predicted to interact with the endogenous orexin peptides).\textsuperscript{16} Almorexant completed Phase III clinical trials in 2009, however its development was stopped (by Actelion and GlaxoSmithKline) in 2011 due to the side effects profile.\textsuperscript{17} Almorexant continued to be used in research as standard to development of new class of orexin antagonists.

GlaxoSmithKline also conduct independently the development of SB-649868 (Kb OX1R = 0.32 nM; Kb OX2R = 0.40 nM)

SB-649868 structure is based on a piperidine core functionalized by amidic bounds with heteroaromatic moieties.\textsuperscript{18}

This compounds showed sleep-promoting effect in rats, good tolerability and half-life in clinical trials.\textsuperscript{19} After the achievement of the Phase II clinical trials its development was stopped.\textsuperscript{20}


Filorexant and Suvorexant are two DORAs developed by Merck that have reached the clinical trails for the treatment of insomnia. Filorexant (MK-6096) is based on a 2-methyl-piperidine scaffold and showed to promoting sleep in rats, mice and dogs.\textsuperscript{21} Filorexant was employed in clinical trials not only for the treatment of insomnia, but also to treat migraine, painful diabetic neuropathy and adjunctive therapy for depression.\textsuperscript{22a-d} The most important DORA developed from Merck is Suvorexant, a substituted diazepam, which has completed three Phase III clinical trials and was approved for sale (trade name Belsomra) by the U. S. Food & drug Administration in August 13\textsuperscript{th}, 2014.\textsuperscript{22e}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{dorastuctures.png}
\caption{Dual Orexin Receptor Antagonists (DORAs)}
\end{figure}

Chapter 2. Chemistry
2.1 Introduction

In this PhD project we concentrate our efforts in designing new DORAs compounds, trying to ameliorate some pharmacological features of Suvorexant, emerged during its clinical development, that induced the FDA to lower its posology.

Some of the orexin receptor antagonists known in literature, contain as a fundamental motif the ethyldiaminic sequence NCCN. For example, Suvorexant (developed by Merck) contains the methyl-diazepine core. SB-649868 developed by GlaxoSmithKline is based on the piperidin-2-ylmethanamine moiety and it has been evaluated in Phase II clinical trials for sleep disorders, Figure 1.

So, we planned to insert the NCCN motif in a bicyclic framework such as the norbornane in order to obtain a markedly rigid structure and to confer specific features to the final derivatives. Three distinct NCCN-containing scaffolds were proposed at the beginning of the Ph.D. project, namely the TYPE I, TYPE II and TYPE III scaffolds reported in Figure 1 and the chemical studies started with an accurate investigation of the literature concerning the synthesis of the three identified scaffolds.

![Figure 1](image)

2.1.1 TYPE I scaffold (2-azabicyclo[2.2.1]heptan-3-ylmethanamine)

After a detailed research in the literature, we found that the TYPE I scaffold was already successfully employed for the preparation of several molecules with orexin antagonism activity by the Actelion Pharmaceuticals research group. In particular, in a patent registered in 2009, the authors describe the synthesis and the biological properties of a series of compounds with the

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general formula depicted in Figure 2. These compounds are in general substituted at the endocyclic nitrogen atom with aryl or heteroaryl (rings A and B, Figure 2), linked to the bicyclic core through an amide linkage. Beside, the exocyclic nitrogen bears heteroaryl substituents (ring $R^1$) that may be linked to the nitrogen atom directly or via an amide bond.

![Figure 2](image-url)

All synthesized compounds were tested as antagonists of human orexin-1 and orexin-2 receptors expressed by engineered cells. Best antagonistic activities (IC$_{50}$ values) of tested compounds were 6 nM with respect to the OX1 receptor and 10 nM with respect to OX2 receptor. Upon this finding, that increases the goodness of our working hypothesis, we decided to focus the research only on TYPE II and TYPE III scaffolds.

### 2.1.2 TYPE II scaffold (7-azabicyclo[2.2.1]heptan-2-amine)

The 7-azabicyclo[2.2.1]heptan-2-amine exists as a couple of diastereoisomers, namely compounds *endo* and *exo* in Figure 3, and each diastereoisomer as a couple of enantiomers.

![Figure 3](image-url)
Thus, we started our investigation on TYPE II scaffold realizing the diastereoselective synthesis of both endo and exo compounds as racemic mixtures. The complete synthesis is reported in Scheme 1.²

**Scheme 1**

The synthetic route involved in the first step a thermal Diels-Alder reaction between methyl 3-bromopropionate (obtained by bromination of methyl propionate with N-bromosuccinimide) and tert-butyl 1H-pyrrole-1-carboxylate, giving rise to the first key intermediate 1. Hydrogenation of 1

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with hydrogen and palladium on carbon, in presence of triethylamine as base, gave the 7-tert-butyl 2-methyl 7-azabicyclo[2.2.1]heptane-2,7-dicarboxylate (2). Compound 2 was isolated as a racemic endo/exo mixture in about 88:12 ratio from $^1$H NMR analysis and represents the common intermediate for the synthesis of both targeted compounds.

Thus, starting from intermediate 2 the TYPE II scaffold with the endo-stereochemistry was prepared following the synthetic route depicted in Scheme 1, path A. Hydrolysis of the methyl ester 2 with lithium hydroxide in THF/water at room temperature furnished quantitatively the free acid 3. Compound 3 preserves the same endo/exo ratio of the starting material 2. Then, in a one-pot reaction, the acid 3 was transformed firstly into the corresponding acylazide in the presence of diphenylphosphoryl azide. In the second step of the one-pot reaction, thermal Curtius rearrangement of the acylazide, in the presence of benzyl alcohol as trapping reagent for the isocyanide intermediate, gave the N-Cbz-protected compound 4 in 80% yield. At this stage of the synthesis, flash chromatographic purification of the crude allowed for the isolation of pure 4 in (+/-)-endo configuration. A final hydrogenation step, performed with hydrogen at 50 psi, in ethanol at room temperature and in the presence of Pd/C (1%), provided the Cbz protecting group cleavage, giving rise to the (+/-)-endo-tert-butyl 2-amino-7-azabicyclo[2.2.1]heptane-7-carboxylate (5) in quantitative yield.

The adopted synthetic strategy to obtain the exo-TYPE II scaffold is depicted in Scheme 1, path B. Intermediate 2 in (+/-)-endo/exo mixture was subjected to complete inversion to (+/-)-exo free acid derivative 6 by treatment with sodium methoxide in refluxing methanol at room temperature (yield 53%). Then, the (+/-)-exo-tert-butyl 2-amino-7-azabicyclo[2.2.1]heptane-7-carboxylate (8) was obtained following the same synthetic strategy described for the endo compound 5.

The structures of the obtained compounds were assigned on the basis of combined 1D ($^1$H NMR, $^{13}$C NMR) and 2D NMR (COSY, HSQC, NOESY) experiments. In particular, the analysis of 1D and 2D experiments allowed for the complete assignment of proton and carbon chemical shifts and coupling constants. The endo/exo stereochemistry at carbon 2 was assigned on the basis of coupling constant values. For illustration, for compounds 3 and 6 the relevant resonance assignments and measured coupling constants for $^1$H NMR are reported in Figure 4.
In compound 3 the angular hydrogen 1 appears as a triplet with a coupling constant of 4.5 Hertz with both equatorial hydrogens 2 and 6. The coupling constant of hydrogen 1 with axial hydrogen 6 is zero. The proton spectra of 3 shows as impurity a 15% of the corresponding exo compound 6.

In compound 6 the angular hydrogen 1 appears as a doublet with a coupling constant of 4.5 Hertz with the equatorial hydrogen 6. The coupling constant of hydrogen 1 with both axial hydrogens 2 and 6 is zero. At the same time in compound 3 the signal of the hydrogen 2 is a double triplet and the same hydrogen in compound 6 is a double doublet with the coupling constant 2-1 equal to zero.

Measured $^3J$ coupling constants are in good agreement with calculated $^3J$ coupling constant ranges, achieved from dihedral angles values by Karplus equation, see Table 1.
**Table 1.** Measured $^1$H–$^1$H coupling constant versus calculated $^1$H–$^1$H coupling constant range (derived from dihedral angles).

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>3</th>
<th>3</th>
<th>6</th>
<th>6</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3J$ H-1/H-2</td>
<td>$\Phi$</td>
<td>$J$ (Hz) exp.</td>
<td>$J$ (Hz) calc.</td>
<td>$\Phi$</td>
<td>$J$ (Hz) exp.</td>
<td>$J$ (Hz) calc.</td>
</tr>
<tr>
<td>$^3J$</td>
<td>41.1$^\circ$</td>
<td>4.5</td>
<td>4.5-5.1</td>
<td>83.5$^\circ$</td>
<td>0</td>
<td>0-1.0</td>
</tr>
<tr>
<td>$^3J$ H-1/H-6ax</td>
<td>79.7$^\circ$</td>
<td>0</td>
<td>0-1.0</td>
<td>80.3$^\circ$</td>
<td>0</td>
<td>0-1.0</td>
</tr>
<tr>
<td>$^3J$ H-1/H-6eq</td>
<td>48.3$^\circ$</td>
<td>4.5</td>
<td>3.5-3.9</td>
<td>40.2$^\circ$</td>
<td>4.5</td>
<td>4.7-5.3</td>
</tr>
</tbody>
</table>

### 2.1.3 Functionalization of TYPE II scaffold (7-azabicyclo[2.2.1]heptan-2-amine)

With both **endo** and **exo** compounds 5 and 8 in hand, we started the search for the more suitable substituents for both endocyclic and exocyclic nitrogen atoms. On the basis of the rationale evaluation of the orexin antagonists known in literature, a “**template structure**” was adopted in order to carry out the **endo**/**exo** TYPE II scaffold rational exploration, Figure 5. The template bears a heteroaromatic ring (ring A in Figure 5) linked to the exocyclic nitrogen atom and a double aromatic/heteroaromatic system (rings B-C in Figure 5) linked through an amide bond to the endocyclic nitrogen atom of the scaffold.

![Figure 5](image)

The functionalization of both nitrogen atoms of compounds 5 and 8 with aromatic and/or heteroaromatic rings allowed for the preparation of a small library of new compounds, Figure 6. Each moiety, A or B-C, was analyzed and modified separately, fixing the other ring, in order to have a direct comparison with the compounds of the same series and a better understanding of the **SAR (Structure Activity Relationship)** after biological activity evaluation.

We started the synthetic work with the exploration of the **endo**-TYPE II scaffold 3. The ring A was the first substituent analyzed, maintaining as B-C moiety the 2-methyl-5-phenylthiazole-4-carbonyl substituent. Five compounds (9a-e) with the ring A ri-elaboration were prepared, Figure 6a. Subsequently, we started with the B-C moiety rational investigation fixing as ring A the 5-(trifluoromethyl)pyridin-2-yl group and we synthesized six derivatives (9f-k), Figure 6b.
The adopted synthetic pathway is reported in Scheme 2 and consisted in the nucleophilic displacement of the appropriate heteroaryl halide (A-X, X = fluoride or chloride) with intermediate in presence of a base (TEA or K$_2$CO$_3$) in dry DMF at 75-100°C affording compounds 10a-k in 45-68% yields. After nearly quantitative $N$-Boc cleavage in the presence of TFA, the intermediates 11a-k were reacted with the selected acids under classical amide coupling conditions in the presence of 2-chloro-4,6-dimethoxy-1,3,5-triazine as carboxylic activating agent. The eleven final products 9a-k were obtained as (+/-)-endo racemic mixture in 55-83% yields.
Reactants and conditions: (a) heteroaryl halides A-X (where X= F or Cl), TEA or K2CO3, DMF, 75-100°C, 44.5-68%; (b) DCM/TFA 4:1, Rt, 89-98%; (c) C-B-COOH, 2-chloro-4,6-dimethoxy-1,3,5-triazine, N-methylmorpholine, Rt then 100°C, 55-83%.

Scheme 2

Then we started the exploration of (+/-)-exo-TYPE II scaffold planning the synthesis of nine compounds (12a-i), Figure 7, and choosing the substituent at the nitrogen atoms with the same rationale adopted in the design of compounds 9a-k.

Figure 7

The synthetic pathway adopted for the synthesis of the exo-TYPE II derivatives was the same used for the endo-TYPE II analogues (reaction conditions and yields are reported in Scheme 3). The nine final products 12a-i were obtained as (+/-)-exo racemic mixture.
Reaction conditions: (a) heteroaryl halides A-X (where X= F or Cl), K$_2$CO$_3$, DMF dry, 85-100°C, 44-68%; (b) phenylacetyl-halide A-X (where X= Cl), pyridine, DCM, Rt, 73%; (c) DCM/TFA 4:1, Rt, 90-quant %; (d) C$_8$-COOH, 2-chloro-4,6-dimethoxy-1,3,5-triazine, N-methylmorpholine, Rt then 100°C, 72-88%.

Scheme 3

Compounds 9 and 12 are diastereoisomers and their structures were assigned on the basis of combined 1D ($^1$H NMR, $^{13}$C NMR) and 2D-NMR (COSY, HSQC, NOESY) experiments. The $^1$H NMR analysis of compounds 9 and 12 reveals the existence in solution of two equilibrating species, two rotamers, for both series of compounds, in 1:1 ratio for 9 and 7:3 ratio for 12. Rotamers are conformational isomers where interconversion by rotation around a single bond is restricted and an energy barrier has to be overcome in order to convert one conformer to another. When this rotation strain barrier is high enough to allow for the isolation of the conformers then the isomers become atropoisomers. The presence of two rotamers complicates the appearance of $^1$H NMR as well as $^{13}$C NMR spectra and variable temperature (VT) NMR is the generally preferred method for studying the equilibration of the rotamers. In fact, at high temperature the two rotamers equilibrate and average simple spectra can be recorded at the coalescence temperature. Nevertheless, in our case working in DMSO-$_d_6$ at 90 °C, the rotation barrier could not be overwhelmed. Recently, Ley and coworkers$^3$ reported a simple NMR experiment that permits to identify protons that are in mutual chemical exchange. The technique involves a 1D gradient NOE experiment in which a selected peak is irradiated during the experiment acquisition and looks like a negative peak (or a null peak depending on settled parameters) in the final spectrum. At the same time also those protons experiencing chemical exchange with the irradiated protons will appear as negative or null peaks in the final spectrum. This experiment is particularly useful also to distinguish between rotamers and diastereoisomers. In our case the two species present in solution are undoubtedly rotamers because none of the frequency of 9 appear in the spectra of its diastereoisomer 12. Moreover, 1D gradient NOE experiment performed for compound 12e (rotamers ratio 7:3) clearly show the existence of two species in chemical exchange process. In Figure 8 the chemical shifts of the protons irradiated in the 1D gradient NOE experiments are reported for both the rotamers.

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Moreover, as clearly shown in the picture in Figures 9, 10 and 11, saturation, respectively, of H-1, H-3\textsubscript{ax} and H-2 proton signals of the major rotamer result in the disappearance of the corresponding resonances of the minor rotamer. This demonstrates that the two detected species are in mutual chemical exchange.

Figure 8

Chemical shifts of diagnostic signals for major and minor* rotamers used in 1D gradient NOE experiments

Figure 9

\textsuperscript{1}H NMR 12e
H-1 irradiated

Arrows show the H-1 chemical shifts of both rotamers
$^1$H NMR 12e
H-3$_{ax}$ irradiated

$^3$H NMR 12e
Arrows show the H-3$_{ax}$ chemical shifts of both rotamers

Figure 10
Moreover, the accurate analysis of 1D and 2D experiments allowed for the complete assignment of proton and carbon chemical shifts and coupling constants for both rotamers of the two series of synthesized compounds (9 and 12). The endo/exo stereochemistry at carbon 2 was assigned on the basis of coupling constant values. For illustration, for compounds 9k and 12i the relevant resonance assignments and measured coupling constants for $^1$H NMR are reported in Figure 12.
In Figure 12 the signals of one rotamer are normally numbered and the second one is marked with an asterisk. In compound 9k the angular hydrogen 1 appears for both rotamers as a triplet with a coupling constant of 4.5 Hertz with both equatorial hydrogens 2 and 6 The coupling constant of hydrogen 1 with axial hydrogen 6 is zero.

In compound 12i the angular hydrogen 1 appears as a doublet with a coupling constant of 4.5 Hertz with the equatorial hydrogen 6. The coupling constant of hydrogen 1 with both axial hydrogens 2 and 6 is zero. As already reported for compounds 3 and 6, measured $^3J$ coupling constants are in good agreement with calculated $^3J$ coupling constant ranges, achieved from dihedral angles values by Karplus equation.

**Figure 12**

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In Figure 12 the signals of one rotamer are normally numbered and the second one is marked with an asterisk. In compound 9k the angular hydrogen 1 appears for both rotamers as a triplet with a coupling constant of 4.5 Hertz with both equatorial hydrogens 2 and 6. The coupling constant of hydrogen 1 with axial hydrogen 6 is zero.

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2.1.4 TYPE III scaffold (2-azabicyclo[2.2.1]heptan-6-amine)

Also the 2-azabicyclo[2.2.1]heptan-6-amine exists as a couple of diastereoisomers, namely compounds \textit{exo} and \textit{endo} in Figure 13, and each diastereoisomer as a couple of enantiomers.

![Figure 13](image)

The complete synthesis of TYPE III scaffold in both (\textit{+/-}-exo- and (\textit{+/-}-endo- configurations is depicted in Scheme 4.)
**Reactants and conditions:** (a) benzylamine hydrochloride, formaldehyde 37%Wt, then cyclopentadiene, water, Rt, 82%; (b) 1) borane-dimethylsulfide complex 2M in THF solution, THF, Rt, 2) NaOH/H₂O₂, THF/Water, 40°C, 49.5%. **Path A:** (c) phthalimide, triphenylphosphine; 1,2-ethoxycarbonyl diazene 40% in Toluene, THF, 0°C then Rt, 72%; (d) hydrazine monohydrate, MeOH/THF 1:1, 65°C, 94%. **Path B:** (e) Pd-C/H₂, 30 psi, BocO, EtOH, Rt, 87%; (f) oxalyl chloride, dimethylsulfoxide, TEA, DCM, -78 then Rt; 94%; (g) benzylamine, sodium triacetoxyhydroborate, 1,2-DCE; Rt; 94%; (h) Pd-C/H₂ 1atm, EtOH, Rt, 83%.

**Scheme 4**

The common intermediate for the synthesis of both desired compounds is the 2-benzyl-2-azabicyclo[2.2.1]heptan-6-ol 16 which was prepared in a two step sequence starting with the azadiels-Alder reaction in water, at room temperature between cyclopentadiene, formaldehyde and benzylamine hydrochloride giving rise to the azanorbornene 15 in 82% yield. Subsequently, hyroboration of 15, followed by a H₂O₂ oxidative workup give the corresponding alcohol 16 in 50% yield as a single regioisomer in (+/-)-exo-configuration, with only trace amounts of the (+/-)-endo alcohol derivative.

Starting from compound 16, the exo-TYPE III scaffold 18 was then prepared following the synthetic pathway depicted in Scheme 4, path A. The first step involves a Mitsunobu reaction with retention

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of configuration giving rise to the (±)-exo-17 in 72% yield. The Mitsunobu reaction usually proceeds with inversion at the carbon atom of the reacting secondary alcohol. This inversion occurs in the final step of the reaction when the reacting nucleophile (in our case the imide) proceeds with inversion at the carbon atom of the reacting secondary alcohol. This inversion of configuration giving rise to the (±)-exo-17 in 72% yield.

The Mitsunobu reaction usually involves the racemic mixture. The group with hydrogen (1 atm) and Pd/C afforded the final compound 5b. The free amino group was finally obtained via phtalimide cleavage with hydrazine to give the desired (±)-exo-18 in 94% yield.

Scheme 5

However, as described by several authors the reaction can occur with complete racemization of the stereocenter through a mechanism involving a carbocationic intermediate instead of an oxyphosphonium ion. Moreover, the intramolecular stabilization of a real or incipient carbocation can influence the stereochemical outcome of the reaction. Probably, in our reaction, the formation of an incipient carbocation at C-6, stabilized by the adjacent nitrogen atom, is responsible for the formation of the observed product with retention of configuration, Scheme 5b. The free amino group was finally obtained via phtalimide cleavage with hydrazine to give the desired (±)-exo-18 in 94% yield.

The adopted synthetic strategy to obtain the endo-TYPE III scaffold 22 is depicted in Scheme 4. Starting from intermediate 16 one-pot nitrogen protecting group exchange from benzyl group to Boc group afforded the (±)-exo-compound 19 in 87% overall yield. Then the hydroxyl group was converted to the corresponding ketone 20 in 94% yield by Swern oxidation. Finally, reductive amination with benzylamine in the presence of sodium triacetoxyhydroborate gave the derivative (±)-endo-21 in 94% yield with excellent selectivity. Reductive cleavage of the benzyl group with hydrogen (1 atm) and Pd/C afforded the final endo-compound 22 in 83% yield as (±)-racemic mixture.

Performing the synthesis of compounds 18 and 22 the appropriate choice of the protecting groups at the endocyclic nitrogen atoms is of outstanding importance in several steps. For instance, the regioselectivity in the oxidative hydroboration reaction is almost complete only using a benzyl residue as protecting group, changing from benzyl to tert-butoxy carbonyl group resulted in the isolation of 1:1 mixture of the two possible regioisomers. Moreover, the benzyl group is essential in the Mitsunobu reaction providing the correct electronic properties to the nitrogen atom stabilizing the incipient carbocation at C-6. On the other hand, for the synthesis of 22 starting from 16, the presence of a benzyl group on N-1 would give, after Swern oxidation ad reductive amination with benzylamine (the better reactant for this reaction), an intermediate with the same protecting group at both nitrogens. On the contrary, mismatched substitution at the two nitrogen atoms is necessary to achieve selective deprotection to compound 22 for the subsequent...

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functionalization. For these reasons a protecting group exchange was realized as first step for the synthesis of 22 starting from 16.

The structures of the obtained compounds were assigned on the basis of combined 1D (1H NMR, 13C NMR) and 2D NMR (COSY, HSQC, NOESY) experiments. In particular, the analysis of 1D and 2D experiments allowed for the complete assignment of proton and carbon chemical shifts and coupling constants. The endo/exo stereochemistry at carbon 2 was assigned on the basis of coupling constant values and NOE interactions. For illustration, for compounds 18 and 22 diagnostic NOE interactions and relevant coupling constant values are reported in Figure 14.

As already reported, the coupling constant between H-1 and H-6 is different from zero only in the endo compound 22. Moreover, NOE interactions between H-6 and both benzylic and aromatic protons in 18 and between H-6 and H-7 in 22 allows for the discrimination of the two diastereoisomers.

2.1.5 Functionalization of TYPE III scaffold (2-azabicyclo[2.2.1]heptan-6-amine)

The functionalization of both nitrogen atoms of compounds 18 and 22 with aromatic and/or heteroaromatic rings was performed following the same template approach reported for TYPE II scaffolds, Figure 15. However in this case we prepared only two derivatives (23a-b) of the exo-18 compound and five derivatives (24a-e) of the endo-22 which would be the most active stereoisomer on the basis of computational studies performed in parallel with the synthetic work (see Chapter 3).
The synthetic pathways employed to obtain compounds 23a-b and 24a-e are summarized in Scheme 6 and parallel those reported for compounds 9 and 12.

Reactants and conditions: (a) 2-fluoro-5-(trifluoromethyl)pyridine, K$_2$CO$_3$, DMF dry, 100°C, 75%; (b) Pd-C/H$_2$ 5 psi, EtOH, Rt, 89%; (c) C-B-COOH, 2-chloro-4,6-dimethoxy-1,3,5-triazine, N-methylmorpholine, Rt then 100°C, 65-76%; (d) 2-fluoro-5-(trifluoromethyl)pyridine, K$_2$CO$_3$, DMF dry, 100°C, 88%; (e) DCM/TFA 4:1, Rt, quant; (f) C-B-COOH, 2-chloro-4,6-dimethoxy-1,3,5-triazine, N-methylmorpholine, Rt then 100°C, 34-79%.

Scheme 6
The structures of the obtained compounds were assigned on the basis of combined 1D (1H NMR, 13C NMR) and 2D-NMR (COSY, HSQC, NOESY) experiments. The 1H NMR analysis of compounds 23 and 24 reveals the existence of two rotamers for both series of compounds, in 7:3 ratio for 23 and in ratios ranging from 97:3 to 80:20 for 24. In particular, the analysis of 1D and 2D experiments allowed for the complete assignment of proton and carbon chemical shifts and coupling constants. The endo/exo stereochemistry at carbon 2 was assigned on the basis of coupling constant values and nOe interactions. For illustration, for compounds 23a and 24a diagnostic NOE interactions and relevant coupling constant values are reported in Figure 16.

![Figure 16](image)

**2.1.6 Preparative chiral HPLC separation**

After biological evaluation assays (see Chapter 4) the most active compounds resulted to pertain, respectively, to the exo-**TYPE II** and endo-**TYPE III** series. In particular, in the first series compound 12a and in the second series compound 24b resulted to be the best candidates and were subjected to chiral preparative HPLC separation in order to evaluate the activity of the two enantiomers, Figure 14. The preparative chromatography was performed for both 12a and 24b on a Chiralpak ADH column (25x2cm, 5μm), eluent heptane (10%) and ethanol (90%) added with diethyleneamine (0.1%), flow rate 10 mL/min. The collected fractions were analyzed by chiral analytical HPLC on a ADH column (250x4.6mm, 5μm), eluent heptane (10%) and ethanol (90%) added with diethyleneamine (0.1%), flow rate 5 mL/min, giving rise to the result summarized in Figure 17.
**Figure 17**

**Compound 12e/E1**
- rt 11.5', optical purity 100%
- $[\alpha]_D = +49.4$

**Compound 12e/E2**
- rt 13.3', optical purity 98.6%
- $[\alpha]_D = -47.6$

**Compound 24b/E1**
- rt 7.5', optical purity 100%
- $[\alpha]_D = -98.6$

**Compound 24b/E2**
- rt 11.4', optical purity 97.3%
- $[\alpha]_D = +96.3$
2.2 General Methods

All the reactions, that involve the use of reagents sensitive to oxygen or hydrolysis, were carried out under inert atmosphere. The glassware was previously dried in an oven at 110 °C and set with cycles of vacuum and nitrogen. Also syringes, used to transfer reagents and solvents, were previously set under nitrogen atmosphere.

When the purity of the obtained product is not indicated means purity >95% (purity determined by 1HNMR and UPLC-Uv detector).

The aromatic and/or heteroaromatic substituents (moieties A and B-C) not commercially available employed for the TYPE II and TYPE III scaffolds functionalization are all note in literature. Their synthesis and characterization are not reported.

Reagents and solvents

Unless otherwise state reagents, solvents and dry solvents were purchased from commercial suppliers and used as received.

Chromatography/purification of compounds

The chromatographic column separations were conducted by automated flash technique, using Isolera-One Biotage apparatus with Bitage HP-SI or KP-NH columns for direct phase separation or RediSep Rf Gold AqC18 columns for reverse phase separations.

For the basic compounds extraction was employed Biotage ISOLUTE SCX columns.

For thin-layer chromatography (TLC), Biotage KP-SIL TLC plates or KP-NH TLC Plates were employed and the detection was performed by irradiation with UV light (λ = 254 nm), by basic solution of KMnO$_4$ (3.0 g KMnO$_4$, 20.0 g K$_2$CO$_3$ and 0.3 g KOH in 300 mL of H$_2$O), ninhydrin, Pancaldi solution or with iodine vapours.

NMR spectroscopy

$^1$H NMR analyses were performed with a Varian-Gemini 200 or with Bruker 300, 400 or 500 Avance spectrometers at room temperature, respectively at 200, 300, 400 or 500 MHz. The coupling constants ($J$) are expressed in Hertz (Hz), the chemical shifts (δ) in ppm. The multiplicities of the proton spectra were described by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), dq (double quartet), dt (double triplet), td (triple doublet), ddd (double double doublet).

$^{13}$C NMR analyses were performed with the same instruments at 50.3, 75.45, 100 and 125.75 MHz; APT sequences were used to distinguish the methine and methyl carbon signals from those arising
from methylene and quaternary carbon atoms. DEPT sequences were adopted for the same purpose.

**Mass spectrometry**

Low resolution MS spectra were recorded by an Acquity UPLC – Waters apparatus with ESI source, with automated sampling system. The values are expressed as mass-charge ratio and the relative intensities of the most significant peaks are shown in brackets.

**HPLC analysis**

The HPLC analysis and the chiral resolution of selected compounds were performed with an Agilent 1200 HPLC and a Shimadzu LC8A assembled HPLC.

**Polarimetry**

Perkin Elmer 342 plus was employed for the analysis of optically active compounds.

**Abbreviations**

- Ac  Acetyl
- AcOEt Ethyl Acetate
- Alk  Alkyl
- APT  Attached Proton Test
- Ar  Aryl
- Boc  t-Butyloxycarbonyl
- brs  Broad signal
- Bu  Butyl
- °C  Celsius degree
- CDMT  2-chloro-4,6-dimethoxy-1,3,5-triazine
- COSY  Correlation spectrometry
- cm  Centimetre
- Cy  Cyclohexyl
- CyHex  Cyclohexane
- δ  Chemical shift
- d  Doublet
- DA  Diels-Alder
- dd  Double doublet
- d.e.  Diastereomeric excess
- DEPT  Distortionless Enhancement by Polarization Transfer
- ddd  Double double doublet
- DME  1,2-Dimethoxyethan
- DCM  Dichloromethane
- DMF  Dimethylformamide
- dt  Double triplet
- ED  Electron-donating
- EI  Electron collision ionization
eq  Equivalents  
ESI  Electronspray ionization  
Et  Ethyl  
eV  Electronvolt  
EW  Electron-withdrawing  
g  Gram  
h  hours  
Hex  Hexyl  
HP-SI High performance silica gel  
Hz  Hertz  
IR  Infrared spectrometry  
i-Pr iso-Propyl  
J  Coupling constant  
L  Ligand  
LA Lewis Acid  
m  Multiplet  
m- meta  
M  Molar  
[M+]  Molecular ion peak  
Me  Methyl  
MeOH Methanol  
mg Milligram  
MHz Megahertz  
min Minutes  
mL Millilitre  
mmol Millimol  
m.p. Melting point  
MS Mass spectrometry  
m/z Mass / Load  
NMM N-methylmorpholine  
N Normal  
NMR Nuclear magnetic resonance  
NOE(SY) Nuclear Overhauser Effect  
o- ortho  
p- para  
Pent Pentyl  
Ph Phenyl  
PMB p-methoxybenzyl  
ppm parts per million  
PPTS Pyridinium p-toluensulphonate  
Pr Propyl  
Py Pyridine  
p-Tol p-Tolyl  
q Quartet  
rt room temperature  
s Singlet  
sat. sol. Saturated solution  
SI Silica gel
T  Temperature
 t  Triplet
 t  Time
 td  Triple doublet
 tert  tertiary
 t-Bu  tert-Butyl
 THF  Tetrahydrofuran
 TFA  Trifluoroacetic acid
 TLC  Thin layer chromatography
 Wt  Weight
2.3 Synthesis of (+/-)-endo- and (+/-)-exo-7-azabicyclo[2.2.1]heptan-2-amine (5 and 8), experimental data

(+/-)-7-tert-buty 2-methyl 3-bromo-7-azabicyclo[2.2.1]hepta-2,5-diene-2,7-dicarboxylate (1)

Step 1: To a stirred solution of methyl propiolate (7.57 mL, 85 mmol) in acetone (50 mL), cooled at 0°C, silver nitrate (1.445 g, 8.50 mmol) and then NBS (18.16 g, 102 mmol) were added. The reaction mixture was stirred at 0 °C for 10 min and then at room temperature for 1 h. The reaction was monitored by 1H NMR analysis (the disappearance of the acetylenic proton was checked, 2.70 δ in CDCl₃). The sample was filtered before the NMR analysis.

Then the reaction mixture was filtered over a celite pad, washing with acetone (20 mL), and the filtrate was concentrated under reduced pressure at room temperature to obtain a final concentration of about 15% Wt (70 mL).

The final concentration of the solution was determined via 1H MNR analysis.

Methyl 3-bromopropiolate= 28.58% Wt, 13.33%mol.

Grams of the solution = 48.83 g.

Grams of methyl 3-bromopropiolate= 13.96 g (theoretical amount = 13.85 g).

1H NMR (400 MHz, CDCl₃) δ = 3.71 (s, 3 H).

Step 2: To the previously obtained solution of methyl 3-bromopropiolate, tert-butyl pyrrol-1-carboxylate (30 mL, 179 mmol) was added. The reaction mixture was stirred at 90 °C, under N₂ atmosphere, for 24 h. The reaction was monitored by SI-TLC (CyHex/AcOEt 8:2; I₂ as resolving agent). Then the reaction mixture was concentrated under reduced pressure and loaded on a silica gel column for purification.

Purification: Isolera-One Biotage, Snap 340g HP-SI Column, sample dissolved in CyHex, eluent: from CyHex/AcOEt 100:0 to 80:20 in gradient.

The containing product fractions were collected and the solvent eliminated under reduced pressure to give 1 as yellow oil (4.31 g, purity 90%, 11.75 mmol, 13.8%).

**ANALYSIS**

**Formula:** C₁₃H₁₆BrNO₄

**Mol. Weight:** 330.17 g/mol
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.15 (brs, 2 H); 5.51 (brs, 1 H); 5.16 (brs., 1 H); 3.82 (s, 3 H); 1.44 (s, 9 H).

MS (ESI+) m/z (%) = 276 (100) [M-56H]$^+$. 
Palladium 10% on activated carbon (1.354 g, 1.272 mmol) was suspended in water (2 mL) and EtOH (10 mL). This mixture was added to a solution of compound 1 (4.20 g, 12.72 mmol) and TEA (3.55 mL, 25.4 mmol) in EtOH (40 mL). The reaction was performed in a hydrogenation apparatus. The reaction mixture was subject to four N$_2$/vacuum cycles and then to four H$_2$/vacuum cycles. Then the mixture was stirred at room temperature under H$_2$ atmosphere (1.1 atm) for 16 h. Then the reaction mixture was filtered through a Celite pad (2 cm height; 9.5 cm diameter) washing with EtOH/MeOH 9:1 (150 mL). The filtrate was concentrated under reduced pressure to give a white solid. The obtained solid was taken up in DCM (50 mL) and the organic layer was washed with water (2x50 mL), dried over anhydrous Na$_2$SO$_4$, filtered and the solvent eliminated under reduced pressure. Compound 2 was isolated as yellow oil (3.07 g, 12.02 mmol, 95%)

**ANALYSIS**

**Note:** Compound 2 was isolated as (+/-)-endo/(+/-)-exo mixture of diastereoisomers in about 88:12 ratio from $^1$H NMR analysis. Chemical shifts of the major diastereoisomer are reported.

**Formula:** C$_{13}$H$_{21}$NO$_4$

**Mol. Weight:** 255.31 g/mol

$^1$H NMR (400 MHz, CDCl$_3$) (endo diastereoisomer) $\delta$ = 4.41 (t, $J$=4.4 Hz, 1 H); 4.23 (t, $J$=4.4 Hz, 1 H); 3.72 (s, 3 H); 3.02 - 3.11 (m, 1 H); 1.93 - 2.05 (m, 1 H); 1.84 - 1.93 (m, 1 H); 1.77 - 1.84 (m, 1 H); 1.66 - 1.77 (m, 1 H); 1.48 - 1.52 (m, 2 H); 1.47 (s, 9 H).

**MS (ESI+)** m/z (%) = 199 (100%) [M-56H]$^+$.
To a stirred solution of compound 2 (1.695 g, 6.64 mmol) in THF/water (33 mL, 3:1), cooled at 0 °C, a solution of lithium hydroxide in water (0.352 g, 8.39 mmol) was added. The mixture was stirred at room temperature for 17 h.

Then the solvent was evaporated under reduced pressure and the residue taken up in water (10 mL) and the pH adjusted to a value of 2 with HCl 2N (4.5 mL). The aqueous phase was then extracted with AcOEt (3x50 mL) and the combined organic layers were dried over anhydrous Na₂SO₄, filtered and the solvent eliminated under reduced pressure to give 3 as an amber oil (1.616 g, 6.70 mmol, quantitative).

**ANALYSIS**

**Note:** Compound 3 was isolated as *endo/exo* mixture in about 87:13 ratio from ¹H NMR analysis. Chemical shifts of the major diastereoisomer are reported.

**Formula:** C₁₂H₁₉NO₄

**Mol. Weight:** 241.28 g/mol

¹H NMR (400 MHz, CDCl₃) (*endo* diastereoisomer) δ = 4.47 (t, J=4.4 Hz, 1 H); 4.26 (t, J=4.6 Hz, 1 H); 3.09 - 3.17 (m, 1 H); 1.97 - 2.07 (m, 1 H); 1.72 - 1.90 (m, 3 H); 1.61 - 1.69 (m, 1 H); 1.52 (dd, J=11.0, 3.7 Hz, 1 H); 1.48 (s, 9 H).

**MS (ESI+)** m/z (%) = 185 (100%) [M-56H]⁺.
(+/-)-endo-tert-butyl 2-(((benzylxy)carbonyl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (4)

To a nitrogen flushed solution of 3 (1.60 g, 6.63 mmol) and TEA (1.1 mL, 7.89 mmol) in dry toluene (25 mL) diphenyl phosphoryl azide (1.5 mL, 7.88 mmol) was added under stirring. After complete addition, the mixture was slowly warmed to 90 °C over 30 min and then stirred at 90 °C for 1h. Then the reaction mixture was cooled at room temperature, benzyl alcohol (1.034 mL, 9.95 mmol) was added and the resulting mixture was further heated at 90 °C for 18 h.

The reaction mixture was cooled at room temperature and then extracted successively with citric acid 5% wt (2x40 mL), water (2x40 mL), NaHCO₃ sat. sol. (2x40 mL) and brine (2x20 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and the solvent eliminated under reduced pressure.

Purification: Isolera-One Biotage, Snap 100g HP-SI Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100:0 to 50:50 in gradient.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 4 as white solid (2.175 g, purity 84%, 5.27 mmol, 80%).

**ANALYSIS**

**Formula:** C₁₉H₂₆N₂O₄

**Mol. Weight:** 346.42

**Rf:** Product: 0.76 (CyHex/AcOEt 5:5; SI-TLC).

**¹H NMR** (400 MHz, CDCl₃) δ = 7.43 - 7.30 (m, 5 H); 5.22 - 5.05 (m, 2 H); 4.82 (brs, 1 H); 4.39 (brs, 1 H); 4.20 (brs, 1 H); 4.16 - 3.99 (m, 1 H); 2.44 - 2.31 (m, 1 H); 1.89 - 1.75 (m, 2 H); 1.75 - 1.64 (m, 1 H); 1.47 (s, 9 H); 1.44 - 1.37 (m, 1 H); 0.91 (dd, J=12.7, 4.4 Hz, 1 H).

**¹³C NMR (APT)** (50 MHz, CDCl₃) δ = 156.17 (C=O), 155.61 (C=O), 136.48 (C), 128.80 (2xCH), 128.47 (CH), 80.09 (C), 67.13 (CH₂), 59.11 (CH), 58.90 (CH), 51.88 (CH), 37.67 (CH₂), 29.92 (CH₂), 28.49 (3 x CH₃), 22.24 (CH₂).

**MS (ESI+) m/z (%) = 347 (100%) [MH]^+.**
(+/-)-endo-tert-butyl 2-amino-7-azabicyclo[2.2.1]heptane-7-carboxylate (5)

Palladium 5% on activated carbon, (0.230 g, 0.054 mmol) was suspended in EtOH (5 mL). This mixture was added to a solution of 4 (84%) (2.150 g, 5.21 mmol) in EtOH (20 mL). The reaction was conducted in a hydrogenation apparatus. The reaction mixture was subject to four H₂/vacuum cycles. Then the solution was stirred at room temperature under H₂ atmosphere (50 psi) for 16 h. The reaction mixture was then filtered through a Celite pad washing with EtOH (30 mL) and MeOH (30 mL). The filtrate was concentrated under reduced pressure to give an oil which was purified by column chromatography.

Purification: Isolera-One Biotage, Snap 55g KP-NH Column, sample dissolved in DCM, eluent: DCM/MeOH 98:2 isocratic.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 5 as clear oil (1.189 g, purity 90%, 5.04 mmol, 97%).

**ANALYSIS**

**Formula:** C₁₁H₁₂O₃N₂O₂

**Mol. Weight:** 212.29 g/mol

**Rf:** 0.9 (DCM/MeOH 95:5; TLC KP-NH).

**¹H NMR** (400 MHz, CDCl₃) δ = 4.13 (brs, 1 H); 4.03 (brs, 1 H); 3.54 - 3.46 (m, 1 H); 2.31 - 2.21 (m, 1 H); 2.05 (ddd, J=12.9, 9.3, 4.4 Hz, 1 H); 1.86 - 1.75 (m, 1 H); 1.72 - 1.61 (m, 1 H); 1.53 - 1.47 (m, 4 H); 1.47 (s, 10 H); 0.84 (dd, J=12.2, 4.40 Hz, 1 H).

**¹³C NMR (APT)** (50 MHz, CDCl₃) δ = 155.22 (C=O), 80.53 (C), 58.41 (CH), 57.28 (CH), 51.18 (CH), 35.91 (CH₂), 29.61 (CH₂), 28.45 (3 x CH₃), 22.79 (CH₂).

**MS (ESI+)** m/z (%) = 213 (100%) [MH]+.
To a nitrogen flushed solution of 2 (250 mg, 0.979 mmol) in dry MeOH (5 mL) solid sodium methoxide (132 mg, 2.448 mmol) was added. The reaction mixture was stirred and heated at reflux (65 °C) for 24 h. Then a second amount of sodium methoxide (397 mg, 7.34 mmol) was added and the reaction mixture was stirred at 65 °C for further 21 h. Then the mixture was cooled to room temperature, water (5 mL) was added and stirring was continued for 1 h at room temperature. The mixture was concentrated in order to eliminate the MeOH and then the pH was adjusted to 4.5 with conc. HCl. The resulting precipitate was collected and dried in vacuo. The obtained solid was subsequently washed with Et₂O/CyHex 6:4 (10 mL) and dried in vacuo to afford compound 6 as off-white solid (125 mg, 0.518 mmol, 53%).

**ANALYSIS**

**Formula:** C₁₂H₁₉NO₄

**Mol. Weight:** 241.28 g/mol

**¹H NMR (400 MHz, CDCl₃)** δ = 4.59 (d, J=4.4 Hz, 1 H); 4.33 (t, J=4.6 Hz, 1 H); 2.62 (dd, J=9.0, 5.1 Hz, 1 H); 2.31 - 2.22 (m, 1 H); 1.92 - 1.75 (m, 2 H); 1.66 (dd, J=12.5, 9.0 Hz, 1 H); 1.56 - 1.46 (m, 2 H); 1.45 (s, 9 H).

**¹³C NMR (APT) (50 MHz, CDCl₃)** δ = 178.23 (C=O), 155.18 (C=O), 80.22 (C), 59.50 (CH), 56.14 (CH), 47.50 (CH), 33.56 (CH₂), 29.71 (CH₂), 29.00 (CH₂), 28.39 (3 x CH₃).

**MS (ESI+)** m/z (%) = 186 (100%) [M-56H]⁺.
(+/-)-exo-tert-butyl 2-{((benzyloxy)carbonyl)amino}-7-azabicyclo[2.2.1]heptane-7-carboxylate (7)

To a nitrogen flushed solution of 6 (110 mg, 0.456 mmol) and TEA (0.076 mL, 0.547 mmol) in dry toluene (2 mL) diphenyl phosphoryl azide (0.104 mL, 0.547 mmol) was added under stirring. After complete addition, the mixture was slowly warmed to 90 °C over 30 min and then stirred at 90 °C for 1h. Then the reaction mixture was cooled at room temperature, benzyl alcohol (0.071 mL, 0.684 mmol) was added and the resulting mixture was further heated at 90 °C for 18 h. The reaction mixture was cooled at room temperature and then extracted successively with citric acid 5%Wt (2x10 mL), water (2x10 mL), NaHCO₃ sat. sol. (2x10 mL) and brine (2x10 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and the solvent eliminated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100: to 50:50 in gradient.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give compound 7 as colorless oil (124 mg, purity 90%, 0.322 mmol, 71%).

**ANALYSIS**

**Formula:** C₁₉H₂₈N₂O₄

**Mol. Weight:** 346.42 g/mol

**¹H NMR** (400 MHz, CDCl₃) δ = 7.42 - 7.30 (m, 5 H); 5.20 - 5.06 (m, 2 H); 4.99 (brs, 1 H); 4.25 (t, J=4.6 Hz, 1 H); 4.14 (d, J=4. 9 Hz, 1 H); 3.84 - 3.76 (m, 1 H); 1.96 (dd, J=13.0, 8.1 Hz, 1 H); 1.85 - 1.65 (m, 2 H); 1.56 - 1.49 (m, 1 H); 1.45 (s, 9 H); 1.43 - 1.31 (m, 2 H).

**¹³C NMR** (APT) (100 MHz, CDCl₃) δ = 156.14 (C=O), 155.56 (C=O), 136.48 (C), 128.50 (2xCH), 128.09 (CH), 80.00 (C), 66.66 (CH₂), 61.59 (CH), 55.61 (CH), 54.67 (CH), 40.27 (CH₂), 28.33 (CH₂), 28.23 (3 x CH₃), 25.82 (CH₂).

**MS (ESI+)** m/z (%) = 347 (100%) [MH]⁺.
**(+/-)-exo-tert-butyl 2-amino-7-azabicyclo[2.2.1]heptane-7-carboxylate (8)**

![Chemical structure diagram]

Palladium 5% on activated carbon (59.6 mg, 0.014 mmol) was suspended in EtOH (0.5 mL). This mixture was added to a solution of 7 (97 mg, 0.280 mmol) in EtOH (5 mL). The reaction was performed in a hydrogenation apparatus. The reaction mixture was subject to four H₂/vacuum cycles and then stirred at room temperature under H₂ atmosphere (1.1 atm) for 16 h.

The reaction mixture was then filtered through a Celite pad washing with EtOH (10 mL) and MeOH (10 mL). The filtrate was concentrated under reduced pressure to give an oil which was purified by column chromatography.

Purification: Isolera-One Biotage, Snap 11g KP-NH Column, sample dissolved in DCM, eluent: DCM/MeOH 98:2 isocratic.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 8 as a clear oil that solidify on standing to give a white solid (48 mg, purity 95%, 0.214 mmol, 76%).

**ANALYSIS**

**Formula:** C₁₁H₂₀N₂O₂  
**Mol. Weight:** 212.29 g/mol

**¹H NMR** (400 MHz, CDCl₃) δ = 4.22 (burs, 1 H); 3.90 (burs, 1 H); 2.98 (dd, J=7.6, 3.2 Hz, 1 H); 1.83 (dd, J=12.7, 7.8 Hz, 1 H); 1.79 - 1.60 (m, 2 H); 1.48 (s, 9 H); 1.41 - 1.26 (m, 3 H).

**¹³C NMR (DEPT)** (100 MHz, CDCl₃) δ = 156.49 (C=O), 79.59 (C), 64.43 (CH), 55.74 (CH), 55.46 (CH), 41.90 (CH₂), 28.31 (3 x CH₃), 28.22 (CH₂), 25.90 (CH₂).

**MS (ESI+)** m/z (%) = 213 (100%) [MH]⁺.
2.4 Synthesis of endo-TYPE II derivatives 9a-k, experimental data

(+/-)-endo-tert-butyl 2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (10a)

A mixture of 5 (100.6 mg, 0.474 mmol), 2-fluoro-5-(trifluoromethyl)pyridine (0.070 mL, 0.580 mmol) and K₂CO₃ (132.5 mg, 0.959 mmol) in DMF (1 mL) was stirred under N₂ atmosphere for 10 min. The reaction was then performed under microwave heating: 3 cycles at 100 °C for 20 min. Then the solvent was evaporated under reduced pressure and the residue was taken up in NaHCO₃ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x10 mL). The organic layers were collected, washed with water (30 mL), dried over anhydrous Na₂SO₄ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 10g HP-Si Column, sample dissolved in DCM, eluent: from CyHex 100% to CyHex/AcOEt 75:25 in gradient.

The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 10a as white solid (84.5 mg, purity 95%, 0.23 mmol, 47%).

**ANALYSIS**

**Formula:** C₁₇H₂₂F₃N₃O₂

**Mol. Weight:** 357.37 g/mol

**Rf:** 0.67 (CyHex/AcOEt 5:5; SI-TLC)

**¹H NMR** (400 MHz, CDCl₃) δ = 8.36 (s, 1 H); 7.65 (dd, J=8.8, 2.4 Hz, 1 H); 6.50 (d, J=8.8 Hz, 1 H); 5.16 (brs, 1 H); 4.49 (brs, 1 H); 4.25 (brs, 1 H); 4.17 - 4.07 (m, 1 H); 2.55 - 2.45 (m, 1 H); 1.92 - 1.81 (m, 2 H); 1.75 - 1.63 (m, 1 H); 1.56 - 1.51 (m, 1 H); 1.50 (s, 9 H); 1.05 (dd, J=12.7, 4.4 Hz, 1 H).

**¹³C NMR (APT)** (50 MHz, CDCl₃) δ = 160.13 (C=O), 155.75 (C), 146.39 (q, J = 4.4 Hz, CH), 134.91 (q, J = 3.2 Hz, CH), 124.67 (q, J = 270.3 Hz, CF₃), 116.38 (q, J = 33.0 Hz, C), 106.04 (CH), 80.19 (C), 58.52 (CH), 56.83 (CH), 52.94 (CH), 38.49 (CH₂), 30.20 (CH₂), 28.50 (3 x CH₃), 22.06 (CH₂).

**MS (ESI+)** m/z (%) = 358 (100) [MH]⁺.
A mixture of 5 (100 mg, 0.471 mmol), 2-chloro-6,7-difluoroquinoxaline (97 mg, 0.485 mmol) and TEA (0.200 mL, 1.435 mmol) in DMF (1 mL) was stirred under N₂ atmosphere for 10 min. The reaction was then performed initially under microwave heating: 3 cycles at 75 °C for 1 h.
To promote the complete conversion of starting 5 further 2-chloro-6,7-difluoroquinoxaline (20 mg, 0.100 mmol) and TEA (0.050 mL, 0.359 mmol) were added. The reaction mixture was subjected to a further microwave cycle at 75°C for 1 h.
The reaction mixture was subsequently further heated at 75 °C for 18 h (thermal reaction).
Then the solvent was evaporated under reduced pressure and the residue taken up in NaHCO₃ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x20 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na₂SO₄ and filtered. Then the solvent was evaporated under reduced pressure.
Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from DCM 100% to DCM/AcOEt 8:2 in gradient.
The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 10b as yellow solid (107.5 mg, 0.27 mmol, 61%).

**ANALYSIS**

**Formula:** C₁₅H₂₂F₂N₄O₂

**Mol. Weight:** 376.40 g/mol

**Rf:** 0.25 (DCM/AcOEt 8:2; SI-TLC)

**¹H NMR** (400 MHz, CDCl₃) δ = 8.22 (s, 1 H); 7.65 (dd, J=10.3, 8.2 Hz, 1 H); 7.46 (dd, J=11.2, 7.8 Hz, 1 H); 5.09 (brs, 1 H); 4.70 (brs, 1 H); 4.44 - 4.35 (m, 1 H); 4.29 (brs, 1 H); 2.61 - 2.48 (m, 1 H); 1.95 - 1.81 (m, 2 H); 1.77 - 1.66 (m, 1 H); 1.65 - 1.47 (m, 10 H); 1.09 (dd, J=12.5, 4.6 Hz, 1 H).

**¹³C NMR** (APT) (50 MHz, CDCl₃) δ 155.79 (C=O), 152.64 (dd, J=253.4, 15.4, CF), 151.66 (C), 148.84 (dd, J=248.6, 15.6), 138.12 (d, J=3.8, CH), 133.38 (d, J=10, C), 115.13 (dd, J=17.3, 2.1, CH), 112.70 (d, J=17.5, CH), 80.22 (C), 58.73 (CH), 56.78 (CH), 52.49 (CH), 38.27 (CH₂), 30.20 (CH₂), 28.54 (3 x CH₃), 22.20 (CH₂).
MS (ESI+) m/z (%) = 377 (100) [MH]^+. 
(+/-)-endo-tert-butyl 2-((4-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (10c)

A mixture of 5 (100 mg, 0.471 mmol), 2-fluoro-4-trifluoromethyl-pyridine (93 mg, 0.565 mmol) and K₂CO₃ (130 mg, 0.942 mmol) in DMF (1 mL) was stirred under N₂ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 22 h. Then the solvent was evaporated under reduced pressure and the residue taken up in NaHCO₃ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x20 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na₂SO₄ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from DCM 100% to DCM/AcOEt 8:2 in gradient. The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 10c as white solid (114 mg, 0.32 mmol, 68%).

**ANALYSIS**

**Formula:** C₁₇H₂₂F₃N₃O₂  
**Mol. Weight:** 357.37 g/mol  
**Rf:** 0.65 (DCM/AcOEt 8:2; SI-TLC)

**¹H NMR** (400 MHz, CDCl₃) δ = 8.24 (d, J=5.4 Hz, 1 H); 6.81 (dd, J=5.4, 1.0 Hz, 1 H); 6.62 (s, 1 H) 4.96 (d, J=5.4 Hz, 1 H); 4.50 (brs, 1 H); 4.28 - 4.19 (m, 1 H); 4.16 - 4.07 (m, 1 H); 2.56 - 2.46 (m, 1 H); 1.94 - 1.81 (m, 2 H); 1.74 - 1.64 (m, 1 H); 1.57 - 1.46 (m, 10 H); 1.04 (dd, J=12.5, 4.6 Hz, 1 H).

**¹³C NMR** (APT) (50 MHz, CDCl₃) δ = 158.68 (C=O), 155.75 (C), 149.75 (CH), 140.12 (q, J=33.4 Hz, C), 123.18 (q, J=273.1 Hz, CF₃), 108.88 (q, J=3.3 Hz, CH), 102.59 (q, J=4.1 Hz, CH), 80.15 (C), 58.57 (CH), 56.82 (CH), 53.02 (CH), 38.51 (CH₂), 30.21 (CH₂), 28.49 (3 x CH₃), 22.07 (CH₂).

**MS (ESI+)** m/z (%) = 358 (100) [MH]+.
(+/-)-endo-tert-butyl 2-((5-(trifluoromethyl)pyrazin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (10d)

A mixture of 5 (100 mg, 0.471 mmol), 2-chloro-5-(trifluoromethyl)pyrazine (103 mg, 0.565 mmol) and K$_2$CO$_3$ (130 mg, 0.942 mmol) in DMF (1 mL) was stirred under N$_2$ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 4 h, then 75 °C for 14 h. Then the solvent was evaporated under reduced pressure and the residue taken up in NaHCO$_3$ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x20 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na$_2$SO$_4$ and filtered. Then the solvent was evaporated under reduced pressure. Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from DCM 100% to DCM/AcOEt 8:2 in gradient. The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 10d as yellow solid (79 mg, purity 95%, 0.21 mmol, 44.5%).

**ANALYSIS**

**Formula**: C$_{16}$H$_{21}$F$_3$N$_4$O$_2$

**Mol. Weight**: 358.36 g/mol

**Rf**: 0.48 (DCM/AcOEt 8:2; Si-TLC)

$^1$H NMR (400 MHz, CDCl$_3$): δ = 8.38 (s, 1 H); 7.98 (s, 1 H); 5.16 (d, J=4.9 Hz, 1 H); 4.55 (brs, 1 H); 4.33 - 4.21 (m, 2 H); 2.58 - 2.47 (m, 1 H); 1.94 - 1.84 (m, 1 H); 1.84 - 1.78 (m, 1 H); 1.77 - 1.66 (m, 1 H); 1.56 - 1.47 (m, 10 H); 1.07 (dd, J=12.7, 4.4 Hz, 1 H).

$^{13}$C NMR (APT) (50 MHz, CDCl$_3$): δ = 155.64 (C=O), 155.33 (C), 140.10 (CH), 132.69 (q, J=35.1, C), 131.69 (CH), 122.4 (q, J=271.8, CF$_3$), 80.33 (C), 58.51 (CH), 56.78 (CH), 52.60 (CH), 38.34 (CH$_2$), 30.14 (CH$_2$), 28.50 (3 x CH$_3$), 22.18 (CH$_2$).

**MS (ESI+)** m/z (%) = 303 (100) [M-56H]$^+$. 
(+/-)-endo-tert-butyl 2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (10e)

A mixture of 5 (100 mg, 0.471 mmol), 2-chloro-4-(trifluoromethyl)pyrimidine (103 mg, 0.565 mmol) and K₂CO₃ (130 mg, 0.942 mmol) in DMF (1 mL) was stirred under N₂ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 4 h, then 75 °C for 14 h. Then the solvent was evaporated under reduced pressure and the residue taken up in NaHCO₃ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x10 mL). The organic layers were collected, washed with water (30 mL), dried over anhydrous Na₂SO₄ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from DCM 100% to DCM/AcOEt 8:2 in gradient. The containing product fraction were collected and the solvent was evaporated under reduced pressure to give 10e as white solid (139 mg, 039 mmol, 82%).

**ANALYSIS**

**Formula:** C₁₈H₂₁F₃N₄O₂

**Mol. Weight:** 358.36 g/mol

**Rf:** 0.63 (DCM/AcOEt 8:2; Si-TLC)

**¹H NMR (400 MHz, CDCl₃)** δ = 8.58 - 8.45 (m, 1 H); 6.89 (d, J=4.9 Hz, 1 H); 5.50 (brs, 1 H); 4.55 (brs, 1 H); 4.35 (dd, J=10.3, 5.4 Hz, 1 H); 4.25 (brs, 1 H); 2.54 - 2.43 (m, 1 H); 1.93 - 1.80 (m, 2 H); 1.75 - 1.66 (m, 1 H); 1.55 - 1.45 (m, 10 H); 1.05 (dd, J=12.5, 4.6 Hz, 1 H).

**¹³C-NMR (APT)** (50 MHz, CDCl₃) δ = 162.20 (C=O), 160.51 (CH), 157.38 (q, J=38.4, C), 155.75 (C), 120.61 (q, J=275.1, CF₃), 106.39 (CH), 80.03 (C), 58.71 (CH), 56.77 (CH), 52.54 (CH), 37.97 (CH₂), 30.15 (CH₂), 28.46 (3 x CH₃), 22.26 (CH₂).

**MS (ESI+)** m/z (%) = 303 (100) [M-56H]⁺.
General Procedure A: synthesis of (+/-)-endo-compounds 11a-e

Compound 10a-e (1eq) was dissolved in a solution of DCM (Ratio: 4.0) and TFA (Ratio: 1.0; 30 eq) and then the mixture was stirred at room temperature for 1 h. Then the solvent was evaporated under reduced pressure.

Purification: the residue was taken up in MeOH and loaded on SCX column; Eluent MeOH then Ammonia (2M in MeOH). The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 11a-e.

(+/-)-endo-N-(5-(trifluoromethyl)pyridin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (11a)

![Chemical Structure of 11a]

General Procedure A was followed using 10a (120 mg, 0.336 mmol) to give 11a as white solid (66.5 mg, 0.26 mmol, 77%).

**ANALYSIS**

**Formula**: C_{12}H_{14}F_{3}N_{3}

**Mol Weight**: 257.25 g/mol

**^{1}H NMR** (400 MHz, Acetone-\textit{d}_{6}) \(\delta = 8.31 \text{ (s, 1 H)}; 7.61 \text{ (dd, } J=8.8, 2.0 \text{ Hz, 1 H}); 6.54 - 6.77 \text{ (m, 2 H)}; 4.16 \text{ (d, } J=5.4 \text{ Hz, 1 H}); 3.83 \text{ (t, } J=4.6 \text{ Hz, 1 H}); 3.47 - 3.58 \text{ (m, 1 H)}; 2.09 - 2.18 \text{ (m, 1 H)}; 1.83 \text{ (ddd, } J=12.6, 8.4, 4.4 \text{ Hz, 1 H}); 1.50 - 1.63 \text{ (m, 1 H)}; 1.41 - 1.50 \text{ (m, 1 H)}; 1.29 - 1.41 \text{ (m, 1 H)}; 1.05 - 1.14 \text{ (m, 1 H)}.

**^{13}C NMR (APT)** (50 MHz, Acetone-\textit{d}_{6}) \(\delta = 161.31 \text{ (C=O)}; 145.93 \text{ (CH)}; 133.49 \text{ (CH)}; 125.48 \text{ (q, } J=273.1 \text{ Hz, CF}_{3}); 113.91 \text{ (q, } J=30.3 \text{ Hz, C); 107.87 \text{ (CH)}; 58.64 \text{ (CH)}; 56.79 \text{ (CH)}; 53.75 \text{ (CH)}; 37.38 \text{ (CH}_{2}); 30.87 \text{ (CH}_{2}); 22.91 \text{ (CH}_{2}).

**MS (ESI+) m/z (%) = 258 (100) [MH]^+.**
**General Procedure A** was followed using 10b (92 mg, 0.244 mmol) to give 11b as clear-yellow solid (68.2 mg, purity 95%, 0.24 mmol, 96%).

**ANALYSIS**

**Formula**: C_{14}H_{14}F_{2}N_{4}

**Mol. Weight**: 276.28 g/mol

**H NMR (400 MHz, CDCl_{3})**: δ = 8.20 (s, 1 H); 7.63 (dd, J=10.8, 8.3 Hz, 1 H); 7.43 (dd, J=11.2, 8.3 Hz, 1 H); 5.06 (d, J=5.4 Hz, 1 H); 4.24 - 4.34 (m, 1 H); 4.08 (t, J=4.6 Hz, 1 H); 3.72 (t, J=4.9 Hz, 1 H); 2.36 (dd, J=12.6, 11.17, 5.1, 2.9 Hz, 1 H); 1.77 - 1.86 (m, 1 H); 1.70 - 1.77 (m, 1 H); 1.46 - 1.59 (m, 2 H); 1.02 (dd, J=12.7, 4.4 Hz, 1 H).

**C-NMR (APT) (50 MHz, CDCl_{3})**: δ = 152.56 (dd, J=257.7, 15.3, CF), 152.09 (C), 148.66 (dd, J=247.9, 15.4, CF), 139.86 (d, J=14.0, C), 138.23 (CH), 133.78 (d, J=10.0, C), 115.09 (d, J=17.3, CH), 112.70 (d, J=17.7, CH), 58.92 (CH), 57.09 (CH), 53.78 (CH), 38.96 (CH_{2}), 31.25 (CH_{2}), 23.11 (CH_{2}).

**MS (ESI+) m/z (%) = 278 (100) [MH]^+.**

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**(+/−)-endo-N-(4-(trifluoromethyl)pyridin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (11c)**

**General Procedure A** was followed using 10c (99 mg, 0.277 mmol) to give 11c as white solid (69.0 mg, 0.27 mmol, 97%).
ANALYSIS
Formula: $C_{12}H_{14}F_3N_3$
Mol. Weight: 257.25 g/mol
$^1H$ NMR (400 MHz, CDCl$_3$) $\delta = 8.23$ (d, $J=4.9$ Hz, 1 H); 6.78 (dd, $J=5.4$, 0.9 Hz, 1 H); 6.60 (s, 1 H); 4.95 (d, $J=5.4$ Hz, 1 H); 3.96 - 4.06 (m, 1 H); 3.91 (t, $J=4.4$ Hz, 1 H); 3.70 (t, $J=4.9$ Hz, 1 H); 2.32 (dddd, $J=12.5$, 10.9, 5.4, 2.9 Hz, 1 H); 1.83 - 1.91 (m, 1 H); 1.62 - 1.75 (m, 1 H); 1.46 - 1.57 (m, 2 H); 0.97 (dd, $J=12.7$, 4.4 Hz, 1 H).
$^{13}$C-NMR (APT) NMR (50 MHz, CDCl$_3$) $\delta = 159.10$ (C), 149.81 (CH), 139.98 (q, $J=33.1$, C), 123.25 (q, $J=273.0$, CF$_3$), 102.55 (q, $J=4.2$, CH), 108.5 (q, $J=3.1$, CH), 58.89 (CH), 57.13 (CH), 54.27 (CH), 39.00 (CH$_2$), 31.24 (CH$_2$), 23.11 (CH$_2$).
MS (ESI+) m/z (%) = 259 (100) [MH]$^+$.

$(+/-)$-endo-N-(5-(trifluoromethyl)pyrazin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (11d)

General Procedure A was followed using 10d (65 mg, 0.181 mmol) to give 11d as clear-yellow solid (45.3 mg, 0.1 mmol, 94%).

ANALYSIS
Formula: $C_{11}H_{13}F_3N_4$
Mol. Weight: 258.24
$^1H$ NMR (400 MHz, CDCl$_3$) $\delta = 8.36$ (s, 1 H); 7.95 (d, $J=1.0$ Hz, 1 H); 5.15 (d, $J=4.9$ Hz, 1 H); 4.11 - 4.20 (m, 1 H); 3.97 (t, $J=4.4$ Hz, 1 H); 3.73 (t, $J=4.9$ Hz, 1 H); 2.30 - 2.40 (m, 1 H); 1.76 - 1.84 (m, 1 H); 1.67 - 1.76 (m, 1 H); 1.47 - 1.60 (m, 2 H); 1.01 (dd, $J=12.5$, 4.6 Hz, 1 H).
$^{13}$C-NMR (APT) (50 MHz, CDCl$_3$) $\delta = 155.73$ (C), 140.33 (q, $J=3.5$, CH), 132.32 (q, $J=35.6$, C), 131.45 (CH), 122.49 (q, $J=271.8$, CF$_3$), 58.80 (CH), 57.09 (CH), 53.89 (CH), 38.91 (CH$_2$), 31.22 (CH$_2$), 23.18 (CH$_2$).
MS (ESI+) m/z (%) = 260 (100) [MH]$^+$. 
**(+/−)-endo-N-(4-trifluoromethyl)pyrimidin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (11e)**

![Chemical structure](image)

**General Procedure A** was followed using 10e (122 mg, 0.340 mmol) to give 11e as white solid (86.0 mg, purity 90%, 0.30 mmol, 89%).

**ANALYSIS**

*Formula:* $C_{11}H_{13}F_{3}N_{4}$

*Mol. Weight:* 258.24 g/mol

**$^1H$ NMR** (400 MHz, CDCl$_3$) $\delta = 8.50$ (brs, 1 H); 6.86 (d, $J=4.9$ Hz, 1 H); 5.51 (brs, 1 H); 4.20 - 4.29 (m, 1 H); 3.95 (t, $J=4.2$ Hz, 1 H); 3.69 (t, $J=4.9$ Hz, 1 H); 2.29 (dddd, $J=12.7$, 11.2, 5.1, 3.2 Hz, 1 H); 1.79 - 1.88 (m, 1 H); 1.64 - 1.71 (m, 1 H); 1.43 - 1.57 (m, 2 H); 0.99 (dd, $J=12.7$, 4.9 Hz, 1 H).

**$^{13}C$-NMR (APT)** (50 MHz, CDCl$_3$) $\delta = 162.59$ (C), 160.63 (CH), 156.19 (q, $J=35.5$, C), 120.68 (q, $J=275.2$, CF$_3$), 105.99 (CH), 58.93 (CH), 57.15 (CH), 53.80 (CH), 38.64 (CH$_2$), 31.05 (CH$_2$), 23.09 (CH$_2$).

**MS (ESI+)** m/z (%) = 260 (100) [MH]$^+$. 

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General Procedure B: synthesis of (+/-)-endo-compounds 9a-k

To a solution of the appropriate carboxylic acid (1.0 eq) in dioxane (1 mL) were added in sequence N-methylmorpholine (3 eq) and CDMT (1.5 eq) under N₂ atmosphere and the resultant solution was stirred for 30 min at room temperature. Then the amine 11a-e (1.2 eq) pre-dissolved in dioxane (1.5 mL) was added and the mixture was stirred at 100 °C for 1 h. The solvent was then evaporated under reduced pressure. The residue was taken up in AcOEt and washed in sequence with NH₄Cl sat. sol., NaHCO₃ sat. sol. and water. Then the organic layer was dried over anhydrous Na₂SO₄, filtered and the solvent was eliminated under reduced pressure.

Purification: Isolera-One Biotage, Flash chromatography by HP-Si Column or Reverse Phase Column. The containing product fractions were collected and the solvent eliminated under reduced pressure to give the final compound 9a-e as (+/-)-endo-racemic mixture.

Analysis of proton and carbon NMR spectra reveals for compounds 9a-k the presence of two rotamers of the molecule in 1:1 ratio. In particular, in proton NMR spectra the two rotamers can be easily detected and described. On the contrary, carbon NMR spectra are complicated by the presence of signals splitted by the presence of C-F coupling and the Csp² carbon atom signals are difficult to distinguish and assign. For these reasons in carbon NMR spectra only the chemical shifts of aliphatic carbons are reported for both rotamers of each compound.

(2-methyl-5-phenylthiazol-4-yl) (+/-)-endo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9a)

![Chemical Structure](image)

General Procedure B was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (40.3 mg, 0.184 mmol), amine 11a (43 mg, 0.167 mmol), NMM (0.055 mL, 0.501 mmol) and CDMT (44.0 mg, 0.251 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100:0 to 0:100 in gradient) 9a was obtained as a white solid (49.5 mg, 0.11 mmol, 65%).
ANALYSIS

Formula: C_{23}H_{21}F_{3}N_{4}OS

Mol. Weight: 458.50

Note: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

Rf: 0.08 (CyHex/AcOEt 5:5; SI-TLC).

$^1$H NMR (400 MHz, Acetone-$d_6$) $\delta = 8.39$ (s, 1 H); 8.29 (s, 1 H); 7.68 - 7.58 (m, 6 H); 7.50 - 7.35 (m, 6 H); 6.78 (m, 2 H); 6.68 (d, $J=8.8$ Hz, 1 H); 6.59 (d, $J=8.8$ Hz, 1 H); 5.03 (t, $J=4.9$ Hz, 1 H); 4.63 (t, $J=4.9$, 1 H,); 4.25 (m, 2 H); 3.98 (m, 2 H); 2.72 (s, 3 H); 2.76 (s, 3 H); 2.30 (m, 1 H); 1.94 (m, 2 H); 1.86 (m, 1 H); 1.74 (m, 1 H); 1.63 - 1.45 (m, 4 H); 1.32 (m, 2 H); 1.20 (m, 1 H).

$^{13}$C-NMR (APT) (MHz, Acetone-$d_6$) $\delta = 59.62$ (CH), 57.97 (CH), 55.92 (CH), 54.01 (CH), 52.97 (CH), 51.94 (CH), 37.49 (CH$_2$), 36.04 (CH$_2$), 30.54 (CH$_2$), 22.62 (CH$_2$), 21.26 (CH$_2$), 18.38 (2 x CH$_3$), (one CH$_2$ signal obscured by acetone-$d_6$ signals).

MS (ESI+) m/z (%) = 460 (100) [MH]$^+$. 

(2-methyl-5-phenylthiazol-4-yl) (+/-)-endo-2-((6,7-difluoroquinoxalin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9b)

General Procedure B was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (45.7 mg, 0.208 mmol), amine 11b (50.5 mg, 0.174 mmol), NMM (0.057 mL, 0.521 mmol) and CDMT (45.7 mg, 0.260 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 98:2 to 95:5 in gradient) 9b was obtained as a white solid (32 mg, 0.07 mmol, 39%).

ANALYSIS

Formula: C_{25}H_{21}F_{3}N_{3}OS

Mol. Weight: 477.53 g/mol

Note: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.
Rf: 0.35 (DCM/MeOH 95:5; SI-TLC).

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 8.33$ (s, 1 H); 8.27 (s, 1 H); 8.01 (m, 2 H); 7.82 (m, 2 H); 7.65 (dd, J=11.9, 8.5 Hz, 1 H); 7.57 - 7.36 (m, 10 H); 7.31 (dd, J= 11.6, 8.3 Hz, 1H); 4.93 (t, J=4.4 Hz, 1 H); 4.59 (t, J=4.4 Hz, 1 H); 4.14 ( s, 1 H); 4.14 ( m, 1 H); 3.87 (t, J=4.4 Hz, 1 H); 3.81 (m, 1 H); 2.76 (s, 3 H); 2.71 (s, 3 H); 2.20 (m, 1 H); 1.86 – 1.65 (m, 4 H); 1.60 – 1.39 (m, 4 H); 1.35 – 1.12 (m, 3 H).

$^{13}$C-NMR (APT) (75 MHz, Pyr-$d_5$) $\delta$ 60.20 (CH), 58.42 (CH), 56.54 (CH), 54.61 (CH), 53.13 (CH), 52.13 (CH), 37.72 (CH$_2$), 36.20 (CH$_2$), 31.17 (CH$_2$), 29.68 (CH$_2$), 23.49 (CH$_2$), 19.17 (CH$_3$), 19.11 (CH$_3$).

MS (ESI+) m/z (%) = 479 (100) [MH]$^+$. (2-methyl-5-phenylthiazol-4-yl) (+/-)-endo-2-((4-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9c)

General Procedure B was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (53.5 mg, 0.244 mmol), amine 11c (52.3 mg, 0.203 mmol), NMM (0.067 mL, 0.610 mmol) and CDMT (53.5 mg, 0.305 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 98:2 to 95:5 in gradient) 9c was obtained as a white solid (62 mg, 0.13 mmol, 65%).

ANALYSIS

Formula: C$_{23}$H$_{21}$F$_3$N$_4$O$_3$

Mol. Weight: 458.50 g/mol

Note: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

Rf: 0.37 (DCM/MeOH 95:5; SI-TLC).

$^1$H NMR (400 MHz, Acetone-$d_6$) $\delta = 8.29$ (d, J=5.1 Hz, 1 H); 8.21 (d, J=5.2 Hz, 1 H); 7.61 (m, 4 H); 7.44 (m, 6 H); 7.57 - 7.64 (m, 2 H); 7.75 (m, 4 H); 7.59 (m, 2H); 5.04 (t, J=4.5 Hz, 1 H); 4.63 (t, J=4.9 Hz, 1 H); 4.26 (m, 1 H); 3.97 (m, 1 H); 2.76 (s, 3 H); 2.74 (s, 3 H); 2.31 (m, 1 H); 1.96 (m, 2 H); 1.88 (m, 1 H); 1.75 (m, 1 H); 1.63 - 1.44 (m, 4 H); 1.29 (m, 2 H); 1.16 (m, 1 H).
**13C-NMR (APT)** (50 MHz, Acetone-$d_6$) $\delta$ = 59.64 (CH), 57.91 (CH), 55.89 (CH), 53.92 (CH), 53.00 (CH), 51.97 (CH), 37.68 (CH$_2$), 36.27 (CH$_2$), 30.58 (CH$_2$), 22.57 (CH$_2$), 21.19 (CH$_2$), 18.37 (2 x CH$_3$), (one CH$_2$ signal obscured by acetone-$d_6$ signals).

**MS (ESI+)** m/z (%) = 479 (100) [MH]$^+$.  

(2-methyl-5-phenylthiazol-4-yl) (+/-)-endo-2-((5-(trifluoromethyl)pyrazin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9d)

**General Procedure B** was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (34.1 mg, 0.156 mmol), amine 11d (34.5 mg, 0.130 mmol), NMM (0.043 mL, 0.389 mmol) and CDMT (34.1 mg, 0.194 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9d was obtained as a white solid (48 mg, 0.10 mmol, 80%).

**ANALYSIS**

**Formula**: C$_{22}$H$_{20}$F$_3$N$_3$OS

**Mol. Weight**: 459.49 g/mol

**Note**: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

**Rf**: 0.36 (DCM/MeOH 95:5; Si-TLC).

**1H NMR** (400 MHz, Acetone-$d_6$) $\delta$ = 8.43 (s, 1 H); 8.34 (s, 1 H); 8.04 (s, 1 H); 7.98 (s, 1 H); 7.61 (m, 4 H); 7.45 (m, 6 H); 7.31 (m; 2 H); 5.03 (t, J=4.0 Hz, 1 H); 4.65 (t, J=4.9 Hz, 1 H); 4.28 (m, 2 H); 4.00 (m, 2 H); 2.76 (s, 3 H); 2.74 (s, 3 H); 2.33 (m, 1 H); 1.97 (m, 2 H); 1.88 (m, 1 H); 1.77 (m, 1 H); 1.64 - 1.45 (m, 4 H); 1.34 (m, 2 H); 1.25 (m, 1 H).

**13C-NMR (APT)** (50 MHz, Acetone-$d_6$) $\delta$ = 59.38 (CH), 57.94 (CH), 55.61 (CH), 53.98 (CH), 52.64 (CH), 51.67 (CH), 37.36 (CH$_2$), 35.96 (CH$_2$), 30.52 (CH$_2$), 22.73 (CH$_2$), 21.33 (CH$_2$), 18.38 (2 x CH$_3$), (one CH$_2$ signal obscured by acetone-$d_6$ signals).

**MS (ESI+)** m/z (%) = 461 (100) [MH]$^+$.  

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(2-methyl-5-phenylthiazol-4-yl) (+/-)-endo-2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9e)

**General Procedure B** was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (60.5 mg, 0.276 mmol), amine 11e (65.3 mg, 0.230 mmol), NMM (0.076 mL, 0.690 mmol) and CDMT (60.6 mg, 0.345 mmol) in dioxane (3 mL). After purification (Snap 25g HP-Si Column, sample dissolved in DCM 100%, eluent: from AcOEt/DCM/MeOH 90:10:0 to AcOEt/DCM/MeOH 60:37:3 in gradient) 9e was obtained as a white solid (80.6 mg, 0.18 mmol, 76%).

**ANALYSIS**

**Formula:** C_{22}H_{20}F_{3}N_{5}OS

**Mol. Weight:** 459.49 g/mol

**Note:** From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

**Rf:** 0.40 (DCM/MeOH 95:5; SI-TLC).

**1H NMR** (400 MHz, Acetone-d_{6}) \( \delta = \) 8.67 (bs, 1 H); 8.58 (bs, 1 H); 7.60 (m, 4 H); 7.43 (m, 6 H); 7.18 (m, 2 H); 7.02 (d, J=4.7, 1 H); 6.97 (d, J=4.7, 1 H); 4.99 (m, 1 H); 4.64 (m, 1 H); 4.27 (m, 2 H); 3.98 (m, 2 H); 2.80 (s, 3 H); 2.73 (s, 3 H); 2.28 (m, 1 H); 1.97 (m, 2 H); 1.84 (m, 1 H); 1.70 (m, 2 H); 1.73 - 1.46 (m, 4 H); 1.40 (m, 1 H); 1.25 (m, 1 H).

**13C-NMR (APT)** (50 MHz, Acetone-d_{6}) \( \delta = \) 59.52 (CH), 58.03 (CH), 55.76 (CH), 53.96 (CH), 53.08 (CH), 51.98 (CH), 36.88 (CH_{2}), 35.28 (CH_{2}), 30.40 (CH_{2}), 28.96 (CH_{2}), 22.67 (CH_{2}), 21.44 (CH_{2}), 18.33 (2 x CH_{3}).

**MS (ESI+)** m/z (%) = 461 (100) [MH]^+. 
General Procedure B was followed using the 6-methyl-3-phenylpicolinic acid (64.7 mg, 0.303 mmol), amine 11a (65 mg, 0.253 mmol), NMM (0.083 mL, 0.758 mmol) and CDMT (66.5 mg, 0.379 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9f was obtained as a white solid (99 mg, purity 95% 0.21 mmol, 83%).

**Analysis**

**Formula:** C$_{25}$H$_{23}$F$_3$N$_4$O  
**Mol. Weight:** 452.47 g/mol  
**Note:** From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.  
$^1$H NMR (400 MHz, Acetone-$d_6$) δ = 8.38 (m, 1 H); 8.27 (m, 1 H); 7.80 (m, 2 H); 7.49 – 7.35 (m, 8 H); 6.75 (m, 2 H); 6.66 (d, J=4.7, 1 H); 6.58 (d, J=4.7, 1 H); 4.97 (t, J=4.0 Hz, 1 H); 4.57 (t, J=4.9 Hz, 1 H); 4.10 (m, 1 H); 3.97 (m, 2 H); 3.61 (m, 1 H); 2.61 (s, 3 H); 2.57 (s, 3 H); 2.18 (m, 1 H); 1.93 – 1.75 (m, 3 H); 1.64 (m, 1 H); 1.56 (m, 1 H); 1.49 - 1.35 (m, 3 H); 1.31 - 1.20 (m, 2 H); 1.12 (m, 1 H).  
$^{13}$C NMR (APT) (50 MHz, Acetone-$d_6$) δ = 58.93 (CH), 57.17 (CH), 55.38 (CH), 53.46 (CH), 52.75 (CH), 52.04 (CH), 37.41 (CH$_2$), 36.15 (CH$_2$), 30.38 (CH$_2$), 29.26 (CH$_2$), 23.39 (2 x CH$_3$), 22.44 (CH$_2$), 21.41 (CH$_2$).  
**MS (ESI+)** m/z (%) = 454 (100) [MH]$^+$.  

![Chemical structure of 9f](image-url)
(5-(4-fluorophenyl)-2-methylthiazol-4-yl) (+/-)-endo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9g)

General Procedure B was followed using the 5-(4-fluorophenyl)-2-methylthiazole-4-carboxylic acid (71.9 mg, 0.303 mmol), amine 11a (65 mg, 0.253 mmol), NMM (0.083 mL, 0.758 mmol) and CDMT (66.5 mg, 0.379 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9g was obtained as a white solid (87 mg, 0.18 mmol, 70%).

ANALYSIS
Formula: C_{23}H_{20}F_{4}N_{4}O_{5}
Mol. Weight: 476.49 g/mol

Note: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

^{1}H NMR (400 MHz, Acetone-d_{6}) δ = 8.39 (s, 1 H); 8.31 (s, 1 H); 7.65 (m, 6 H); 7.23 (m, 4 H); 6.80 (m, 2 H); 6.68 (d, J=8.8 Hz, 1 H); 6.62 (d, J=8.9 Hz, 1 H); 5.02 (t, J=4.0 Hz, 1 H); 4.63 (t, J=5.0 Hz, 1 H); 4.34 (t, J=4.5 Hz, 1 H); 4.27 (m, 1 H); 4.02 (m, 2 H); 2.80 (s, 3 H); 2.76 (s, 3 H); 2.31 (m, 1 H); 2.09 (m, 1 H); 1.90 – 1.85 (m, 2 H); 1.76 (m, 1 H); 1.63 - 1.48 (m, 4 H); 1.41 – 1.29 (m, 2 H); 1.24 (m, 1 H).

^{13}C-NMR (APT) (75 MHz, Acetone-d_{6}) δ = 59.78 (CH), 58.14 (CH), 56.14 (CH), 54.22 (CH), 53.11 (CH), 52.06 (CH), 37.73 (CH_{2}), 36.14 (CH_{2}), 30.75 (CH_{2}), 29.24 (CH_{2}), 22.84 (CH_{2}), 21.39 (CH_{2}), 18.55 (2 x CH_{3}).

MS (ESI+) m/z (%) = 479 (100) [MH]^{+}. 
(3-(cyclopropylmethoxy)-6-methylpyridin-2-yl) (+/-)-endo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9h)

**General Procedure B** was followed using the 3-(cyclopropylmethoxy)-6-methylpicolinic acid (50 mg, 0.241 mmol), amine 11a (51.7 mg, 0.201 mmol), NMM (0.066 mL, 0.603 mmol) and CDMT (53.0 mg, 0.302 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9h was obtained as a white solid (70.4 mg, 0.16 mmol, 78%).

**ANALYSIS**

**Formula:** C_{23}H_{25}F_{3}N_{4}O_{2}

**Mol. Weight:** 446.47 g/mol

**Note:** From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

**^1H NMR** (400 MHz, Acetone-\(d_6\)) \(\delta = 8.41\) (s, 1 H); \(8.22\) (s, 1 H); \(7.67\) (dd, \(J=8.8, 2.3\) Hz, 1 H); \(7.60\) (dd, \(J=8.8, 2.4\) Hz, 1 H); \(7.41\) (t, \(J= 8.7\) Hz, 2 H); \(7.23\) (dd, \(J= 8.2, 7.4\) Hz, 2 H); \(6.87\) (m, 2 H); \(6.73\) (d, \(J=8.8\) Hz, 1 H); \(6.64\) (d, \(J=8.8\) Hz, 1 H); \(5.08\) (t, \(J=4.6\) Hz, 1 H); \(4.69\) (t, \(J=4.9\) Hz, 1 H); \(4.57\) (m, 1 H); \(4.45\) (m, 1 H); \(4.04\) (t, \(J=4.5\) Hz, 1 H); \(4.00 – 3.91\) (m, 4 H); \(3.73\) (t, \(J=5.0\) Hz, 1 H); \(2.55 – 2.35\) (m, 2 H); \(2.46\) (s, 3 H); \(2.43\) (s, 3 H); \(2.10 – 1.80\) (m, 4 H); \(1.74 – 1.56\) (m, 4 H); \(1.41 – 1.21\) (m, 4 H); \(0.58\) (m, 4 H); \(0.37\) (m, 4 H).

**^13C-NMR (APT)** (50 MHz, Acetone-\(d_6\)) \(\delta = 73.55\) (CH\(_2\)), \(59.13\) (CH), \(57.50\) (CH), \(55.70\) (CH), \(53.81\) (CH), \(52.99\) (CH), \(52.14\) (CH), \(37.63\) (CH\(_2\)), \(36.17\) (CH\(_2\)), \(30.74\) (CH\(_2\)), \(29.39\) (CH\(_2\)), \(22.75\) (CH\(_2\)), \(22.62\) (CH), \(21.50\) (CH\(_2\)), \(10.22\) (CH), \(2.86\) (CH\(_2\)).

**MS (ESI+)** m/z (%) = 448 (100) [MH]^+.
(6-methyl-3-(pyrimidin-2-yl)pyridin-2-yl) (+/-)-endo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9i)

General Procedure B was followed using the 6-methyl-3-(pyrimidin-2-yl)picolinic acid (65.3 mg, 0.303 mmol), amine 11a (65 mg, 0.253 mmol), NMM (0.083 mL, 0.758 mmol) and CDMT (66.5 mg, 0.379 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9i was obtained as a white-pink solid (65.4 mg, 0.14 mmol, 56%).

ANALYSIS
Formula: C\textsubscript{23}H\textsubscript{21}F\textsubscript{3}N\textsubscript{6}O
Mol. Weight: 454.45 g/mol
Note: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

\textsuperscript{1}H NMR (400 MHz, Acetone-d\textsubscript{6}) \(\delta = 8.88\) (m, 4 H); \(8.41\) (m, 3H); \(8.21\) (m, 1 H); \(7.67\) (dd, J= 8.8, 2.4 Hz, 1 H); \(7.59\) (dd, J= 8.9, 2.4 Hz, 1 H); \(7.49 - 7.38\) (m, 4 H); \(6.89\) (m, 1H); \(6.84\) (m, 1H); \(6.74\) (d, J= 8.8 Hz, 1 H); \(6.62\) (d, J= 8.8 Hz, 1 H); \(5.02\) (t, J=4.6 Hz, 1 H); \(4.66\) (t, J=4.8 Hz, 1 H); \(4.63\) (m, 1 H); \(4.33\) (t, J=4.6 Hz, 1 H); \(3.99\) (t, J=4.7 Hz, 1 H); \(2.64 - 2.49\) (m, 2 H); \(2.65\) (s, 3 H); \(2.61\) (s, 3 H); \(2.12 - 1.92\) (m, 5 H); \(1.86 - 1.75\) (m, 2 H); \(1.73 - 1.56\) (m, 2 H); \(1.45 - 1.39\) (m, 1 H); \(1.33 - 1.29\) (m, 1 H).

\textsuperscript{13}C-NMR (APT) (50 MHz, Acetone-d\textsubscript{6}) \(\delta = 59.30\) (CH), \(57.64\) (CH), \(55.62\) (CH), \(53.64\) (CH), \(52.86\) (CH), \(52.10\) (CH), \(37.67\) (CH\textsubscript{2}), \(36.57\) (CH\textsubscript{2}), \(30.62\) (CH\textsubscript{2}), \(29.42\) (CH\textsubscript{2}), \(23.70\) (2 x CH\textsubscript{3}), \(22.65\) (CH\textsubscript{2}), \(21.64\) (CH\textsubscript{2}).

MS (ESI+) m/z (%) = 456 (100) [MH]\textsuperscript{+}.
(5-methyl-2-(pyrimidin-2-yl)phenyl) (+/-)-endo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9j)

**General Procedure B** was followed using the 5-methyl-2-(pyrimidin-2-yl)benzoic acid (65.0 mg, 0.303 mmol), amine 11a (65 mg, 0.253 mmol), NMM (0.083 mL, 0.758 mmol) and CDMT (66.5 mg, 0.379 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9j was obtained as a brown solid (74.7 mg, 0.16 mmol, 64.5%).

**ANALYSIS**

**Formula:** C$_{24}$H$_{22}$F$_3$N$_5$O  
**Mol. Weight:** 453.46 g/mol  
**Note:** From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

$^1$H NMR (400 MHz, Acetone-$_d_6$) $\delta$ = 8.85 (m, 4 H); 8.41 (m, 1H); 8.21 (m, 1 H); 8.07 (d, J= 7.9 Hz, 2 H); 7.67 (dd, J=8.8, 2.3 Hz, 1 H); 7.58 (dd, J=8.9, 2.3 Hz, 1 H); 7.38 (m, 6 H); 6.85 (m, 1H); 6.80 (m, 1H); 6.72 (d, J= 8.8 Hz, 1 H); 6.59 (d, J= 8.9 Hz, 1 H); 4.96 (t, J=4.6 Hz, 1 H); 4.61 (t, J=4.7 Hz, 1 H); 4.53 (m, 1 H); 4.23 (t, J=4.6 Hz, 1 H); 3.87 (t, J=4.9 Hz, 1 H); 2.54 – 2.45 (m, 3 H); 2.48 (s, 3 H); 2.46 (s, 3 H); 2.03 (m, 1 H); 1.93 (m, 3 H); 1.81 – 1.52 (m, 4 H); 1.37 (m, 1 H); 1.25 (m, 1 H).

$^{13}$C-NMR (APT) (50 MHz, CDCl$_3$) $\delta$ 59.90 (CH), 58.20 (CH), 55.47 (CH), 53.67 (CH), 53.11 (CH), 52.36 (CH), 39.18 (CH$_2$), 37.77 (CH$_2$), 31.00 (CH$_2$), 29.72 (CH$_2$), 22.70 (CH$_2$), 21.67 (CH$_2$), 21.46 (CH$_3$).

**MS (ESI+)** m/z (%) = 454 (100) [MH]$^+$.
(5-chloro-2-(pyrimidin-2-yl)phenyl) (+/-)-endo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (9k)

General Procedure B was followed using the 5-chloro-2-(pyrimidin-2-yl)benzoic acid (71.1 mg, 0.303 mmol), amine 11a (65 mg, 0.253 mmol), NMM (0.083 mL, 0.758 mmol) and CDMT (66.5 mg, 0.379 mmol) in dioxane (2.5 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 9k was obtained as a white-purple solid (88.4 mg, 0.19 mmol, 73.8%).

ANALYSIS
Formula: C_{23}H_{19}ClF_3N_5O
Mol. Weight: 473.88 g/mol
Note: From NMR spectra were observed two signal patterns in about 1:1 ratio due to the two rotamers of the molecule. Chemical shifts of both rotamers are reported.

\(^{1}\text{H NMR}\) (400 MHz, Acetone-\(d_6\)) \(\delta = 8.88\) (m, 4 H); \(8.41\) (m, 1H); \(8.20\) (m, 3 H); \(7.70 - 7.52\) (m, 6 H); \(7.46\) (m, 2 H); \(6.87\) (m, 1H); \(6.82\) (m, 1H); \(6.73\) (d, J= 8.8 Hz, 1 H); \(6.61\) (d, J= 8.9 Hz, 1 H); \(4.97\) (t, J=4.6 Hz, 1 H); \(4.62\) (t, J=4.6 Hz, 1 H); \(4.53\) (m, 1 H); \(4.25\) (t, J=4.5 Hz, 1 H); \(3.91\) (t, J=4.9 Hz, 1 H); \(2.57 - 2.47\) (m, 2 H); \(2.05\) (m, 2 H); \(1.95\) (m, 3 H); \(1.81 - 1.55\) (m, 4 H); \(1.39\) (m, 1 H); \(1.29\) (m, 1 H).

\(^{13}\text{C-NMR (APT)}\) (75 MHz, Acetone-\(d_6\)) \(\delta = 60.34\) (CH); \(58.67\) (CH); \(55.92\) (CH); \(53.99\) (CH); \(52.99\) (CH); \(52.01\) (CH); \(37.79\) (CH2), \(36.45\) (CH2), \(30.80\) (CH2), \(22.74\) (CH2), \(21.63\) (CH2), (one CH2 signal obscured by acetone-\(d_6\) signals).

\(\text{MS (ESI)}\) m/z (%) = 474 (100) [MH]+.
2.5 Synthesis of exo-TYPE II derivatives 12a-i, experimental data

(+/-)-exo-tert-butyl 2-((4-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (13a)

A mixture of 8 (90 mg, 0.339 mmol), 2-fluoro-4-trifluoromethyl-pyridine (85 mg, 0.515 mmol) and K$_2$CO$_3$ (120 mg, 0.868 mmol) in DMF (1 mL) was stirred under N$_2$ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 13 h.

The solvent was then evaporated under reduced pressure and the residue taken up in NaHCO$_3$ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x20 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na$_2$SO$_4$ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 10g HP-Si Column, sample dissolved in DCM/MeOH, adsorbed on silica gel and loaded dry on column, eluent: from CyHex/AcOEt 100:0 to 70:30 in gradient.

The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 13a as white solid (88.7 mg; purity 92%; 0.23 mmol; 67.3%).

**ANALYSIS**

**Formula:** C$_{17}$H$_{22}$F$_3$N$_3$O$_2$

**Mol. Weight:** 357.37 g/mol

**Rf:** 0.75 (CyHex/AcOEt 5:5; SI-TLC)

**Note:** From $^1$H NMR analysis traces of endo-compound were observed (about 8%mol).

**$^1$H NMR** (400 MHz, CDCl$_3$) δ = 8.23 (d, J=5.3 Hz, 1 H); 6.76 (d, J=5.4 Hz, 1 H); 6.52 (s, 1 H); 4.93 (d, J=5.9 Hz, 1 H); 4.31 (brs, 1 H); 4.22 (d, J=4.9 Hz, 1 H); 3.97 (td, J=7.4, 3.0 Hz, 1 H); 2.05 (dd, J=13.0, 7.6 Hz, 1 H); 1.93 - 1.81 (m, 1 H); 1.81 - 1.70 (m, 1 H); 1.59 - 1.51 (m, 2 H); 1.45 (s, 9 H); 1.43 - 1.39 (m, 1 H).

**$^{13}$C-NMR (DEPT)** (100 MHz, CDCl$_3$) δ = 157.55 (C), 156.28 (C), 149.00 (CH), 139.63 (q, J=33.4, C), 122.93 (q, J=273.0, CF$_3$), 108.06 (CH), 103.95 (CH), 79.98 (C), 60.87 (CH), 55.73 (CH), 55.33 (CH), 40.62 (CH$_2$), 28.26 (CH$_2$), 28.22 (3 x CH$_3$), 25.94 (CH$_2$).

**MS (ESI+)** m/z (%) = 358 (100) [MH]$^+$. 
A mixture of 8 (90 mg, 0.339 mmol), 2-chloro-4-(trifluoromethyl)pyrimidine (95 mg, 0.520 mmol) and K$_2$CO$_3$ (115 mg, 0.832 mmol) in DMF (1 mL) was stirred under N$_2$ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 13 h.

The solvent was then evaporated under reduced pressure and the residue taken up in NaHCO$_3$ sat. sol. (10 mL) and the mixture was extracted with AcOEt (3x10 mL). The organic layers were collected, washed with water (30 mL), dried over anhydrous Na$_2$SO$_4$ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from DCM/AcOEt 100:0 to 80:20 in gradient.

The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 13b as white solid (66.8 mg; purity 80%; 0.15 mmol; 44.0%).

**ANALYSIS**

**Formula:** C$_{16}$H$_{21}$F$_3$N$_4$O$_2$

**Mol. Weight:** 358.36 g/mol

**Rf:** 0.63 (CyHex/AcOEt 5:5; SI-TLC)

$^1$H NMR (400 MHz, CDCl$_3$) δ = 8.51 (d, J=4.9 Hz, 1 H); 6.85 (d, J=4.9 Hz, 1 H); 5.55 (brs, 1 H); 4.32 (brs, 1 H); 4.23 (d, J=4.9 Hz, 1 H); 4.08 (td, J=7.7, 3.2 Hz, 1 H); 2.05 (dd, J=13.0, 7.6 Hz, 1 H); 1.92 - 1.73 (m, 2 H); 1.65 - 1.59 (m, 1 H); 1.57 - 1.50 (m, 1 H); 1.45 (s, 9 H); 1.43 - 1.38 (m, 1 H).

$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) δ = 161.47 (C), 160.35 (CH), 156.07 (C), 155.81 (q, J=32.8, C), 120.44 (q, J=276.3, CF$_3$), 105.75 (CH), 79.95 (C), 61.01 (CH), 55.54 (CH), 55.01 (CH), 40.22 (CH$_2$), 28.48 (CH$_2$), 28.19 (3 x CH$_3$), 25.90 (CH$_3$).

**MS (ESI+)** m/z (%) = 304 (100) [M-56H]^+. 
(+/-)-exo-tert-butyl 2-((5-(trifluoromethyl)pyrazin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (13c)

A mixture of 8 (90 mg, 0.339 mmol), 2-chloro-5-(trifluoromethyl)pyrazine (95 mg, 0.520 mmol) and K$_2$CO$_3$ (115 mg, 0.832 mmol) in DMF (1 mL) was stirred under N$_2$ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 13 h. The solvent was then evaporated under reduced pressure and the residue taken up in NaHCO$_3$ sat. sol. (10 mL) and the mixture extracted with AcOEt (3x20 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na$_2$SO$_4$ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-Si Column, sample dissolved in DCM, eluent: from DCM/AcOEt 100:0% to 80:20 in gradient. The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 13c as white-yellow solid (54.7 mg; 0.153 mmol; 45%).

**ANALYSIS**

**Formula:** C$_{15}$H$_{21}$F$_3$N$_4$O$_2$

**Mol. Weight:** 358.36 g/mol

$^1$H NMR (400 MHz, CDCl$_3$) δ = 8.34 (s, 1 H); 7.87 (d, J=1.0 Hz, 1 H); 5.36 (brs, 1 H); 4.33 (brs, 1 H); 4.24 (d, J=4.4 Hz, 1 H); 4.07 (td, J=7.3, 3.0 Hz, 1 H); 2.09 (dd, J=13.2, 7.8 Hz, 1 H); 1.93 - 1.83 (m, 1 H); 1.83 - 1.73 (m, 1 H); 1.58 - 1.48 (m, 2 H); 1.46 (s, 9 H); 1.43 (d, J=3.4 Hz, 1 H).

$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) δ = 156.45 (C), 154.50 (C), 139.84 (CH), 132.45 (CH), 131.78 (q, J=35.1, C), 122.32 (q, J=271.7, CF$_3$), 80.27 (C), 60.85 (CH), 56.01 (CH), 54.86 (CH), 40.49 (CH$_2$), 28.54 (CH$_2$), 28.23 (3 x CH$_3$), 25.88 (CH$_2$).

**MS (ESI+)** m/z (%) = 359 (100) [MH]$^+$. 
(+/-)-exo-tert-butyl 2-(2-phenylacetamido)-7-azabicyclo[2.2.1]heptane-7-carboxylate (13d)

To 8 (70 mg, 0.330 mmol) in dry DCM (2 mL) was added pyridine (0.080 mL, 0.989 mmol) followed by phenacetyl chloride (0.052 mL, 0.396 mmol). The resulting mixture was left to stir for 18 hours at room temperature.

Then the reaction was quenched by addition of NaHCO₃ sat. sol. (10 mL) and DCM (10 mL). After brief mixing, the organic layer was separated, dried over anhydrous Na₂SO₄ and then the solvent was eliminated under reduced pressure.

Purification: Isolera-One Biotage, Snap 10g HP-Si Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100:0 to 50:50 in gradient.

The containing product fractions were collected and the solvent eliminated under reduced pressure to give 13d as colorless oil (84 mg; purity 95%; 0.24 mmol; 73.2%).

**ANALYSIS**

**Formula:** C₁₃H₂₆N₂O₃  
**Mol. Weight:** 330.42 g/mol  

**H NMR** (400 MHz, CDCl₃) δ = 7.39 - 7.32 (m, 2 H); 7.32 - 7.29 (m, 1 H); 7.28 - 7.23 (m, 2 H); 5.74 (brs, 1 H); 4.20 (t, J=4.6 Hz, 1 H); 4.05 (d, J=5.4 Hz, 1 H); 4.03 – 3.98 (m, 1 H); 3.54 (s, 2 H); 1.94 (dd, J=13.2, 7.8 Hz, 1 H); 1.81 - 1.63 (m, 2 H); 1.48 (d, J=3.9 Hz, 1 H); 1.43 (s, 9 H); 1.41 - 1.30 (m, 2 H).

**C NMR (DEPT)** (100 MHz, CDCl₃) δ = 170.30 (C), 156.05 (C), 134.73 (C), 129.23 (CH), 128.91 (CH), 127.24 (CH), 79.95 (C), 61.23 (CH), 55.76 (CH), 53.12 (CH), 43.72 (CH₂), 40.47 (CH₂), 28.30 (CH₂), 28.24 (3 x CH₃), 26.02 (CH₂).

**MS (ESI+)** m/z (%) = 331 (100) [M-56H]⁺.
(+/-)-exo-tert-butyl 2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (13e)

A mixture of 8 (300 mg, 1.131 mmol), 2-fluoro-5-(trifluoromethyl)pyridine (0.190 mL, 1.574 mmol) and potassium carbonate (360 mg, 2.60 mmol) in DMF (3 mL) was stirred under N₂ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 2 h then at 85 °C for 18 h. The solvent was then evaporated under reduced pressure and the residue taken up in NaHCO₃ sat. sol. (30 mL) and the mixture extracted with AcOEt (3x30 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na₂SO₄ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 50g HP-SI Column, sample dissolved in DCM/MeOH, adsorbed on silica gel and loaded dry on column, eluent: from CyHex/AcOEt 100:0 to 70:30 in gradient.

The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 13e as white solid (295 mg, purity 93%, 0.767 mmol, 67.9%).

**ANALYSIS**

**Formula:** C₁₃H₂₂F₃N₃O₂

**Mol. Weight:** 357.37 g/mol

**Ref:** 0.70 CyHex/AcOEt 5:5 (TLC-SI)

**H NMR** (400 MHz, CDCl₃) δ = 8.35 (s, 1 H); 7.58 (dd, J=8.8, 2.4 Hz, 1 H); 6.38 (d, J=8.8 Hz, 1 H); 5.16 (brs, 1 H); 4.31 (brs, 1 H); 4.23 (d, J=4.9 Hz, 1 H); 4.00 (brs, 1 H); 2.07 (dd, J=13.0, 7.6 Hz, 1 H); 1.92 - 1.71 (m, 2 H); 1.62 - 1.47 (m, 3 H); 1.45 (s, 9 H); 1.44 - 1.38 (m, 1 H).

**C-NMR (DEPT)** NMR (100 MHz, CDCl₃) δ = 159.09 (C), 156.33 (C), 145.95 (CH), 134.11 (CH), 124.46 (q, J=270.2, CF₃), 115.59 (q, J=33.0, C), 107.35 (CH), 80.01 (C), 60.90 (CH), 55.80 (CH), 55.24 (CH), 40.55 (CH₂), 28.56 (CH₂), 28.22 (3 x CH₃), 25.95 (CH₃).

**MS (ESI+)** m/z (%) = 358 (100) [MH]+.
General Procedure C: synthesis of compounds 14a-e

Compound 13a-e (1eq) was dissolved in a solution of DCM (Ratio: 4.0) and TFA (Ratio: 1.0; 30 eq) and then the mixture was stirred at room temperature for 1 h. Then the solvent was evaporated under reduced pressure. Purification: the residue was taken up in MeOH and loaded on SCX column, eluent MeOH then Ammonia (2M in MeOH). The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 14a-e.

(+/-)-exo-N-(4-(trifluoromethyl)pyridin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (14a)

General Procedure C was followed using 13a (70 mg, 0.180 mmol) to give 14a as white solid (46.5 mg; purity 90%; 0.163 mmol, 90%).

ANALYSIS
Formula: C_{12}H_{14}F_{3}N_{3}
Mol. Weight: 257.25 g/mol

$^1$H NMR (400 MHz, CDCl$_3$) δ = 8.23 (d, J=5.4 Hz, 1 H); 6.75 (d, J=5.4 Hz, 1 H); 6.53 (s, 1 H); 4.94 (d, J=6.4 Hz, 1 H); 3.82 (td, J=7.5, 3.2 Hz, 1 H); 3.73 (t, J=4.4 Hz, 1 H); 3.58 (d, J=4.9 Hz, 1 H); 1.98 (dd, J=12.7, 7.8 Hz, 1 H); 1.67 - 1.33 (m, 5 H).

$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) δ = 158.15 (C), 149.42 (CH), 139.40 (q, J=33.3, C), 123.03 (q, J=123.03, CF$_3$), 108.02 (CH), 103.65 (CH), 61.38(CH), 55.62 (CH), 54.71 (CH), 40.51 (CH$_2$), 29.47 (CH$_2$), 26.67 (CH$_2$).

MS (ESI+) m/z (%) = 258 (100) [MH]$^+$. 

(+/-)-exo-N-(4-(trifluoromethyl)pyrimidin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (14b)

ANALYSIS
Formula: C_{12}H_{14}F_{3}N_{3}
Mol. Weight: 257.25 g/mol

$^1$H NMR (400 MHz, CDCl$_3$) δ = 8.23 (d, J=5.4 Hz, 1 H); 6.75 (d, J=5.4 Hz, 1 H); 6.53 (s, 1 H); 4.94 (d, J=6.4 Hz, 1 H); 3.82 (td, J=7.5, 3.2 Hz, 1 H); 3.73 (t, J=4.4 Hz, 1 H); 3.58 (d, J=4.9 Hz, 1 H); 1.98 (dd, J=12.7, 7.8 Hz, 1 H); 1.67 - 1.33 (m, 5 H).

$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) δ = 158.15 (C), 149.42 (CH), 139.40 (q, J=33.3, C), 123.03 (q, J=123.03, CF$_3$), 108.02 (CH), 103.65 (CH), 61.38(CH), 55.62 (CH), 54.71 (CH), 40.51 (CH$_2$), 29.47 (CH$_2$), 26.67 (CH$_2$).

MS (ESI+) m/z (%) = 258 (100) [MH]$^+$. 

(+/-)-exo-N-(4-(trifluoromethyl)pyrimidin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (14b)
**General Procedure C** was followed using 13b (55 mg, 0.123 mmol) to give 14b as white-yellow solid (40.0 mg; purity 80%; 0.124 mmol, quant).

**ANALYSIS**

**Formula**: C\(_{11}\)H\(_{13}\)F\(_3\)N\(_4\)

**Mol. Weight**: 258.24 g/mol

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 8.57 - 8.42\) (m, 1 H); 6.83 (d, J=4.9 Hz, 1 H); 5.68 (brs, 1 H); 4.02 (td, J=7.7, 3.2 Hz, 1 H); 3.78 (t, J=4.4 Hz, 1 H); 3.66 (d, J=4.9 Hz, 1 H); 2.17 (brs, 1 H); 1.99 (dd, J=13.2, 7.8 Hz, 1 H); 1.78 - 1.36 (m, 5 H).

\(^{13}\)C-NMR (DEPT) (100 MHz, CDCl\(_3\)) \(\delta = 161.67\) (C), 160.33 (CH), 156.37 (q, J=33.6, C), 120.49 (q, J=275.02, CF\(_3\)), 105.52 (CH), 61.55 (CH), 55.65 (CH), 54.49 (CH), 40.28 (CH\(_2\)), 29.40 (CH\(_2\)), 26.71 (CH\(_2\)).

**MS (ESI+)** m/z (%) = 259 (100) [MH\(^+\)].

\((\pm\righthook)\)-exo-N-(5-(trifluoromethyl)pyrazin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (14c)

![Chemical Structure]

**General Procedure C** was followed using 13c (50 mg, 0.140 mmol) to give 14c as white solid (34.0 mg; 0.130 mmol, 93%).

**ANALYSIS**

**Formula**: C\(_{11}\)H\(_{13}\)F\(_3\)N\(_4\)

**Mol. Weight**: 258.24 g/mol

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 8.33\) (s, 1 H); 7.88 (s, 1 H); 5.44 (d, J=7.3 Hz, 1 H); 4.01 (td, J=7.8, 2.9 Hz, 1 H); 3.81 (t, J=4.4 Hz, 1 H); 3.65 (d, J=4.9 Hz, 1 H); 2.38 (brs, 1 H); 2.00 (dd, J=12.7, 7.8 Hz, 1 H); 1.74 - 1.55 (m, 3 H); 1.55 - 1.44 (m, 2 H).

\(^{13}\)C-NMR (DEPT) (100 MHz, CDCl\(_3\)) \(\delta = 154.76\) (C), 139.86 (CH), 131.59 (q, J=35.1), 132.57 (CH), 122.36 (q, J=271.53, CF\(_3\)), 61.57 (CH), 55.75 (CH), 53.80 (CH), 39.83 (CH\(_2\)), 29.57 (CH\(_2\)), 26.71 (CH\(_2\)).

**MS (ESI+)** m/z (%) = 260 (100) [MH\(^+\)].
N-((+/-)-exo-7-azabicyclo[2.2.1]heptan-2-yl)-2-phenylacetamide (14d)

General Procedure C was followed using 13d (67 mg, 0.193 mmol) to give 14d as white-yellow solid (46 mg; purity 90%; 0.180 mmol, 93%).

ANALYSIS
Formula: C_{14}H_{18}N_{2}O
Mol. Weight: 230.31 g/mol

^{1}H NMR (400 MHz, CDCl_{3}) δ = 7.39 - 7.32 (m, 2 H); 7.32 - 7.29 (m, 2 H); 7.27 (s, 1 H); 5.93 - 5.67 (m, 1 H); 3.93 (td, J=8.1, 2.9 Hz, 1 H); 3.68 - 3.63 (m, 1 H); 3.54 (s, 2 H); 3.47 (d, J=4.4 Hz, 1 H); 2.02 (broad, 1 H); 1.83 (dd, J=13.0, 8.1 Hz, 1 H); 1.61 - 1.42 (m, 3 H); 1.42 - 1.33 (m, 1 H); 1.30 - 1.21 (m, 1 H).

^{13}C-NMR (DEPT) (100 MHz, CDCl_{3}) δ = 170.31 (C), 135.08 (C), 129.25 (CH), 128.82 (CH), 127.11 (CH), 62.01 (CH), 55.51 (CH), 52.14 (CH), 43.79 (CH_{2}), 39.71 (CH_{2}), 29.54 (CH_{2}), 27.07 (CH_{2}).

MS (ESI+): m/z (%) = 232.1 (100) [MH^{+}].

(+/-)-exo-N-(5-(trifluoromethyl)pyridin-2-yl)-7-azabicyclo[2.2.1]heptan-2-amine (14e)

General Procedure C was followed using 13e (275 mg, 0.770 mmol) to give 14e as white solid (186 mg, 0.716 mmol, 93%).

ANALYSIS
Formula: C_{12}H_{14}F_{3}N_{3}
Mol. Weight: 257.25 g/mol

^{1}H NMR (400 MHz, CDCl_{3}) δ = 8.35 (s, 1 H); 7.56 (dd, J=8.8, 2.45 Hz, 1 H); 6.39 (d, J=8.8 Hz, 1 H); 5.10 (d, J=6.4 Hz, 1 H); 3.87 (td, J=7.5, 3.2 Hz, 1 H); 3.75 (t, J=4.4 Hz, 1 H); 3.60 (d, J=4.9 Hz, 1 H); 1.98 (dd, J=13.0, 7.6 Hz, 1 H); 1.68 - 1.38 (m, 5 H).
$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) $\delta =$ 159.58 (C), 146.12 (CH), 134.07 (CH), 124.63 (q, J=270.2, CF$_3$), 115.36 (q, J=32.9, C), 106.93 (CH), 61.23(CH), 55.47 (CH), 54.75 (CH), 40.77 (CH$_2$), 29.77 (CH$_2$), 26.96 (CH$_2$).

**MS (ESI+)** m/z (%) = 258 (100) [MH]$^+$. 
General Procedure D: synthesis of (+/-)-exo-compounds 12a-i

To a solution of the appropriate carboxylic acid (1.0 eq) in dioxane (1 mL) were added in sequence N-methylmorpholine (3 eq) and CDMT (1.5 eq) under N₂ atmosphere and the resultant solution was stirred for 30 min at room temperature. Then the amine 14a-e was added (1.2 eq) pre-dissolved in dioxane (1 mL) and the mixture was stirred at 100 °C for 1 h. The solvent was then evaporated under reduced pressure. The residue was taken up in AcOEt and washed in sequence with NH₄Cl sat. sol., NaHCO₃ sat. sol. and water. Then the organic layer was dried over anhydrous Na₂SO₄, filtered and the solvent was eliminated under reduced pressure.

Purification: Isolera-One Biotage, flash chromatography by HP-SI Column or Reverse Phase Column. The containing product fractions were collected and the solvent eliminated under reduced pressure to give the final compound 12a-i as (+/-)-exo-racemic mixture.

Analysis of proton and carbon NMR spectra reveals for compounds 12a-i the presence of two rotamers of the molecule in ratio ranging from 8:3 to 7:3. In particular, in proton NMR spectra the major rotamer can be easily detected and described. On the contrary, carbon NMR spectra are complicated by the presence of signals splitted by the presence of C-F coupling and the Cₛ⁻² carbon atom signals are difficult to distinguish and assign. For these reasons in carbon NMR spectra only the chemical shifts of aliphatic carbons are reported for both rotamers of each compound.

(2-methyl-5-phenylthiazol-4-yl) (+/-)-exo-2-((4-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (12a)

General Procedure D was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (35 mg, 0.160 mmol), amine 14a (30 mg, 0.105 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (33 mg, 0.188 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 12a was obtained as white solid (43 mg; 0.092 mmol; 88%).

ANALYSIS

Formula: C₂₃H₂₁F₃N₄OS
**Mol. Weight:** 458.50 g/mol  

**Note:** From NMR spectra two signal patterns were observed (about 7:3 ratio in Acetone-$d_6$) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

**$^1$H NMR (400 MHz, Acetone-$d_6$) (major rotamer)** $\delta = 8.18$ (d, $J=4.8$, 1H); 7.61 - 7.55 (m, 2H); 7.46 - 7.37 (m, 3H); 6.97 (d, $J=4.8$, 1H); 6.44 (brs, 1H); 4.75 (t, $J=4.7$, 1H); 4.29 (m, 1H); 4.03 (m, 1H); 2.63 (s, 3H); 2.10 (m, 1H); 1.87 - 1.35 (m, 5H).

**$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) (major rotamer)** $\delta = 61.46$ (CH), 58.31 (CH), 57.28 (CH), 55.34 (CH), 54.31 (CH), 53.28 (CH), 40.71 (CH$_2$), 40.25 (CH$_2$), 29.35 (CH$_2$), 27.98 (CH$_2$), 27.4 (CH$_2$)$_2$, 24.98 (CH$_2$), 19.15 (CH$_3$), 18.76 (CH$_3$).

**MS (ESI+) m/z (%) = (100) [MH]$^+$**

(2-methyl-5-phenylthiazol-4-yl) (+/-)-exo-2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)-7-azabicyclo[2.2.1]heptane-7-carboxylate (12b)

**General Procedure D** was followed using 2-methyl-5-phenylthiazole-4-carboxylic acid (30 mg, 0.137 mmol), amine 14b (25 mg, 0.097 mmol), NMM (0.032 mL, 0.290 mmol) and CDMT (30 mg, 0.171 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) **12b** was obtained as white solid (32mg; 0.070 mmol; 71.9%).

**ANALYSIS**

**Formula:** $C_{22}H_{20}F_3N_3OS$  

**Mol. Weight:** 459.49 g/mol  

**Note:** From NMR spectra were observed two signal patterns (about 7:3 ratio in Acetone-$d_6$) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

**$^1$H NMR (400 MHz, Acetone-$d_6$) (major rotamer)** $\delta = 8.59$ (d, $J=4.8$, 1H); 7.61 - 7.55 (m, 2H); 7.46 - 7.37 (m, 3H); 6.97 (d, $J=4.8$, 1H); 6.44 (brs, 1H); 4.75 (t, $J=4.7$, 1H); 4.29 (m, 1H); 4.03 (m, 1H); 2.63 (s, 3H); 2.10 (m, 1H); 1.87 - 1.35 (m, 5H).

**$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) (major rotamer)** $\delta = 61.46$ (CH), 58.31 (CH), 57.28 (CH), 55.34 (CH), 54.31 (CH), 53.28 (CH), 40.71 (CH$_2$), 40.25 (CH$_2$), 29.35 (CH$_2$), 27.98 (CH$_2$), 27.4 (CH$_2$)$_2$, 24.98 (CH$_2$), 19.15 (CH$_3$), 18.76 (CH$_3$).
**General Procedure D** was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (30 mg, 0.137 mmol), amine 14c (25 mg, 0.097 mmol), NMM (0.032 mL, 0.290 mmol) and CDMT (30 mg, 0.171 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) **12c** was obtained as white solid (36.0 mg; 0.078 mmol; 81%).

**ANALYSIS**

**Formula**: C_{22}H_{20}F_{3}N_{3}O_{5}

**Mol. Weight**: 459.49 g/mol

**Note**: From NMR spectra in Acetone-d₆ there were two signal patterns in about 75:25 ratio due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

**1H NMR** (400 MHz, Acetone-d₆) (major rotamer) δ = 8.30 (s, 1H); 7.99 (s, 1H); 7.52 - 7.48 (m, 2H); 7.45 - 7.39 (m, 3H); 7.10 (brs, 1H); 4.71 (t, J=4.8, 1H); 4.19 (d, J=5.3 Hz, 1H); 3.95 (m, 1H); 2.48 (s, 3H); 2.10 (m, 1H); 1.82 – 1.21 (m, 5H).

**13C-NMR (DEPT)** (100 MHz, CDCl₃) (major rotamer) δ = 61.10 (CH), 58.22 (CH), 57.67 (CH), 55.08 (CH), 54.42 (CH), 53.19 (CH), 41.01 (CH₂), 39.31 (CH₂), 29.10 (CH₂), 28.32 (CH₂), 26.14 (CH₂), 25.30 (CH₂), 18.79 (CH₃), 18.63 (CH₃).

**MS (ESI+)** m/z (%) = 461 (100) [MH]⁺.
General Procedure D was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (37.1 mg, 0.169 mmol), amine 14d (30 mg, 0.130 mmol), NMM (0.043 mL, 0.391 mmol) and CDMT (36.6 mg, 0.208 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 12d was obtained as white-yellow solid (43.5 mg; 0.097 mmol; 74.3%).

ANALYSIS
Formula: C_{25}H_{25}N_{3}O_{2}S
Mol. Weight: 431.55 g/mol
Note: From NMR spectra were observed two signal patterns (about 7:3 ratio in Acetone-\textit{d}_6) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

^{1}H NMR (400 MHz, Acetone-\textit{d}_6) (major rotamer) $\delta = 7.61 - 7.55$ (m, 2H); 7.47 - 7.36 (m, 3H); 7.36 - 7.17 (m, 5H); 4.66 (t, J=4.8 Hz, 1H); 4.00 (d, J=5.1 Hz, 1H); 3.80 (m, 1H); 3.47 (d, J=14.5 Hz, 1H); 3.39 (d, J=14.5 Hz, 1H); 2.72 (s, 3H); 1.97 (m, 1H); 1.69 – 1.25 (m, 5H).

^{13}C-NMR (DEPT) (100 MHz, CDCl\textsubscript{3}) (major rotamer) $\delta = 62.09$ (CH), 58.94 (CH), 57.54 (CH), 53.68 (CH), 52.92 (CH), 52.62 (CH), 43.53 (CH\textsubscript{2}), 43.29 (CH\textsubscript{2}), 40.48 (CH\textsubscript{2}), 39.63 (CH\textsubscript{2}), 28.87 (CH\textsubscript{2}), 28.01 (CH\textsubscript{2}), 26.42 (CH\textsubscript{2}), 25.33 (CH\textsubscript{2}), 19.00 (2 x CH\textsubscript{3}).

MS (ESI+) m/z (%) = 433.2 (100) [MH]\textsuperscript{+}. 

\textbf{N-\textit{(+/-)-exo-7-(2-methyl-5-phenylthiazole-4-carbonyl)-7-azabicyclo[2.2.1]heptan-2-yl)-2-phenylacetamide (12d)}
(2-methyl-5-phenylthiazol-4-yl)((+/-)-exo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptan-7-yl)methanone (12e)

\[
\text{General Procedure D was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (35 mg, 0.160 mmol), amine 14e (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (33 mg, 0.188 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) was obtained 12e as white solid (46.5 mg, 0.100 mmol, 86%).}
\]

**ANALYSIS**

**Formula:** C_{23}H_{21}F_{3}N_{4}OS

**Mol. Weight:** 458.50 g/mol

**Note:** From NMR spectra were observed two signal patterns (about 7:3 ratio in CDCl\textsubscript{3}) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

\( ^{1}\text{H NMR} \) (400 MHz, CDCl\textsubscript{3}) (major rotamer) \( \delta = 8.29 \) (s, 1H); 7.54 - 7.36 (m, 6H); 6.35 (d, J=8.8 Hz, 1H); 5.93 (brs, 1H); 4.86 (t, J=4.9, 1H); 4.06 (m, 2H); 2.64 (s, 3H); 2.12 (m, 1H); 1.85 – 1.20 (m, 5H).

\( ^{13}\text{C-NMR (DEPT)} \) (1001 MHz, CDCl\textsubscript{3}) (major rotamer) \( \delta = 61.61 \) (CH), 55.13 (CH), 53.07 (CH), 39.99 (CH\textsubscript{2}), 28.22 (CH\textsubscript{2}), 26.42 (CH\textsubscript{2}), 18.96 (CH\textsubscript{3}).

**MS (ESI+) m/z (%) = 460 (100) [MH]+**

(6-methyl-3-phenylpyridin-2-yl)((+/-)-exo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptan-7-yl)methanone (12f)

86
General Procedure D was followed using the 6-methyl-3-phenylpicolinic acid (30 mg, 0.141 mmol), amine 14e (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (31 mg, 0.177 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 12f was obtained as white solid (43 mg; 0.093 mmol; 80%).

**ANALYSIS**

**Formula:** C_{25}H_{23}F_{3}N_{4}O  
**Mol. Weight:** 452.47 g/mol

**Note:** From NMR spectra analysis two signal patterns were observed (about 7:3 ratio in CDCl₃) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

**¹H NMR** (400 MHz, CDCl₃) (major rotamer) δ = 8.25 (s, 1H); 7.67 (d, J=8.0 Hz, 1H); 7.52 - 7.39 (m, 6H); 7.22 (d, J=8 Hz, 1H); 6.45 (d, J=8.8 Hz, 1H); 6.33 (brs, 1H); 4.81 (t, J=4.9, 1H); 4.07 (m, 1H); 3.67 (d, J=5.3 Hz 1H); 2.62 (s, 3H); 2.07 (m, 1H); 1.88 – 1.10 (m, 5H).

**³¹C-NMR (DEPT)** (100 MHz, CDCl₃) (major rotamer) δ = 60.94 (CH), 58.06 (CH), 56.31 (CH), 55.07 (CH), 53.84 (CH), 52.51 (CH), 40.64 (CH₂), 40.27 (CH₂), 29.02 (CH₂), 28.39 (CH₂), 26.36 (CH₂), 25.3 (CH₂)7, 24.12 (CH₃), 23.97 (CH₃).

**MS (ESI+)** m/z (%) = 454 (100) [MH]^+.

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(6-methyl-3-(pyrimidin-2-yl)pyridin-2-yl)((+/-)-exo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptan-7-yl)methanone (12g)

General Procedure D was followed using the 6-methyl-3-(pyrimidin-2-yl)picolinic acid (30 mg, 0.141 mmol), amine 14e (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (32 mg, 0.182 mmol) in dioxane (2 mL). After purification (RediSep Rf Gold AqC18 30g Column, sample dissolved in DMSO+AcOH, eluent additive 0.1% AcOH, eluent: from water/CH₃CN 100:0 to 0:100 in gradient) 12g was obtained as white solid (37 mg; 0.081 mmol; 69.8%).

**ANALYSIS**

**Formula:** C_{23}H_{21}F_{3}N_{6}O  
**Mol. Weight:** 454.45 g/mol
Note: From NMR spectra were observed two signal patterns (about 7:3 ratio in Acetone-\(d_6\)) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

\(^1\)H NMR (400 MHz, Acetone-\(d_6\)) (major rotamer) \(\delta = 8.92\) (d, J=4.9 Hz, 2H); 8.26 (m, 2H); 7.53 (dd, J=8.8, 1.9 Hz, 1H); 7.49 (t, J=4.9 Hz, 1H); 7.37 (d, J=8.0 Hz, 1H); 6.45 (d, J=8.8 Hz, 1H); 6.33 (brs, 1H); 4.72 (t, J=4.9 Hz, 1H); 4.26 (m, 1H); 4.18 (d, J=5.3 Hz, 1H); 2.52 (s, 3H); 2.19 (m, 1H); 1.90 – 1.57 (m, 5H).

\(^13\)C-NMR (DEPT) (100 MHz, DMSO-\(d_6\)) (major rotamer) \(\delta = 60.79\) (CH), 58.20 (CH), 56.36 (CH), 39.87 (CH2), 38.01 (CH2), 28.73 (CH2), 28.47 (CH2), 24.42 (CH3), 23.80 (2 x CH3).

\(\text{MS (ESI+)}\) m/z (%) = 456 (100) [MH]+.

(5-methyl-2-(pyrimidin-2-yl)phenyl)((+/-)-exo-2-((5-(trifluoromethyl)pyridin-2-yl)amino)-7-azabicyclo[2.2.1]heptan-7-yl)methanone (12h)

General Procedure D was followed using the 5-methyl-2-(pyrimidin-2-yl)benzoic acid (30.0 mg, 0.140 mmol), amine 14e (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (32 mg, 0.182 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 12h was obtained as white-yellow solid (47 mg; 0.103 mmol; 88%).

ANALYSIS

Formula: \(\text{C}_{24}\text{H}_{22}\text{F}_{3}\text{N}_{3}\text{O}\)

Mol. Weight: 453.46 g/mol

Note: From NMR spectra analysis two signal patterns were observed (about 8:2 ratio in CDCl\(_3\)) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) (major rotamer) \(\delta = 8.85\) (d, J=4.9 Hz, 2H); 8.24 (s, 1H); 7.87 (d, J=8.5 Hz, 1H); 7.57 (m, 1H); 7.43 – 7.359 (m, 2H); 7.24 (s, 1H); 6.54 (brs, 1H); 4.68 (t, J=4.6 Hz, 1H); 4.21 (m, 1H); 4.02 (d, J=4.9 Hz, 1H); 2.20 (s, 3H); 2.14 (m, 1H); 1.90 – 1.53 (m, 5H).

\(^13\)C-NMR (DEPT) (100 MHz, DMSO-\(d_6\)) (major rotamer) \(\delta = 61.45\) (CH), 57.94 (CH), 57.05 (CH), 55.08 (CH), 54.13 (CH), 52.11 (CH), 39.70 (CH2), 37.40 (CH2), 28.88 (CH2), 28.42 (CH2), 25.61 (2 x CH3), 21.33 (CH3), 20.79 (CH3).
**General Procedure D** was followed using the 5-chloro-2-(pyrimidin-2-yl)benzoic acid (33 mg, 0.141 mmol), amine 14e (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (32 mg, 0.182 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 12h was obtained as white-yellow solid (43 mg; purity 95%, 0.086 mmol; 73.9%).

**ANALYSIS**

**Formula:** C_{23}H_{19}ClF_{3}N_{3}O  
**Mol. Weight:** 473.88 g/mol  
**Note:** From NMR spectra analysis two signal patterns were observed (about 75:25 ratio in CDCl$_3$) due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.  
$^{1}$H NMR (400 MHz, CDCl$_3$) (major rotamer) δ = 8.85 (d, J=4.9 Hz, 2H); 8.26 (s, 1H); 7.99 (d, J=8.3 Hz, 1H); 7.46 (dd, J=8.4, 2.0 Hz, 1H); 7.34 (m, 2H); 6.01 (brs, 1H); 4.87 (t, J=4.2 Hz, 1H); 4.40 (m, 1H); 4.05 (d, J=3.7 Hz, 1H); 2.23 (m, 1H); 2.20 - 1.50 (m, 5H).  
$^{13}$C-NMR (DEPT) (100 MHz, CDCl$_3$) (major rotamer) δ = 63.55 (CH), 58.46 (CH), 57.67 (CH), 55.06 (CH), 54.88 (CH), 53.16 (CH), 41.58 (CH$_2$), 39.77 (CH$_2$), 29.04 (CH$_2$), 28.04 (CH$_2$), 26.72 (CH$_2$), 25.71 (CH$_2$).  
**MS (ESI+)** m/z (%) = 474 (100) [MH]$^+$. 

\[
\text{MS (ESI+)} \text{ m/z (\%)} = 454.2 \ (100) \ [\text{MH}]^+.
\]

\[(5\text{-chloro-2-(pyrimidin-2-yl)phenyl})(\pm\text{-exo-2-}(5\text{-trifluoromethyl}\text{pyridin-2-yl)amino})\text{-7-azabicyclo[2.2.1]heptan-7-yl)methanone (12i)}\]
2.6 Synthesis of (+/-)-endo- and (+/-)-exo-2-azabicyclo[2.2.1]heptan-6-amine (18 and 22), experimental data

(+/-)-2-benzyl-2-azabicyclo[2.2.1]hept-5-ene (15)

Benzylamine hydrochloride (6.54 g, 45.5 mmol) and formaldehyde 37% Wt in water (4.77 mL, 63.7 mmol) were dissolved in water (25 mL) and the resulting solution was stirred under N₂ for 5 min. Freshly distilled cyclopenta-1,3-diene (7.4 ml, 91 mmol) was then added and the resulting mixture was allowed to stir vigorously for 18 h at room temperature. Then the reaction mixture was extracted with CyHex/ Et₂O 1:1 (4x50 mL). The aqueous layer was made basic (pH about 11-12) with NaOH 2M and extracted with Et₂O (3x30 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated.

Purification: isolera-One Biotage, Snap 340g HP-Si Column, sample dissolved in DCM, eluent: from CyHex/ AcOEt 80:20 to 0:100 in gradient. The containing product fractions were collected and the solvent eliminated under reduced pressure to give 15 as pale yellow oil (7.25g, purity 95%, 37.2 mmol, 82%)

ANALYSIS

Formula: C₁₃H₁₅N
Mol. Weight: 185.26

¹H NMR (400 MHz, CDCl₃) δ = 7.39 - 7.29 (m, 4 H); 7.29 - 7.21 (m, 1 H); 6.40 (dd, J=4.9, 3.3 Hz); 6.11 (dd, J=5.7, 1.9 Hz, 1 H); 3.84 (d, J=1.2 Hz, 1 H); 3.60 (d, J=13.2 Hz, 1 H); 3.36 (d, J=13.2 Hz, 1 H); 3.19 (dd, J=8.8, 3.0 Hz, 1 H); 2.95 (brs, 1 H); 1.66 (d, J=8.8 Hz, 1 H); 1.55 (dd, J=8.6, 2.0 Hz, 1 H); 1.43 (dd, J=8.0, 1.5 Hz, 1 H).

¹³C NMR (DEPT) (100 MHz, CDCl₃) δ = 139.93 (C), 136.38 (CH), 130.95 (CH), 128.73 (CH), 128.20 (CH), 126.72 (CH), 64.40 (CH), 59.19 (CH₂Ph), 52.60 (CH₂), 48.17 (CH₂), 43.99 (CH).

MS (ESI+) m/z (%) = 186 (100%) [MH]⁺.
ANALYSIS

Formula: C_{13}H_{17}NO

Mol. Weight: 203.28 g/mol

$^1$H NMR (400 MHz, CDCl$_3$) δ = 7.41 - 7.21 (m, 5 H); 4.13 (d, J=6.8 Hz, 1 H); 3.72 (m, 2 H); 3.06 (s, 1 H); 2.53 (dt, J=8.8, 3.0 Hz, 1 H); 2.43 (brs, 1 H); 2.37 (d, J=8.8 Hz, 1 H); 1.87 (dd, J=13.0, 7.1 Hz, 1 H); 1.58 (s, 2 H); 1.49 (brs, 1 H); 1.35 (ddt, J=12.8, 4.5, 2.3, 2.3 Hz, 1 H).

$^{13}$C NMR (DEPT) (100 MHz, CDCl$_3$) δ = 139.33 (C), 128.65 (CH), 128.29 (CH), 126.97 (CH), 72.12 (CH), 65.84 (CH), 59.45 (CH$_2$Ph), 58.51 (CH$_2$), 40.21 (CH$_2$), 36.50 (CH), 31.17 (CH$_2$).

MS (ESI+) m/z (%) = 204 (100%) [MH]$^+$. 

To a stirred solution of 15 (90%) (5.4 g, 26.23 mmol) in dry THF (50 mL), cooled at 0 °C under N$_2$ atmosphere, borane-dimethylsulfide complex 2M in THF solution (24.29 mL, 48.6 mmol) was added dropwise. The stirring was continued for 4 h at room temperature.

The excess of BH$_3$ was destroyed by careful addition of a 1:1 mixture of THF-water (15 mL) at 0 °C. Subsequently sodium hydroxide 2N (9.72 mL, 19.43 mmol) was added in one portion and hydrogen peroxide 35% in water (9.57 mL, 109 mmol) dropwise. The resulting mixture was kept at 40 °C for 1.5 h with stirring.

Then, after cooling to room temperature, K$_2$CO$_3$ (2.5 g) was added and the THF was removed under reduced pressure. The remaining solution was extracted with DCM (3x 40 mL), and the combined extracts were washed with water (50 mL). The organic extract was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure.

Purification: Isolera-One Biotage, Snap 340g HP-Si Column, sample dissolved in DCM, eluent: DCM/MeOH/NaOH 95:4.5:0.5 isocratic.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 16 as clear oil (2.95 g, purity 90%, 13.07 mmol, 50%).

(+/-)-exo-2-benzyl-2-azabicyclo[2.2.1]heptan-6-ol (16)
2-\((\pm)\)-exo-2-benzyl-2-azabicyclo[2.2.1]heptan-6-yl)isoindoline-1,3-dione (17)

To a mixture of 16 (50 mg, 0.246 mmol), phthalimide (57.9 mg, 0.394 mmol) and triphenylphosphine (103 mg, 0.394 mmol) in THF (2 mL), 1,2-ethoxycarbonyl diazene 40% in toluene (0.179 mL, 0.394 mmol) was added under N\(_2\) atmosphere at 0 °C. The reaction mixture was then stirred at room temperature overnight. The reaction mixture was diluted with Et\(_2\)O (15 mL) and the precipitate that formed was filtered off. The filtrate was concentrated under reduced pressure. Purification: Isolera-One Biotage, Snap HP-Si 10g Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100:0 to 35:65 in gradient. The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 17 as white solid (48 mg, purity 95%, 0.14 mmol, 56%)

**ANALYSIS**

**Chemical Formula:** C\(_{21}\)H\(_{20}\)N\(_2\)O\(_2\)

**Mol. Weight:** 332.40 g/mol

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta =\) 7.81 (m, 2 H); 7.70 (m, 2 H); 7.42 (m, 2 H); 7.31 (m, 2 H); 7.23 (m, 1 H); 4.66 (ddd, J=8.7, 5.3, 1.1 Hz, 1 H); 3.82 (m, 2 H); 3.38 (s, 1 H) 2.84 (dt, J=8.9, 3.4, 1 H); 2.57 (brs, 1 H); 2.37 (m, 1 H); 2.27 (t, J=9.4, 2 H); 1.89 (ddd, J=11.9, 9.0, 2.1 Hz, 1 H); 1.77 (m, 1 H).

\(^{13}\)C NMR (DEPT) (100 MHz, CDCl\(_3\)) \(\delta =\) 168.74 (C=O), 134.23 (CH), 133.86 (CH), 131.86 (C), 128.54 (CH), 128.25 (CH), 126.83 (CH), 122.99 (CH), 64.36 (CH), 58.09 (CH\(_2\)), 57.72 (CH\(_2\)Ph), 51.58 (CH), 37.54 (CH) 35.05 (CH\(_2\)) 34.69 (CH\(_2\)).

**MS (ESI+)** m/z (%) = 334.4 (100%) [MH]+.
(+/-)-exo-2-benzyl-2-azabicyclo[2.2.1]heptan-6-amine (18)

Compound 17 (920 mg, 2.77 mmol) was dissolved in MeOH/THF 1:1 (40 mL). Subsequently hydrazine monohydrate (0.537 mL, 11.07 mmol) was added and the mixture was heated at 65 °C for 2 h. The formed precipitate was collected by filtration using THF (20 mL), and the filtrate was concentrated under reduced pressure. The residue was treated with NaOH 1M solution (30 mL) and then extracted with DCM (3x30 mL). The organic layers were collected dried over anhydrous Na₂SO₄, filtered, and the solvent was eliminated under reduced pressure to give 18 as white-yellow solid (585 mg, purity 90%, 2.60 mmol, 94%).

**ANALYSIS**

**Formula**: C₁₃H₁₈N₂  
**Mol. Weight**: 202.30 g/mol  
**Note**: -NH₂ signal was detected only in ¹H NMR spectra in DMSO-d₆.

¹H NMR (400 MHz, DMSO-d₆) δ = 7.30 (m, 4 H); 7.21 (m, 1 H); 3.66 (d, J=13.7 Hz, 1H); 3.59 (d, J=13.7 Hz, 1H); 3.07 (dd, J=7.6, 3.2 Hz, 1 H); 2.74 (s, 1 H); 2.43 (dt, J=8.6, 3.3 Hz, 1 H); 2.25 (brs, 1 H); 2.12 (d, J=8.8 Hz, 1 H); 1.62 (ddd, J=12.2, 7.6, 2.2 Hz, 1 H); 1.50 (m, 1 H); 1.45 (brs, 2 H); 1.39 (m, 1 H); 0.98 (dq, J=11.9, 3.4 Hz, 1 H).

²H NMR (400 MHz, CDCl₃) δ = 7.43 - 7.38 (m, 2 H); 7.36 - 7.31 (m, 2 H); 7.27 - 7.23 (m, 1 H); 3.81 - 3.67 (m, 2 H); 3.35 (m, 1 H); 2.93 (s, 1 H); 2.62 (dt, J=9.1, 3.1 Hz, 1 H); 2.41 (brs, 2 H); 1.87 (ddd, J=12.6, 7.9, 2.0 Hz, 1 H); 1.61 (d, J=10.3 Hz, 1 H); 1.52 (d, J=10.3 Hz, 1 H); 1.11 (dq, J=12.5, 3.4 Hz, 1 H).

¹³C NMR (DEPT) (100 MHz, CDCl₃) δ = 140.17 (C), 128.44 (CH), 128.20 (CH), 126.68 (CH), 67.40 (CH), 59.29 (CH²), 58.50 (CH₂), 52.69 (CH), 40.59 (CH₂), 37.16 (CH), 31.23 (CH₂).

**MS (ESI+) m/z (%) = 203 (100%) [MH]⁺.**
Palladium 10% on activated carbon (0.733 g, 0.689 mmol) was suspended in EtOH (10 mL) and water (2 mL). This mixture was added to a solution of 16 (1.4 g, 6.89 mmol) and di-tert-butyl dicarbonate (3.01 g, 13.77 mmol) in EtOH (30 mL). The reaction was conducted in a hydrogenation apparatus. The reaction mixture was subject to four H₂/Vacum cycles. Then the solution was stirred at room temperature under H₂ atmosphere (30 psi) for 1.5 h.

Then the reaction mixture was filtered through a filter septum washing with EtOH (30 mL) and MeOH (5mL). The filtrate was concentrated under reduced pressure to give an oily residue.

Purification: Isolera-One Biotage, Snap HP-SI 100g Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 75:25 to 0:100 in gradient.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 19 as white solid (1.28 g, 6.00 mmol, 87%).

**ANALYSIS**

Formula: C₁₁H₁₉NO₃

Mol. Weight: 213.27 g/mol

**¹H NMR** (400 MHz, DMSO-d₆) δ = 4.92 (brs, 1 H); 3.89 (d, J=19.0 Hz, 1 H); 3.68 (brs, 1 H); 3.01 (dd, J=19.1, 3.4 Hz, 1 H); 2.69 (d, J=9.0 Hz, 1 H); 2.43 (brs, 1 H); 1.75 - 1.65 (m, 1 H); 1.65 - 1.57 (m, 1 H); 1.45 - 1.33 (m, 10 H); 1.31 - 1.22 (m, 1 H).

**¹³C NMR (DEPT)** (100 MHz, CDCl₃) δ = 154.50 (NC=O), 79.38 (C); 72.20 (CH), 60.76 (CH), 51.51 (CH₂), 39.40 (CH₂), 35.80 (CH), 33.44 (CH₂) 28.54 (3 x CH₃).

**MS (ESI+)** m/z (%) = 214 (100%) [MH]⁺.
(+/-)-tert-butyl 6-oxo-2-azabicyclo[2.2.1]heptane-2-carboxylate (20)

To a stirred solution of oxalyl chloride (0.502 mL, 5.93 mmol) in dry DCM (20 mL), cooled at -78°C, a solution of dimethyl sulfoxide (0.919 mL, 12.94 mmol) in DCM (1 mL) was added dropwise. The mixture was stirred for 10 min, then a solution of 19 (1.15 g, 5.39 mmol) in DCM (10 mL) was added dropwise. The reaction mixture was then quenched with brine (40 mL), the organic layer was separated and the reaction mixture was added and stirring continued for 5 min at -78°C and for 10 min at room temperature. The reaction mixture was stirred at -78°C for 3 h, then TEA (2.71 mL, 19.41 mmol) was added dropwise. The reaction mixture was stirred at -78°C for 3 h, then TEA (2.71 mL, 19.41 mmol) was added. Then, stirring continued for 5 min at -78°C and for 10 min at room temperature. The reaction mixture was then quenched with brine (40 mL), the organic layer was separated and the aqueous phase was extracted with DCM (3 x 40 mL). The organic layers were collected dried over anhydrous Na2SO4, filtered, and the solvent removed under reduced pressure.

Purification: Isolera-One Biotage, Snap HP-SI 50 g Column, sample dissolved in DCM, eluent: from DCM/MeOH 100:0 to 95:5 in gradient.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 20 as off-white solid (1.70 g, 5.06 mmol, 94%).

**ANALYSIS**

Note: NMR spectra show some signals split by the presence of two rotamers of the molecule. Chemical shifts of both rotamers are reported.

**Formula:** C11H17NO3

**Mol. Weight:** 211.26 g/mol

**1H NMR (400 MHz, DMSO-δ6)** δ = 3.96 (brs, 1 H); 3.31 (d, J=11.3 Hz, 1 H); 3.06 (d, J=9.3 Hz, 1 H); 2.77 (brs, 1 H); 2.23 (dd, J=17.8, 3.5 Hz, 1 H); 1.97 (dd, J=17.9, 3.5 Hz, 1 H); 1.81 (brs, 1 H); 1.74 (d, J=10.7 Hz, 1 H); 1.38 (s, 9 H).

**13C NMR (DEPT) (100 MHz, DMSO-δ6)** δ = 205.95 (C=O), 154.58 - 153.94 (C=O), 79.59 (C); 61.58 - 61.62 (CH), 50.54 - 50.94 (CH2), 41.59 (CH2), 35.69 - 36.20 (CH2), 34.27 - 34.84 (CH), 28.51 (3 x CH3).

**MS (ESI+)** m/z (%) = 156 (100%) [M-56H]⁺.

**Mp:** 83.5°C.
A solution of 20 (980 mg, 4.64 mmol) and benzylamine (0.600 mL, 5.49 mmol) in 1,2-dichloroethane (25 mL) was stirred at room temperature under N₂ atmosphere for 30 min. Then sodium triacetoxyhydroborate (1430 mg, 6.75 mmol) was added and the reaction mixture was stirred at room temperature, under N₂ atmosphere for 1 h.

NaHCO₃ sat. sol. (40 mL) was added to the reaction mixture, the organic layer was separated and the aqueous phase was extracted with DCM (3x40 mL). The organic layers were collected, dried over anhydrous Na₂SO₄, filtered, and the solvent was eliminated under reduced pressure.

Purification: Isolera-One Blotage, Snap HP-Si 50g Column, sample dissolved in DCM, eluent: from DCM/MeOH 100:0 to 95:5 in gradient.

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 21 as off-white solid (1.39 g, purity 95%, 4.35 mmol, 94%).

**ANALYSIS**

**Note:** NMR spectra show some signals split by the presence of two rotamers of the molecule. Chemical shifts of both rotamers are reported.

**Formula:** C₁₈H₂₆N₂O₂

**Mol. Weight:** 302.41 g/mol

**¹H NMR** (400 MHz, CDCl₃) δ = 7.33 (m, 5 H); 4.43 (m, 1 H); 4.01 (m, 1 H); 3.70 (m, 1 H); 3.36 (m, 1 H); 2.98 (m, 1 H); 2.47 (m, 1 H); 2.10 (m, 1 H); 1.68 (m, 1 H); 1.53 (m, 10 H); 0.89 (m, 1 H).

**¹³C NMR (DEPT)** (100 MHz, CDCl₃) δ = 155.48 - 155.09 (C=O), 140.42 (C), 128.72 (CH), 128.31 (CH), 128.22 (CH), 127.86 (CH), 126.81 (CH), 79.38 - 79.02 (C); 61.45 - 61.13 (CH), 58.12 - 56.80 (CH), 53.63 - 53.15 (CH₂), 52.18 - 51.89 (CH₃), 37.23 - 36.71 (CH) 36.99 - 36.55 (CH₂), 36.38 - 36.30 (CH₂), 28.55 (3 × CH₃).

**MS (ESI⁺)** m/z (%) = 303 (100%) [MH]⁺.
**(+/−)-endo-tert-butyl 6-amino-2-azabicyclo[2.2.1]heptane-2-carboxylate (22)**

Palladium 10% on activated carbon (0.457 g, 0.430 mmol) was suspended in EtOH (5 mL) and some drops of water. This mixture was added to a solution of 21 (1.30 g, 4.30 mmol) in EtOH (20 mL). The reaction was performed in a hydrogenation apparatus. The reaction mixture was subject to four N₂/Vaccum cycles and then to four H₂/Vacum cycles. Then the solution was stirred at room temperature under H₂ atmosphere (1.1 atm) for 4 h. The reaction mixture was filtered through a filter septum washing with EtOH (20 mL) and MeOH (5 mL). The filtrate was concentrated under reduced pressure to give an oily residue containing trace amount of Pd/C, so it was taken up in EtOH (5 mL), re-filtered through Celite pad washing with further EtOH (5 mL). The filtrate was concentrated under reduced pressure to give 22 as a colorless oil (0.84 g, purity 90%, 3.56 mmol, 83%).

**ANALYSIS**

**Note:** NMR spectra show some signals splitted by the presence of two rotamers of the molecule. Chemical shifts of both rotamers are reported.

**Formula:** C₁₁H₂₀N₂O₂

**Mol. Weight:** 212.29 g/mol

**¹H NMR** (400 MHz, CDCl₃) δ = 4.04 (m, 1 H); 3.36 (brs, 2 H); 3.01 (m, 1 H); 2.45 (brs, 1 H); 2.11 (ddddd, J=13.0, 10.6, 4.8, 3.0 Hz, 1 H); 1.64 (m, 2 H); 1.66 - 1.55 (m, 1 H); 1.51 (d, J=10.3 Hz, 1 H); 1.47 (s, 9 H); 0.81 (m, 1 H).

**¹³C NMR (DEPT)** (100 MHz, CDCl₃) δ = 155.57 (C=O), 79.22 (C); 62.15 - 61.19 (CH), 55.37 - 55.19 (CH) 53.69 - 52.99 (CH₂), 37.81 - 37.35 (CH₂), 37.74 - 37.25 (CH), 36.95 (CH₂), 28.55 (3 x CH₃).

**MS (ESI+)** m/z (%) = 213 (100%) [MH]⁺.
2.7 Synthesis of exo-TYPE III derivatives 23a-b, experimental data

(+/-)-exo-2-benzyl-N-(5-(trifluoromethyl)pyridin-2-yl)-2-azabicyclo[2.2.1]heptan-6-amine (25)

A mixture of 18 (300 mg, 1.483 mmol), 2-fluoro-5-(trifluoromethyl)pyridine (0.190, 1.574 mmol) and K$_2$CO$_3$ (360 mg, 2.60 mmol) in DMF (3 mL) was stirred under N$_2$ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 2.5 h.

The solvent was then evaporated under reduced pressure and the residue was taken up in NaHCO$_3$ sat. sol. (30 mL) and the mixture was extracted with AcOEt (3x30 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na$_2$SO$_4$ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-SI Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100:0 to 0:100 in gradient.

The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 25 as white solid (408 mg; purity 95%; 1.116 mmol; 75%).

**ANALYSIS**

**Formula:** C$_{19}$H$_{20}$F$_3$N$_3$

**Mol. Weight:** 347.38 g/mol

**Rf:** 0.29 (CyHex/AcOEt 5:5; SI-TLC)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ = 8.26 (s, 1 H); 7.57 (dd, $J$=8.8, 2.1 Hz, 1 H); 7.38 (d, $J$=7.4 Hz, 2 H); 7.32 (t, $J$=7.4 Hz, 2 H); 7.22 (t, $J$=7.1 Hz, 1 H); 7.15 (d, $J$=6.7 Hz, 1 H); 6.48 (d, $J$=8.6 Hz, 1 H); 4.13 (brs, 1 H); 3.74 (m, 2 H); 3.05 (s, 1 H); 2.58 (d, $J$=8.6 Hz, 1 H); 2.38 (brs, 1 H); 2.23 (d, $J$=8.6 Hz, 1 H); 1.84 (m, 1 H); 1.53 (d, $J$=9.8 Hz, 1 H); 1.46 (d, $J$=9.8 Hz, 1 H); 1.39 (d, $J$=11.7 Hz, 1 H).

**$^{13}$C-NMR (DEPT)** (100 MHz, CDCl$_3$) $\delta$ = 159.60 (C), 146.09 (q, $J$= 4.6 Hz, CH), 134.52 (q, $J$= 3.8 Hz, CH), 128.63 (CH), 128.32 (CH), 127.06 (CH), 124.6 (q, $J$= 270.7 Hz, CF$_3$), 115.53 (q, $J$= 33.6 Hz, C), 105.16 (CH), 62.63 (CH), 59.11 (CH$_2$), 58.61 (CH$_2$), 53.51 (CH), 38.82 (CH$_2$), 36.89 (CH), 32.30 (CH$_2$).

**MS (ESI+)** m/z (%) = 349 (100) [MH$^+$].
(±)-exo-N-(5-(trifluoromethyl)pyridin-2-yl)-2-azabicyclo[2.2.1]heptan-6-amine (26)

Palladium 10% on activated carbon (107 mg, 0.101 mmol) was dissolved in EtOH (2 mL) and some drops of water. This mixture was added to a solution of 25 (350 mg, 1.008 mmol) in EtOH (4 mL). The reaction was conducted in a hydrogenation apparatus. The reaction mixture was subject to four N₂/Vacum cycles and then four H₂/Vacum cycles. Then the solution was stirred at room temperature under H₂ atmosphere (1 atm) for 1.5 h.

The reaction mixture was then filtered through a filter septum and a Celite pad in order to eliminate the Pd/C. The Pd/C was washed with EtOH (5 mL). The filtrate was concentrated under reduced pressure to give 26 as an oily residue (248 mg; 0.935 mmol; 89%)

**ANALYSIS**

**Formula:** C₁₂H₁₄F₃N₃

**Mol. Weight:** 257.25 g/mol

**Note:** in ¹H NMR spectrum in CDCl₃ the RR’NH signal was not observed (probably covered by the water signal at 1.61 ppm).

**¹H NMR** (400 MHz, DMSO-d₆) δ = 8.28 (s, 1 H); 7.61 (dd, J=9.3, 2.0 Hz, 1 H); 7.11 (d, J=6.8 Hz, 1 H); 6.55 (d, J=8.8 Hz, 1 H); 3.76 (brs, 1 H); 3.13 (s, 1 H); 2.66 (dt, J=8.8, 2.9 Hz, 1 H); 2.39 (d, J=8.8 Hz, 1 H); 2.33 (brs, 1 H); 2.04 (brs, 1 H); 1.79 (ddd, J=12.7, 8.3, 2.0 Hz, 1 H); 1.48 (d, J=9.23 Hz, 1 H); 1.38 (m, 1 H); 1.30 (d, J=9.3 Hz, 1 H).

**¹³C-NMR (DEPT)** (100 MHz, CDCl₃) δ = 159.43 (C), 146.13 (q, J= 4.7 Hz, CH), 134.52 (q, J= 3.2 Hz, CH), 124.5 (q, J= 268.9 Hz, CF₃), 115.72 (q, J= 32.1 Hz, C), 105.84 (CH), 58.61 (CH), 56.28 (CH), 49.96 (CH₂), 39.08 (CH₂), 35.69 (CH), 34.88 (CH₂).

**MS (ESI+) m/z (%) = 257 (100) [MH]⁺**
General Procedure F: synthesis of exo-compounds 23a-b

To a solution of the appropriate carboxylic acid (1.2 eq) in dioxane (1 mL) were added in sequence N-methylmorpholine (3 eq) and CDMT (1.5 eq) under N\textsubscript{2} atmosphere and the resultant solution was stirred for 30 min at room temperature. Then was added the amine 26 (1.0 eq) pre-dissolved in dioxane (1 mL) and the mixture was stirred at 100 °C for 1 h. The solvent was then evaporated under reduced pressure and the residue taken up in AcOEt and washed in sequence with NH\textsubscript{4}Cl sat. sol., NaHCO\textsubscript{3} sat. sol. and water. Then the organic layer was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent was eliminated under reduced pressure.

Purification: Isolera-One Biotage, flash chromatography by HP-SI Column or Reverse Phase Column. The containing product fractions were collected and the solvent eliminated under reduced pressure to give the final compound 23a-b as (+/-)-exo-racemic mixture.

Analysis of proton and carbon NMR spectra reveals for compounds 23a-b the presence of two rotamers of the molecule in ratio ranging from 7:3 to 6:4. In particular, in proton NMR spectra the major rotamer can be easily detected and described. On the contrary, carbon NMR spectra are complicated by the presence of signals splitted by the presence of C-F coupling and the C\textsubscript{sp2} carbon atom signals are difficult to distinguish and assign. For these reasons in carbon NMR spectra only the chemical shifts of aliphatic carbons are reported for both rotamers of each compound.

(2-methyl-5-phenylthiazol-4-yl) (+/-)-exo-6-[(5-(trifluoromethyl)pyridin-2-yl)amino]-2-azabicyclo[2.2.1]heptan-2-carboxylate (23a)

\[
\begin{align*}
\text{F}_3\text{C} & \quad \text{N} \\
\text{H} & \quad \text{N} \\
\text{O} & \quad \text{N} \\
\text{S} & \quad \text{N} \\
\end{align*}
\]

General Procedure E was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (44.3 mg, 0.202 mmol), amine 26 (40 mg, 0.155 mmol), NMM (0.050 mL, 0.455 mmol) and CDMT (44 mg, 0.251 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/AcOEt 100:0 to 0:100 in gradient) 23a was obtained as white solid (54 mg, 0.118 mmol, 76%).

**ANALYSIS**

**Formula:** C\textsubscript{23}H\textsubscript{21}F\textsubscript{3}N\textsubscript{4}OS

**Mol. Weight:** 458.50 g/mol

**Note:** From NMR spectra were observed two signal patterns in about 70:30 ratio due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.
Rf: 0.35 (DCM/MeOH 95:5; SI-TLC).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) (major rotamer) \(\delta = 8.03\) (s, 1 H); 7.57 (dd, \(J=9.0, 2.7\) Hz, 1 H); 7.51 - 7.32 (m, 5 H); 7.14 (d, \(J=5.9\) Hz, 1 H); 6.33 (d, \(J=8.8\) Hz, 1 H); 3.90 (s, 1 H); 3.65 (brs, 1 H); 3.30 - 3.24 (m, 1 H); 3.11 (d, \(J=10.8\) Hz, 1 H); 2.68 (s, 3 H); 2.61 (brs, 1 H); 1.89 (dd, \(J=12.0, 9.0\) Hz, 1 H); 1.56 - 1.46 (m, 2 H); 1.32 (d, \(J=10.3\) Hz, 1 H).

\(^13\)C NMR (DEPT) (100 MHz, CDCl\(_3\)) (major rotamer) \(\delta = 60.64\) (CH), 57.90 (CH), 55.25 (CH), 53.90 (CH), 52.85 (CH\(_2\)), 51.59(CH\(_2\)), 38.09(CH\(_2\)), 37.63(CH\(_2\)), 36.08 (CH), 35.21 (CH), 34.84(CH\(_2\)), 33.22(CH\(_2\)), 19.22(CH\(_3\)), 19.18(CH\(_3\)).

MS (ESI+) m/z (%) = 460 (100) [MH]\(^+\)

(6-methyl-3-phenylpicolin-2-yl) (+/-)-exo-6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptan-2-carboxylate (23b)

\[\text{F}_3\text{C} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{F}_3 \]

23b

General Procedure E was followed using the 6-methyl-3-phenylpicolinic acid (40 mg, 0.188 mmol), amine 26 (40.2 mg, 0.156 mmol), NMM (0.050 mL, 0.455 mmol) and CDMT (44 mg, 0.251 mmol) in dioxane (2 mL). After purification (Snap 10g HP-Si Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 23b was obtained as white solid (55 mg, 0.122 mmol, 64.8 %).

ANALYSIS

Formula: C\(_{25}\)H\(_{23}\)F\(_3\)N\(_4\)O

Mol. Weight: 452.47 g/mol

Note: From NMR spectra were observed two signal patterns in about 60:40 ratio due to the two rotamers of the molecule. Chemical shifts of the major rotamer are reported.

Rf: 0.32 (DCM/MeOH 95:5; SI-TLC).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) (major rotamer) \(\delta = 8.14\) (s, 1 H); 7.74 (d, \(J=8.3\) Hz, 1 H); 7.81 - 7.30 (m, 7 H); 5.73 (d, \(J=8.8\) Hz, 1 H); 4.54 (d, \(J=5.9\) Hz, 1 H); 3.62 (brs, 1 H); 3.53 (s, 1 H); 3.25 - 3.16 (m, 2 H); 2.75 (s, 3 H); 2.54 (brs, 1 H); 2.07 (ddd, \(J=13.4, 8.1, 2.4\) Hz, 1 H); 1.31 - 1.24 (m, 2 H); 0.75 (d, \(J=10.8\) Hz, 1 H).

\(^13\)C NMR (DEPT) (100 MHz, CDCl\(_3\)) (major rotamer) \(\delta = 59.94\) (CH), 57.37 (CH), 54.81 (CH), 53.69 (CH), 52.25 (CH\(_2\)), 50.79 (CH\(_2\)), 37.92 (CH\(_2\)), 37.47 (CH\(_2\)), 36.03 (CH), 35.05 (CH), 34.37 (CH\(_2\)), 33.19 (CH\(_2\)), 24.22 (CH\(_3\)), 24.14 (CH\(_3\)).

MS (ESI+) m/z (%) = 454.2 (100) [MH]\(^+\)
2.8 Synthesis of endo-TYPE III derivatives 24a-e, experimental data

(+/-)-endo-tert-butyl 6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptane-2-carboxylate (27)

A mixture of 22 (300 mg, 1.272 mmol), 2-fluoro-5-(trifluoromethyl)pyridine (0.190, 1.574 mmol) and K₂CO₃ (360 mg, 2.60 mmol) in DMF (3 mL) was stirred under N₂ atmosphere for 10 min. The reaction mixture was heated at 100 °C for 1.5 h then at 80 °C for 18 h. The solvent was then evaporated under reduced pressure and the residue taken up in NaHCO₃ sat. sol. (30 mL) and the mixture extracted with AcOEt (3x30 mL). The organic layers were collected, washed with water (50 mL), dried over anhydrous Na₂SO₄ and filtered. Then the solvent was evaporated under reduced pressure.

Purification: Isolera-One Biotage, Snap 25g HP-SI Column, sample dissolved in DCM, eluent: from CyHex/AcOEt 100:0 to 50:50 in gradient. The containing product fractions were collected and the solvent was evaporated under reduced pressure to give 27 as white solid (398 mg; 1.114 mmol; 88%).

ANALYSIS

Formula: C₁₇H₂₂F₃N₃O₂
Mol. Weight: 357.37g/mol
Rf: 0.43 (CyHex/AcOEt 5:5; SI-TLC)
Note: NMR spectra show some signals splitted by the presence of two rotamers of the molecule. Chemical shifts of both rotamers are reported.

¹H NMR (400 MHz, CDCl₃) (mixture of rotamers) δ = 8.36 (brs, 1 H); 7.56 (m, 1 H); 6.37 (m, 1 H); 5.41 (brs, 1 H); 4.43 (brs, 1 H); 4.19 (m, 1 H); 3.48 (m, 1 H); 3.11 (m, 1 H); 2.61 (brs, 1 H); 2.35 (m, 1 H); 1.74 (m, 2 H); 1.56 (m, 9 H); 1.05 (m, 1 H).

¹³C-NMR (DEPT) (100 MHz, CDCl₃) (mixture of rotamers) δ = 160.04 (C), 154.95 (C), 145.83 (CH), 133.98 (CH), 124.59 (q, J=269.1 Hz, CF₃), 115.55 (q, J=35.8 Hz, C), 107.25 (CH), 79.35 (C), 58.58 (CH), 56.08 (CH), 54.92 (CH), 53.69 (CH₂), 52.83 (CH₂), 37.73 (CH), 36.98 (CH), 36.92 (CH₂), 36.72 (CH₂), 36.36 (CH₂), 36.08 (CH₂), 28.42 (3 x CH₃), 28.15 (3 x CH₃).

MS (ESI+) m/z (%) = 358 (100) [MH]⁺.
(+/-)-endo-N-(5-(trifluoromethyl)pyridin-2-yl)-2-azabicyclo[2.2.1]heptan-6-amine (28)

Compound 27 (350 mg, 0.979 mmol) was dissolved in a solution of DCM (Ratio: 4, Volume: 9.00 mL) and TFA (Ratio: 1.000, Volume: 2.25 mL) and then the mixture was stirred at room temperature for 1 h.

The solvent was then evaporated under reduced pressure.

Purification: the residue was taken up in MeOH and loaded on SCX 5g, eluent MeOH then ammonia (2M in MeOH).

The containing product fractions were collected and the solvent was eliminated under reduced pressure to give 28 as white solid (260 mg, 0.979 mmol, quantitative).

ANALYSIS

Formula: C12H14F3N3

Mol. Weight: 257.25 g/mol

^1H NMR (400 MHz, CDCl3) δ = 8.33 (bs, 1 H); 7.54 (m, 1 H); 6.38 (d, J=8.8 Hz, 1 H); 6.14 (bs, 1 H); 4.10 - 4.00 (m, 1 H); 3.48 (brs, 1 H); 3.00 (dt, J=9.7, 3.2 Hz, 1 H); 2.63 (d, J=9.8 Hz, 1 H); 2.47 (brs, 1 H); 2.21 - 2.10 (m, 1 H); 1.68 - 1.63 (m, 1 H); 1.59 - 1.53 (m, 1 H); 0.97 (dt, J=12.7, 3.2 Hz, 1 H).

^13C-NMR (DEPT) (100 MHz, CDCl3) δ = 160.15 (C), 146.07 (CH), 133.84 (CH), 124.77 (q, J= 269.9 Hz, CF3), 114.75 (q, J=33.2 Hz, C), 107.25 (CH), 58.38 (CH), 52.80 (CH), 51.62 (CH2), 38.46 (CH2), 37.61 (CH), 37.35 (CH2).

MS (ESI+) m/z (%) = 258 (100) [MH]^+. 
General Procedure E: synthesis of endo-compounds 24a-e

To a solution of the appropriate carboxylic acid (1.0 eq) in dioxane (1 mL) were added in sequence N-methylmorpholine (3 eq) and CDMT (1.5 eq) under N\textsubscript{2} atmosphere and the resultant solution was stirred for 30 min at room temperature. Then was added the amine 28 (1.2 eq) pre-dissolved in dioxane (1 mL) and the mixture was stirred at 100 °C for 1 h.

The solvent was then evaporated under reduced pressure and the residue taken up in AcOEt and washed in sequence with NH\textsubscript{4}Cl sat. sol., NaHCO\textsubscript{3} sat. sol. and water. Then the organic layer was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent was eliminated under reduced pressure.

Purification: Isolera-One Biotage, HP-SI Column or Reverse Phase Column.

The containing product fractions were collected and the solvent eliminated under reduced pressure to give the final compound 24a-e as (+/-)-endo-racemic mixture.

(2-methyl-5-phenylthiazol-4-yl) (+/-)-endo-6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptan-2-carboxylate (24a)

General Procedure E was followed using the 2-methyl-5-phenylthiazole-4-carboxylic acid (44.3 mg, 0.202 mmol), amine 28 (40 mg, 0.155 mmol), NMM (0.050 mL, 0.455 mmol) and CDMT (44 mg, 0.251 mmol) in dioxane (2 mL). After purification (Snap 10 g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 24a was obtained as white solid (52.5 mg; 0.115 mmol; 73.6%).

**ANALYSIS**

**Formula:** C\textsubscript{23}H\textsubscript{21}F\textsubscript{3}N\textsubscript{4}OS

**Mol. Weight:** 458.50 g/mol

**Note:** From NMR spectra were observed two signal patterns in about 93:7 ratio due to the two rotamers of the molecule. The signal of 1 CH of the 2-azabicyclo[2.2.1]heptan-6-amine is covered by DMSO solvent signal. The chemical shifts of the major rotamer are reported.

\textsuperscript{1}H NMR (400 MHz, DMSO-\textsubscript{d6}) (major rotamer) \(\delta = 8.05\) (s, 1 H); 7.52 - 7.31 (m, 7 H); 6.36 (d, \(J=8.8\) Hz, 1 H); 4.38 (s, 1 H); 3.85 (brs, 1 H); 3.37 - 3.22 (m, 2 H); 2.32 (s, 3 H); 2.07 (m, 1 H); 1.46 (d, \(J=9.8\) Hz, 1 H); 1.22 (m, 1 H); 1.16 (m, 1 H).

\textsuperscript{13}C NMR (DEPT) (100 MHz, DMSO-\textsubscript{d6}) (major rotamer) \(\delta = 163.35\) (C), 163.06 (C), 160.40 (C), 145.22 (CH), 144.38 (C), 136.06 (C), 132.57 (CH), 130.79 (C), 129.30 (2 x CH), 128.85 (CH), 128.45 (2
105

x CH), 128.34 (q, J = 270.7 Hz, CF₃), 112.70 (q, J=31.3 Hz, C), 109.42 (CH), 60.09 (CH), 54.54 (CH), 53.13 (CH₂), 36.60 (CH₂), 36.24 (CH), 33.44 (CH₂), 18.73 (CH₃).

**MS (ESI+)** m/z (%) = 460 (100) [MH]⁺.

(6-methyl-3-phenylpicolin-2-yl) (+/-)-endo-6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptan-2-carboxylate (24b)

![Structure of 24b](image)

**General Procedure E** was followed using the 6-methyl-3-phenylpicolinic acid (40 mg, 0.188 mmol), amine 28 (40 mg, 0.155 mmol), NMM (0.050 mL, 0.455 mmol) and CDMT (44 mg, 0.251 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 24b was obtained as white solid (53 mg; 0.117 mmol; 75%).

**ANALYSIS**

**Formula:** C₂₅H₂₃F₃N₄O

**Mol. Weight:** 452.47 g/mol

**Note:** From NMR spectra were observed two signal patterns in about 80:20 ratio due to the two rotamers of the molecule. The chemical shifts of the major rotamer were reported.

**¹H NMR** (400 MHz, DMSO-d₆) (major rotamer) δ = 7.90 (brs, 1 H); 7.66 (d, J=8.3 Hz, 1 H); 7.57 (dd, J=9.3, 2.4 Hz, 1 H); 7.52 (brs, 1 H); 7.50 - 7.45 (m, 2 H); 7.42 - 7.36 (m, 3 H); 7.09 (d, J=7.8 Hz, 1 H); 6.52 (m, 1 H); 3.81 (brs, 1 H); 3.76 (m, 1 H); 3.14 – 3.05 (m, 2 H); 2.38 (brs, 1 H); 2.11 (s, 3 H); 2.00 (m, 1 H); 1.22 (m, 1 H); 1.12 (d, J=12.7 Hz, 1 H); 0.29 (brs, 1 H).

**¹³C NMR (DEPT)** (100 MHz, DMSO-d₆) (major rotamer) δ = 166.59 (C), 160.50 (C), 156.53 (C), 151.98 (C), 145.30 (CH), 137.81 (C), 137.33 (CH), 133.03 (CH), 130.95 (C), 129.06 (2 x CH), 128.69 (2 x CH), 125.67 (q, J= 269.9 Hz, CF₃); 128.23 (CH), 123.38 (CH), 112.53 (q, J= 32.3 Hz, C), 107.43 (CH), 60.13 (CH), 54.12 (CH), 52.29 (CH₂), 36.06 (CH), 35.97 (CH₂), 33.75 (CH₂), 23.56 (CH₃).

**MS (ESI+)** m/z (%) = 454 (100) [MH]⁺.
(6-methyl-3-(pyrimidin-2-yl)pyridin-2-yl) (+/-)-endo-6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptan-2-carboxylate (24c)

General Procedure E was followed using the 6-methyl-3-(pyrimidin-2-yl)picolinic acid (30 mg, 0.139 mmol), amine 28 (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (33 mg, 0.188 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) 24c was obtained as pink solid (18 mg; 0.040 mmol; 34.0%).

ANALYSIS
Formula: C_{23}H_{21}F_{3}N_{6}O
Mol. Weight: 454.45 g/mol
Note: From NMR spectra were observed two signal patterns in about 90:10 ratio due to the two rotamers of the molecule. The chemical shifts of the major rotamer are reported.

^1H NMR (400 MHz, DMSO-d_6) (major rotamer) δ = 8.92 (d, J=4.9 Hz, 2 H); 8.30 (d, J=7.8 Hz, 1 H); 8.05 (brs, 1 H); 7.59 (d, J=8.8 Hz, 2 H); 7.49 (t, J=4.9 Hz, 1 H); 7.26 (d, J=7.8 Hz, 1 H); 6.49 (d, J=7.8 Hz, 1 H); 4.03 (brs, 1 H); 3.95 (brs, 1 H); 3.47 (d, J=9.8 Hz, 1 H); 3.15 (d, J=10.23 Hz, 1 H); 2.62 (brs, 1 H); 2.27 (brs, 3 H); 2.19 (brs, 1 H); 1.59 - 1.39 (m, 2 H); 1.18 (d, J=12.7 Hz, 1 H).

^13C NMR (DEPT) (100 MHz, DMSO-d_6) (major rotamer) δ = 167.96 (C), 163.30 (C), 159.11 (C), 157.17 (2 x CH), 154.40 (C), 145.19 (CH), 138.65 (CH), 133.78 (CH), 129.60 (C), 127.07 (q, J=275.9, CF_3), 123.73 (CH), 119.60 (CH), 114.62 (q, J=33.3 Hz, C), 108.50 (CH), 61.49 (CH), 54.42 (CH), 52.60 (CH_2), 37.40 (CH_2), 37.01 (CH_2), 36.77 (CH), 24.25 (CH_3).

MS (ESI+) m/z (%) = 455 (100) [MH]^+. 

(5-methyl-2-(pyrimidin-2-yl)phenyl) (+/-)-endo-6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptan-2-carboxylate (24d)
General Procedure E was followed using the 5-methyl-2-(pyrimidin-2-yl)benzoic acid (30 mg, 0.140 mmol), amine 28 (30 mg, 0.117 mmol), NMM (0.040 mL, 0.364 mmol) and CDMT (33 mg, 0.188 mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/Acetone 100:0 to 5:5 in gradient. Sample was further purified by SCX column) 24d was obtained as pink solid (16 mg; 0.035 mmol; 30.3%).

ANALYSIS
Formula: C_{24}H_{22}F_{3}N_{5}O
Mol. Weight: 453.46 g/mol

Note: From NMR spectra were observed two signal patterns in about 90:10 ratio due to the two rotamers of the molecule. The chemical shifts of the major rotamer are reported. The signal of a bridged CH was partially covered by DMSO solvent signal.

\[^1\text{H NMR} \ (400 \text{ MHz, DMSO-d}_6)\] (major rotamer) δ = 8.85 (d, J=4.9 Hz, 2 H); 7.97 (brs, 1 H); 7.89 (d, J=7.8 Hz, 1 H); 7.63 (d, J=8.3 Hz, 1 H); 7.56 (d, J=6.8 Hz, 1 H); 7.40 - 7.44 (m, 1 H); 7.08 (d, J=6.8 Hz, 1 H); 6.74 (s, 1 H); 6.66 (brs, 1 H); 4.06 (brs, 1 H); 3.77 (brs, 1 H); 3.39 (dt, J=10.6, 3.0 Hz, 1 H); 3.18 (d, J=10.3 Hz, 1 H); 2.53 - 2.58 (m, 1 H); 2.09 (t, J=11.5 Hz, 1 H); 1.88 (s, 3 H); 1.40 (d, J=9.8 Hz, 1 H); 1.29 - 1.10 (m, 2 H).

\[^1\text{H NMR} \ (400 \text{ MHz, CD}_3\text{OD})\] (major rotamer) δ = 8.83 (d, J=4.9 Hz, 2 H); 8.03 (d, J=7.8 Hz, 1 H); 7.94 (brs, 1 H); 7.59 (dd, J=8.8, 2.4 Hz, 1 H); 7.37 (t, J=4.9 Hz, 1 H); 7.18 - 7.11 (m, 1 H); 6.87 (s, 1 H); 6.68 - 6.48 (m, 1 H); 4.15 - 3.99 (m, 2 H); 3.54 (dt, J=10.8, 3.2 Hz, 1 H); 3.35 (s, 1 H); 2.63 (brs, 1 H); 2.31 - 2.18 (m, 1 H); 1.98 (s, 3 H); 1.55 - 1.42 (m, 1 H); 1.38 - 1.21 (m, 2 H).

\[^{13}\text{C NMR (DEPT)} \ (100 \text{ MHz, CD}_3\text{OD})\] (major rotamer) δ = ppm 171.98 (C), 164.17 (C), 160.30 (C), 157.15 (CH), 144.87 (CH), 143.13 (C), 140.29 (C), 136.09 (C), 133.33 (CH), 129.76 (CH), 129.33 (CH), 129.05 (CH), 128.70 (CH), 128.09 (CH), 127.88 (q, J= 269.2 Hz, CF_{3}), 119.26 (CH), 114.02 (q, J=32.4 Hz, C), 61.37 (CH), 53.82 (CH), 52.64 (CH), 36.46 (CH), 35.85 (CH), 33.86 (CH), 19.70 (CH).

MS (ESI+) m/z (%) = 454 (100) [MH]^+.

(5-chloro-2-(pyrimidin-2-yl)phenyl) (+/-)-endo-6-((5-(trifluoromethyl)pyridin-2-yl)amino)-2-azabicyclo[2.2.1]heptan-2-carboxylate (24e)

\[
\begin{align*}
\text{H} & \text{N} \\
F_{3}C & \text{N} \\
\text{Cl} & \text{O} \\
\end{align*}
\]

General Procedure E was followed using the 5-chloro-2-(pyrimidin-2-yl)benzoic acid (44 mg, 0.188 mmol), amine 28 (40 mg, 0.155 mmol), NMM (0.050 mL, 0.455 mmol) and CDMT (44 mg, 0.251 mmol)
mmol) in dioxane (2 mL). After purification (Snap 10g HP-SI Column, sample dissolved in DCM 100%, eluent: from DCM/MeOH 100:0 to 95:5 in gradient) **24e** was obtained as purple solid (29 mg; 0.061 mmol; 39.4%).

**ANALYSIS**

*Formula:* $C_{23}H_{19}ClF_3N_5O$

*Mol. Weight:* 473.88 g/mol

**Note:** From NMR spectra were observed two signal patterns in about 85:15 ratio due to the two rotamers of the molecule. The chemical shifts of the major rotamer are reported.

$^1H$ NMR (400 MHz, DMSO-$_d$6) (major rotamer) $\delta$ = 8.90 (d, $J$=4.9 Hz, 2 H); 7.99 (d, $J$=8.5 Hz, 1H); 7.95 (m, 1 H); 7.61 (m, 1 H); 7.52 (d, $J$=6.8 Hz, 1 H); 7.48 (t, $J$=4.9 Hz, 1 H); 7.31 (dd, $J$=8.6, 2.2 Hz, 1 H); 6.94 (s, 1 H); 6.56 - 6.69 (m, 1 H); 4.07 (brs, 1 H); 3.73 (brs, 1 H); 3.37 (dt, $J$=10.4, 2.9 Hz, 1H); 3.23 - 3.16 (m, 1 H); 2.53 (brs, 1 H); 2.09 (m, 1 H); 1.40 (d, $J$=9.8 Hz, 1 H); 1.14 (brs, 2 H).

$^1H$ NMR (400 MHz, CD$_3$OD) (major rotamer) $\delta$ = 8.86 (d, $J$=4.9 Hz, 2 H); 8.13 (d, $J$=8.3 Hz, 1 H); 7.93 (bs, 1 H); 7.56 - 7.63 (m, 1 H); 7.41 (t, $J$=4.9 Hz, 1 H); 7.31 (dd, $J$=8.6, 2.2 Hz, 1 H); 7.04 (d, $J$=2.0 Hz, 1 H); 6.57 (d, $J$=7.8 Hz, 1 H); 3.88 - 4.12 (m, 2 H); 3.54 (dt, $J$=10.9, 3.1 Hz, 1 H); 3.36 (bs, 1 H); 2.63 (bs, 1 H); 2.25 (m, 1 H); 1.51 (d, $J$=9.8 Hz, 1 H); 1.27 (m, 2 H).

$^{13}C$ NMR (DEPT) (100 MHz, CD$_3$OD) (major rotamer) $\delta$ = 170.06 (C), 163.24 (C), 160.14 (C), 157.31 (2 x CH) 144.88 (CH), 137.61 (C), 135.87 (C), 133.83 (CH), 133.49 (C), 130.59 (CH), 128.77 (CH), 128.38 (CH), 127.56 (C), 124.82 (q; $J$= 269.7; CF$_3$), 119.71 (CH), 114.44 (q, $J$= 32.8 Hz; C), 108.27 (CH), 61.30 (CH), 53.90 (CH), 52.76 (CH$_2$), 36.37 (CH), 35.84 (CH$_2$), 33.64 (CH$_2$).

**MS (ESI+)** m/z (%) = 474 (100) [MH]$^+$.
3.1. Drug-like Properties Evaluation

The drug-like properties of a molecule or of a set of molecules, considered for pharmaceutical purposes (drugs), are qualitative indications that describe how "drug-like" a substance is with respect to several parameters and these properties can be extrapolated from the molecular structure.\(^1\) Nevertheless, it should be noted that the drug-like properties are referred only to the bioavailability of a drug-candidate and not to its biological properties.

A smart method to evaluate the drug-like properties of a compound is to apply specific and rational “rules” based on a set of guidelines and able to predict, from the structural properties of a molecule, the probability of being well absorbed after oral administration. These guidelines are not absolute, nor are they intended to give severe cut-off values, however they are often quite efficacious.

In the design of the new TYPE II and TYPE III scaffolds derivatives and in particular in the rationale evaluation of the best substitution patterns for the two nitrogen atoms embedded in the core structures, we apply the so-called “Lipinski rules” (Lipinski et al.).\(^2\) These rules are an assets of values deduced from the analysis and classification of the key physiochemical properties of a series of drug-like compounds taken from the literature.

The Lipinski rules were originally studied and employed at Pfizer Inc. in the US for some years and after their publication in 1997 become largely used by the medicinal chemists community. In the drug discovery fields, the impact of these rules was very significant. The Lipinsky rules are also known as the “rule of 5” because the proposed values for each of the parameters are all near to 5 or to a multiple of 5. The intended purpose of the “rule of 5” is to analyze specific physicochemical properties of compounds, in order to predict their absorption or permeation. In particular, good absorption or permeation are more likely when a drug-like compound possesses:

- less than 5 H-bond donors (sum of all OHs and NHs);
- has a molecular weight (MW) < 500;
- the Log P is between 2 and 5 (octanol/water partition coefficient);
- less than 10 H-bond acceptors (sum of all Ns and Os);
- rotatable bonds < 8;
- compounds that are substrates for biological transporters and natural compounds are exceptions to the rule.

The Lipinsky rules were extrapolated by the structural properties of compounds that passed the phase I clinical trials and moved on to phase II studies. Phase I clinical trials entail human administration of the drug candidate in order to define the pharmacokinetic profile and the toxicity. When a compound moves on Phase II studies it means that sufficient adsorption in humans and low toxicity were observed and consequently it is liable for further investments for his

development. More than 2200 molecules were analyzed in order to extrapolate and build up the general rules of drug likeness. Validation of the Lipinsky rules demonstrated that the 90% of the compounds possessing molecular property values that fall within the guidelines show sufficient absorption after oral dosing.

The rules were building up on the basis of robust physicochemical evaluations:

- Hydrogen bonds increase solubility in water of a compound and they must be broken in order to permit lipid bilayer membrane permeation. Thus, a high number of hydrogen bonds decrease the partition from the aqueous phase into the lipid bilayer membrane decreasing the membrane permeation by passive diffusion.
- Molecular weight (MW) is related to the dimension of the molecule. Increasing MW reduces the compound concentration at the surface of the intestinal epithelium, thus reducing absorption. Increasing size also inhibits passive diffusion through the tightly packed aliphatic side chains of the bilayer membrane.
- Increasing Log P also decreases aqueous solubility and reduces absorption.
- Finally, membrane transporters can either enhance or reduce compound absorption by either active uptake transport or efflux, respectively. Thus, transporters can have a strong impact on increasing or decreasing absorption and molecules that are substrates for membrane transporters represent an exception to the rule of 5. Natural products are another important exception to the rule of 5. In this case natural evolution leads to the optimization of these compounds in terms of active transport, developing conformationally and configurationally useful features for passive transport.

However, when a drug-like compound is recognized, often the lead optimization procedure (achievement of a drug candidate) requires structural modifications that increase both molecular weight and lipophilicity. So, the activity improvement can decrease the drug-like properties of a compound. These drawbacks can be overcome introducing the concept of lead-like molecules. A lead-like molecule possesses less severe requirements with respect to a drug-like compound. In particular:

- less than 3 H-bond donors (sum of all OHs and NHs);
- has a molecular weight (MW) < 300;
- the Log P is between 1 and 3 (octanol/water partition coefficient);
- less than 3 H-bond acceptors (sum of all Ns and Os);
- rotatable bonds < 3.
- compounds that are substrates for biological transporters and natural compounds are exceptions to the rule.

Thus, when a lead-like compound is identified, the lead optimization is less difficult because the introduction of hydrophobic groups to increase potency has a little effect on MW and logP as

---

defined by the rule of 5 and compounds can become larger and more lipophilic without loss of their drug-like properties.

It should be pointed out that, from a biological point of view, a drug-like and a lead-like compound can show diverse levels of activity towards the selected target, nM vs mM activity, because a lead-like compound is open to a wider margin of improvement with respect to a drug-like compound on the basis of reported rules.

Moreover, over the last years, these descriptors have been modified and improved to obtain an indication on the physiochemical features required for compound targeting particular human body districts. For example, the polar surface area of a molecule (PSA) expressed in squared Å, is stated as the surface occupied by all polar atoms (mainly oxygen and nitrogen and their hydrogens) and indicates the ability of a molecule to permeate cell membranes. Molecules with a polar surface area higher than 140 squared Å have the tendency to be scarce in permeating cell membranes.

In this PhD project, we focused our attention on the search for new antagonist of the orexinic receptors that are mainly located in the Central Nervous System (CNS). As a consequence, particular physiochemical features are required for the potential orexin antagonists for the blood-brain barrier (BBB) permeation. In fact, the commercial CNS drugs have fewer hydrogen bond donors, higher Log P, lower PSA (polar surface area), and fewer rotatable bonds than the corresponding non-CNS drugs. Pardridge proposed a set of physiochemical BBB rules for molecules with potential effects on CNS. In particular, he postulated that a molecule should have:

- Total H-bonds < 8–10;
- MW < 400-500;
- No acid groups.

More restrictive features were also proposed suggesting that the H-bond donors must be < 2 and H-bond acceptors < 6. This is in agreement with general consideration that H-bond donors are more limiting than H-bond acceptors. Moreover, molecules able to penetrate the BBB (and thus act on receptors in the central nervous system) normally have a PSA of 60-70 squared Å. All these features are useful for evaluating BBB permeability of a class of compounds prior to their synthesis and can suggest which structural modifications provide better BBB permeation.

These considerations led us to perform an In Silico filtering phase for the evaluation of the physiochemical properties of the two series of compounds we planned to synthesize as antagonists for the orexin receptors.

Actually, we choose to evaluate the molecular weight (MW), the log P, the total polar surface area (tPSA) and the total H-bonds (HBA + HBD).

---

The first parameter evaluated in the final 20 endo/exo TYPE II compounds (9a-k and 12a-i) was the molecular weight. In general, the molecular weights of the designed molecules were above the flag level of 500 g/mol proposed by Lipinski and Partrige.\textsuperscript{2,6}

The LogP parameter, calculated with the ChemBioDrow Ultra software, was evaluated to obtain an indication about the lipophilic nature of the final compounds obtained. The LogP values are under the reported value of 5 reported by Lipinski ranging from the 3.51 of compound 9h to 4.84 of compound 9g. The peculiar feature of compound 9h is the presence of the cyclopropylmethoxy group that displaces the phenyl or the pyrimidin-2-yl rings present in the other derivatives. While the compound 9g contains an additional fluorine atom in the 2-methyl-5-phenylthiazol-4-yl group.

The tPSA (expressed as Å\textsuperscript{2}) values are comprised between 57.06 (compounds 9f, 9g and 12f) and 81.78 (compounds 9i and 12g). These latter compounds contain the 6-methyl-3-(pyrimidin-2-yl)pyridin-2-yl group that increase the tPSA over the reference values proposed by Clark and Lobell.\textsuperscript{7}

Finally, the total number of H-bonds (HBD+HBA) present in the structure of the final compounds was evaluated. The total number of H-bonds of the TYPE II derivatives ranging between 6 and 7, only the compounds 9i and 12g present 8 H-bonds in the structure in agreement with the requirements reported by Partrige.\textsuperscript{6}

The collected data TYPE II derivatives 9a-k and 12a-i are reported in Figure 1.
**Figure 1.** Drug-like properties evaluated for the endo/exo TYPE II scaffold derivatives 9a-k and 12a-i: 1) molecular weight (MW); 2) Log P; 3) total polar surface area (tPSA); 4) total H-bonds (HBA + HBD).
TYPE III Scaffold derivatives 23a-b and 24a-e

The functionalization employed for the design of the 7 endo/exo TYPE III scaffold derivatives (23a-b and 24a-e) are selected moieties already used for the TYPE II final compounds, so the evaluated drug-like properties are quite similar, Figure 2.

The molecular weights ranged from 458.50 g/mol of compounds 23a and 24a to 473.88 g/mol of compound 24e that contain the trifluoromethyl)pyridin-2-yl moiety and the 5-chloro-2-(pyrimidin-2-yl)phenyl group as substituents.

Also in this case, the LogP values are under the value of 5 of Lipinski and ranging from the 3.59 of compound 24c to 4.73 of compounds 23a and 24a. The compounds 24c contain the 6-methyl-3-(pyrimidin-2-yl)pyridin-2-yl group that lowers the LogP value and consequently increase the tPSA value (81.78), that is the higher of the TYPE III series. Compound 24c also shows the higher number of total H-bonds (8) with respect to the other compounds that contain 6 or 7 total H-bonds.
Figure 2. Drug-like properties evaluated for the endo/exo TYPE III scaffold derivatives 23a-b and 24a-e: 1) molecular weight (MW); 2) Log P; 3) total polar surface area (tPSA); 4) total H-bonds (HBA + HBD).
3.2 Pharmacophore Model Hypothesis

3.2.1 Introduction

In this part of the PhD work we focused our attention on the key structural features and key residues of OX receptors involved in the synthesized antagonists binding. In principle, these studies can be performed in different ways. For example, molecular modelling and docking are useful methods to explore the preferred orientation of small molecules to their target ligands (receptors) when they form a stable complex. In particular, docking techniques are often used to predict the binding orientation of small molecules, such as drug candidates, to the protein target in order to predict their affinity and activity. Therefore, docking techniques play a central role in the rational design of drugs. To perform a docking screen, it is mandatory to define the 3D structure of the protein/receptor of interest and this can be done using biophysical techniques such as X-ray crystallography or NMR spectroscopy. Unfortunately, the 3D structures of both OX1 and OX2 receptors are not available in the literature. Recently, some research groups attempted to address this lack by constructing the homology models of OX1 and OX2 receptors by using the available high-resolution crystal structures of correlated class GPCRs-A (in particular the dopamine-3 receptor and the β2-adrenergic receptor).\(^1\) This Homology models were then used to conduct studies of Molecular Dynamics and Docking. Unfortunately, this kind of approach cannot be applied in our project because the homology models of OX1 and OX2 receptors constructed by Heifetz and Gotter are not available to the scientific community. Moreover, we evaluated that the re-construction of the homology model of OX1 and OX2 receptors would be too much expensive and onerous in terms of resources and time. Therefore, the computational study undertaken as a part of this PhD project was devoted to identify a Pharmacophoric Model able to indicate compounds with potential biological activity toward the orexin receptors. The software used to perform this In Silico study was MOE 2010.10 (Chemical Computing Group).\(^2\) The scope of the pharmacophore modelling procedure is the determination of the chemical features and their mutual spatial arrangements, necessary for the binding of a ligand to its receptor and consequently for the drug activity of the ligand. Pharmacophore models can be build up from the known structural data of ligand-protein complexes, starting from the analysis of known ligands (when receptor information are not available) or from the receptor structure (when ligands are not available). The pharmacophore model obtained can be subsequently used for the virtual screening of compounds libraries, in order to individuate potentially active molecules.

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\(^2\) *Molecular Operating Environment (MOE)*; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7.
Already in 1909, Paul Ehrlich coined the term *Pharmacohore* in order to indicate “a molecular framework that carries (*phoros*) the essential features responsible for a drug’s (*pharmacon*) biological activity”.

More recently, the IUPAC (IUPAC 1998) gave a more precise definition: “A pharmacophore is an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response”.

With the term supramolecular are indicated the non-covalent interactions and consequently key pharmacophoric features include ionized areas (positively and negatively), hydrogen bond donors and acceptors, hydrophobic areas (*Figure1*). A pharmacophore feature is referred to a particular property and is not tied to a specific chemical moiety. Indeed, the same property may be shared from diverse chemical groups that consequently can be represented by the same feature.

*Figure 1*. Representative Pharmacophoric features for a set of aligned molecules. Picture taken from the MOE 2010.10 contents.

The *Pharmacophore modelling* procedure consists in the generation of a pharmacophore hypothesis for the binding interactions in a particular active site.

In MOE program, the representation of a theorised pharmacophore is called a *pharmacophore query* and consists is a set of *query features* that are generally created from ligand *annotation points*. Annotation points are indicators in space that spot the location and the nature of atoms and functional groups that are biologically important (aromatic centres, charged groups, hydrogen donors and acceptors, projected positions of possible interaction partners).

For a ligand, the annotation points correspond to the potential location of the pharmacohore query features. The annotation points that are chosen for the pharmacophore building are converted in query features (addition of non-zero radius parameter for the variation of the pharmacophore query’s geometry).

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The pharmacophore query generated can be subsequently employed for the virtual screening of compound libraries and can also be used to filter conformer databases, for example for the biologically active conformations identification. The study performed for the individuation of a pharmacopore model for the orexin receptors and subsequent TYPE II and TYPE III derivatives evaluation can be summarized in the following items: flexible alignment of known active molecules, pharmacophore query generation, pharmacophore validation and TYPE II/TYPe III scaffold derivatives testing.

3.2.2 Flexible alignment

Flexible Alignment is an application for flexibly aligning small molecules (Labute 2001). The method employs as input a collection of small molecules and computes a collection of alignments. To each alignment a score is given that quantifies the quality of the alignment in terms of both internal strain and overlap of molecular features. Flexible Alignment is a stochastic search procedure that simultaneously searches the conformation space of a collection of molecules and the space of alignments of those molecules. The scoring of alignments is based upon a Gaussian density representation of features.

As in our case, when atomic-level details of the structures of pharmaceutically relevant receptors are not available, the 3D alignment (or superposition) of putative ligands can be used to deduce structural requirements for biological activity.

For the Flexible Alignment procedure, we employed a set of selective orexin receptor 2 antagonists (SORAs 2). We choose to use the SORAs 2 because these compounds are present in a greater number and greater chemical diversity in literature with respect to SORAs 1. However, the pharmacophore model subsequently obtained on the basis of SORAs 2 alignment, may be able to individuate also the DORAs (dual orexin receptor antagonists) molecules.

Thus, we select 5 arrays of SORAs 2, each containing six different molecules, that were superimposed and aligned. As an example, in table 1 is reported the array_1 of SORAs 2 employed in the Flexible Alignment procedure.

For the molecules compared in the same array the same biological data values ($K_i$, $fK_i$ or $IC_{50}$) were available in the literature. For example, for the array_1 of 6 molecules reported in Table 1, the $IC_{50}$ values are known.

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Table 1. IC_{50} = IC50 determined by FLIPR assay.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound Structure</th>
<th>OX1 Activity IC_{50} (nM)</th>
<th>OX2 Activity IC_{50} (nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Compound Structure" /></td>
<td>1500</td>
<td>3,3</td>
<td><em>Bioorg. Med. Chem. Lett.</em> 2011, 21, 6409-6413</td>
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<td>2300</td>
<td>30</td>
<td><em>J. Med. Chem.</em> 2009, 52, 891-903</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Compound Structure" /></td>
<td>1130</td>
<td>25</td>
<td><em>J. Med. Chem.</em> 2009, 52, 891-903</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Compound Structure" /></td>
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<td>5</td>
<td><em>J. Med. Chem.</em> 2009, 52, 891-903</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Compound Structure" /></td>
<td>7023</td>
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<td><em>ChemMedChem</em> 2010, 5, 1197-1214 WO2009016560A2</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6" alt="Compound Structure" /></td>
<td>3784</td>
<td>1</td>
<td>WO2011138266A1</td>
</tr>
</tbody>
</table>

Before the alignment, the SORAs 2 molecules were minimized in their energy and an appropriate force field was selected: for the small molecules alignment, the MMFF94 force field was employed. The alignments obtained for an arrays of molecules were sorted and selected on the bases of the subsequent parameters:

- **U** parameter. The average strain energy of the molecules in the alignment in kcal/mol. This is calculated as the sum of the individual force field potential energies (possibly with solvent model) divided by the number of molecules.
• \( F \) parameter. The similarity measure of the configuration. \( F \) is the negative value of the P-density overlap function (P= probability). Lower values indicate greater similarity.

• \( S \) parameter. The grand alignment score. This is simply the sum of the \( U \) and the \( F \) columns. Lower values are intended to indicate better alignments.

Typically, the top scoring few alignments should be investigated. The best alignment obtained for the array_1 is depicted in Figure 1, and its \( U \), \( F \) and \( S \) parameters are:

\[ \begin{align*} 
U \text{ parameter} & : 90.3672 \text{ Kcal/ml}; \\
F \text{ parameter} & : -165.8883; \\
S \text{ parameter} & : -75.5211.
\end{align*} \]

Figure 1. Representation of the best alignment obtained for the array_1 of SORAs OX2 described in Table 1.

Also the other 4 arrays of SORAs 2 considered in this study were aligned giving rise to \( U \), \( F \) and \( S \) parameters quite similar to those reported for the first array and were subsequently employed in Pharmacophore Query procedure.

### 3.2.3 Pharmacophore query

The purpose of the Pharmacophore Query Editor in MOE is to create a query consisting of a set of constraints on the location and type of ligand annotations which can then be used to search and filter a database of molecules in their deepest energy conformations.

Thus, the best alignments obtained for each of the 5 arrays of molecules in the flexible alignment phase, were employed to build up five Pharmacophoric models. In each identified pharmacophore common features were identified, in particular:

• 2 Aromatic features (Aro);
• 1 hydrophobic or Aromatic feature (Hyd/Aro);
• 1 hydrogen bond acceptor feature (Acc);
• 1 hydrogen bond acceptor projection feature (Acc2); *Projected* annotations are typically located along implicit lone pair or implicit hydrogen directions and are used to annotate the location of possible hydrogen bond. This means that in this area of the receptor active site there may be a donor of H-bond. The 5 features individuated using the array_1 are shown in Figure 2 as spheres within the 6 molecules of the array_1.

*Figure 2.* Representation of the 5 features individuated with the pharmacophore query procedure and the aligned molecules of the array_1.

The radius (in angstroms) of each spherical volume of the feature specifies both the radius of the displayed sphere and the maximum permitted distance between the feature and a matched ligand annotation point.

### 3.2.4 Pharmacophore validation

All the obtained Pharmacophore Models were validated with an array of 14 potent DORAs (*Ki*, *fKi* and IC$_{50}$ values under 50 nM for both the orexin receptors) selected from 133 DORAs taken from literature and an array of 6 non-active molecules. The structure and relative data of selected DORAs and inactive molecules are reported in Table 2 and Table 3 respectively.
Table 2. $K_i$ = binding constant. $fK_i$ = functional $K_i$ determined by FLIPR assay. $IC_{50}$ = IC50 determined by FLIPR assay.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound Structure</th>
<th>OX1 Activity (nM)</th>
<th>OX2 Activity (nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>$fK_i$</td>
<td>$IC_{50}$</td>
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<td>106</td>
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<td>Ref.</td>
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**ChemMedChem**

W02009133522A1

W02008117241A2

W02009104155A1

W02009022311A2

W02009016564A2

W02009016560A2

**Biorg. Med. Chem. Lett.**

Lett. 21 (2011) 5562 - 5567
Table 3. \( K_i \) = binding constant. \( fK_i \) = functional \( K_i \) determined by FLIPR assay. \( IC_{50} \) = IC50 determined by FLIPR assay.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound Structure</th>
<th>OX1 Activity (nM)</th>
<th>OX2 Activity (nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( K_i )</td>
<td>( fK_i )</td>
<td>( IC_{50} )</td>
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<td>&gt;10000</td>
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</table>

For the DORAs and the non-active molecules a database of their normalized and energetically allowed conformers was generated. These conformers were “introduced and fitted” in the proposed Pharmacophoric Models.

The pharmacophore model build up on the array_01 alignment emerged as the best in the discrimination among the DORAs and the inactive molecules. It was able to identify all the active molecules and only one molecule considered inactive (but showing \( K_i \) OX1 = 880 nM and \( K_i \) OX2 = 4100 nM). The other 5 inactive compounds don’t fit with the pharmacophore model.

The relative MOE molecular database, generated after the pharmacophore validation process, is reported in Figure 3.
Figure 3. MOE molecular database generated after the validation of the pharmacophore model based on array_01 alignment. The compounds that match in the pharmacophore query are reported in the database. For each compound, only the conformation with lower \textit{rmsd} parameter is displayed. \textit{mol} (molecule): conformation found in the input databases possibly rotated and translated to match the pharmacophore query. \textit{rmsd}: root of the mean square distance between the query features and their matching ligand annotation points. \textit{mseq} (sequence): molecule sequence number. \textit{row}: the entry number of the matched conformation in the searched input database. \textit{hitmap}: a numerical representation of the mapping between the query features and their matching ligand annotation points. All annotation points are distinguished, regardless of their symmetry. For each feature of the query, the field stores the index of the matched ligand annotation point or 0 if the feature is not matched.

The others four pharmacophoric models obtained in the pharmacophore query step resulted less prone to distinguish between active and inactive molecules. In particular, they were able to recognize all the active compounds together with two or more inactive molecules. The \textit{Validated Pharmacophoric Model} is shown in Figure 4 and the distances (in angstroms) among the different features are reported.
**Figure 4.** Validated Pharmacophore model arising from the array_1 alignment. The pharmacophore features are shown as spherical volumes and the distances in angstroms (in green) are reported. **Aro:** aromatic feature; **Hyd/Aro:** hydrophobic or aromatic feature; **Acc:** hydrogen bond acceptor feature; **Acc2:** hydrogen bond acceptor projection feature.

### 3.2.5 TYPE II and TYPE III derivative analysis

**TYPE II Scaffold**

After the *Pharmacophore Validation* step we test all possible four stereoisomers of the designed TYPE II scaffold derivatives: the two enantiomers of the *endo* compounds 9 and the two enantiomers of *exo* compounds 12. As for the validation step also in this case a conformers database for the tested molecules was first generated. The derivatives showed as example are compounds *endo*-9a and *exo*-12e. For this two compounds both the enantiomers were analyzed and indicated as *enant1* and *enant2*, Figure 5. The first remark emerged was the different arrangement in the pharmacophore of the *endo*-TYPE II and *exo*-TYPE II derivatives. At a first glance it appears that the two enantiomers of the *exo*-12e derivative fit better than the two enantiomers of the *endo*-9a in the features described in the *Pharmacophoric Model*. Moreover, the great importance of NCCN-containing bicyclic core for the fitting with the Hydrophilic/Aromatic feature is well recognized.

In addition, both the enantiomers *endo*-9a seem direct the 2-methyl-5-phenylthiazole-4-carbonyl substituent (B-C moiety) in a not-allowed zone of the orexin receptors, leaving the shape tolerated for the correct fitting.

Finally, an accurate analysis of the behavior of the two enantiomers 12e*_enant1* and 12e*_enant2* clearly shows that the 2-methyl-5-phenylthiazole-4-carbonyl substituent accommodate in diverse manner with the hydrogen acceptor feature. Consequently, the two enantiomer assume diverse spatial assessment that confer only to one enantiomer (*enant1* or *enant2*) the spatial requirements to accommodate in the binding site of both the orexin receptors (see Chapter 4).
Figure 5. Pharmacophore model matching representation for compounds 9a and 12e (TYPE II scaffold). For the two compounds, both the enantiomers (enant1 and enant2) were assayed in the model. The conformers with best rmsd (root of the mean square distance between the query features and their matching ligand annotation points) parameter are reported.

**TYPE III Scaffold**

The procedure was identical to that used for TYPE II scaffolds and the examples reported in Figure 6 are endo-24b beside compound exo-23b. Also in this case, both the enantiomers were analyzed and labeled as enant1 and enant2. First, a conformer database of the selected derivatives was generated. In this case, the obtained results indicate that the two enantiomers of the endo-24b derivative fit better than the two enantiomers of the exo-23b in the features described in the Pharmacophoric Model.

Also in this case, the two enantiomers of compound 24b (24b_enant1 and 24b_enant2) direct in different manner the bi-aromatic moiety connected by amide bond to the NCCN motif, in this case the 6-methyl-3-phenylpyridin-2-yl substituent. It seems that 6-methylpyridin-2-yl group assessment in a postulated pocket is important for the interaction in the active site of the
receptor. Consequently, only one of the two enantiomers properly interact with the orexin receptor.

![23b_enant1](image1)

![23b_enant2](image2)

![24b_enant1](image3)

![24b_enant2](image4)

*Figure 6.* Pharmacophore model matching representation for compounds 23b and 24b (TYPE III scaffold). For the two compounds, both the enantiomers (enant1 and enant2) were assayed in the model. The conformers with best *rmsd* (root of the mean square distance between the query features and their matching ligand annotation points) parameter are reported.

It is worth to note that the information achieved by computational methods are in perfect agreement with the biological data obtained and described in the Chapter 4.
Chapter 4. Biological activity evaluation
4.1. **Biological functional activity evaluation**

4.1.1. **Materials and Methods**

To evaluate the biological functional activity toward the orexin receptors, the *TYPE II* and *TYPE III* scaffolds derivatives were assayed into CHO (Chinese Hamster Ovary) cell line and HEK-293 (Human Embryonic Kidney) cell line engineered to overexpress, respectively, the human orexin 1 receptor and the human orexin 2 receptor, Table 1.1

**Table 1.** In Vitro pharmacology: cellular receptor functional assay.

<table>
<thead>
<tr>
<th>Assay Receptors</th>
<th>Source</th>
<th>Stimulus</th>
<th>Incubation</th>
<th>Measured Component</th>
<th>Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX1 (h) (antagonist effect)</td>
<td>Human recombinant (CHO cells)</td>
<td>orexin-A (3nM)</td>
<td>RT</td>
<td>intracellular [Ca^{2+}]</td>
<td>Fluorimetry</td>
</tr>
<tr>
<td>OX2 (h) (antagonist effect)</td>
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<td>orexin-B (10nM)</td>
<td>RT</td>
<td>intracellular [Ca^{2+}]</td>
<td>Fluorimetry</td>
</tr>
</tbody>
</table>

Orexin receptors activity was studied by using a FLIPR (Fluorometric Imaging Plate Reader) assay.2 The FLIPR protocol consists in a calcium flux measurement for functional determination of orexin antagonism. While endogenous orexin peptides induce an intracellular [Ca^{2+}] increasing, a substance with an antagonist activity towards the OX1R/OX2R decreases the calcium flux.

The samples were dissolved in DMSO as solvent to give a stock solution of 1E-02M. For In Vitro pharmacology assays, depending on the assay volume and solvent tolerance, the stock solutions were diluted to [100x], [333x] or [1000x] in 100% solvent, then added directly or further diluted to [10x] or [5x] in H_{2}O or assay buffer before addition to the assay vial (final solvent concentration was kept constant).

The results are expressed as percent of control agonist response:

\[
\frac{\text{measured response}}{\text{control response}} \times 100
\]

and as percent inhibition of control antagonist response:

\[
100 - \left(\frac{\text{measured response}}{\text{control response}} \times 100\right)
\]

obtained in the presence of the tested compounds.

---


The IC₅₀ values (concentration causing a half-maximal inhibition of the control agonist response) were determined by non-linear regression analysis of the concentration-response curves generated with mean replicate (two times) values using Hill equation curve fitting:

\[
Y = D + \frac{A-D}{1+(C/C_{50})^{nH}}
\]

where \(Y\) = response, \(A\) = left asymptote of the curve, \(D\) = right asymptote of the curve, \(C\) = compound concentration, \(C_{50}\) = EC₅₀ or IC₅₀ and \(nH\) = slope factor.

For the antagonists, the binding constant (\(K_b\), expressed in nM) were calculated using the modified Cheng-Prusoff equation:

\[
K_b = \frac{IC_{50}}{1+(A/EC_{50A})}
\]

where \(A\) = concentration of reference agonist in the assay, and \(EC_{50A}\) = EC₅₀ value of the reference agonist.

Results showing an inhibition higher than 50% are considered to represent significant effects of the tested compounds. 50% is the most common cut-off value for further investigation (determination of IC₅₀ values from concentration-response curves). Results showing a stimulation or an inhibition between 25% and 50% are indicative of weak to moderate effects (in some assays, they may be confirmed by further testing as they are within a range where more inter-experimental variability can occur).

Results showing a stimulation or an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level.

In each experiment and if applicable, the respective reference compound was tested concurrently with the test compounds, and the data were compared with historical values determined.

The reference compound adopted for the antagonist effect toward the human OX1R was the SORA 1 SB-334867, and the reference compound for the OX2 antagonist effect was JNJ-10397049. The structure of the two reference compounds are reported in Figure 1.

![Figure 1: Structures of the reference compounds in the FLIPR assay. SB-334867 was employed for the OX1 antagonist effect, while JNJ-10397049 for the OX2 antagonist effect.](image-url)
4.1.2. **TYPE II Scaffold Derivatives 9a-k and 12a-i**

The rational exploration (substitution pattern at the two nitrogen atoms of the bicyclic core) of the TYPEII scaffold started with the employment of heterocycles which respond to the bioisosterism rules, as the bioisosteric replacement approach of cyclic systems plays an important role in drug design. Also, we employed “analogy approaches” by implementing ring contractions or expansions and “conjunctive approaches”, based on the creation or addition of further rings. This rationale resulted in the construction of two small libraries of compounds, namely the (+/-)-endo-TYPE II derivatives 9a-k and the (+/-)-exo-TYPE II derivatives 12a-i.

The *in vitro* biological data (binding constant, $K_b$) of compounds 9a-k are reported in Table 2. From this evaluation it emerged that all the 11 (+/-)-endo derivatives tested showed no antagonism toward both the orexin receptors at the tested concentration. Only the compound 9j showed a weak signal of activity at the concentrations tested ($K_b>1000$ nM) toward OX1 receptor.

**Table 2.** Functional $K_b$ at OX1 and OX2 receptors (FLIPR assay) for the TYPE II scaffold derivatives. The $K_b$ data were determined from the IC$_{50}$. The IC$_{50}$ values were extrapolated from a 5 points curves (1, 3, 10, 100, 1000 nM). N.C.: IC$_{50}$ value not calculable. Concentration-response curve shows less than 25% effect at the highest tested concentration. > 1000: concentration-response curve shows an effect lower than 50 % but higher than 25% at the highest tested concentration.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cmpd</th>
<th>A</th>
<th>B-C</th>
<th>OX1R activity ($K_b$ nM)</th>
<th>OX2R activity ($K_b$ nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9a</td>
<td><img src="image1" alt="Structure image" /></td>
<td><img src="image2" alt="Structure image" /></td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
<tr>
<td>2</td>
<td>9b</td>
<td><img src="image3" alt="Structure image" /></td>
<td><img src="image4" alt="Structure image" /></td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
<tr>
<td>3</td>
<td>9c</td>
<td><img src="image5" alt="Structure image" /></td>
<td><img src="image6" alt="Structure image" /></td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

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<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>9d</td>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Structure" /></td>
<td>N.C.</td>
</tr>
<tr>
<td>5</td>
<td>9e</td>
<td><img src="image3" alt="Structure" /></td>
<td><img src="image4" alt="Structure" /></td>
<td>N.C.</td>
</tr>
<tr>
<td>6</td>
<td>9f</td>
<td><img src="image5" alt="Structure" /></td>
<td><img src="image6" alt="Structure" /></td>
<td>N.C.</td>
</tr>
<tr>
<td>7</td>
<td>9g</td>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Structure" /></td>
<td>N.C.</td>
</tr>
<tr>
<td>8</td>
<td>9h</td>
<td><img src="image9" alt="Structure" /></td>
<td><img src="image10" alt="Structure" /></td>
<td>N.C.</td>
</tr>
<tr>
<td>9</td>
<td>9i</td>
<td><img src="image11" alt="Structure" /></td>
<td><img src="image12" alt="Structure" /></td>
<td>N.C.</td>
</tr>
<tr>
<td>10</td>
<td>9j</td>
<td><img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Structure" /></td>
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<tr>
<td>11</td>
<td>9k</td>
<td><img src="image15" alt="Structure" /></td>
<td><img src="image16" alt="Structure" /></td>
<td>N.C.</td>
</tr>
</tbody>
</table>

The in vitro biological data (binding constant, \( K_b \)) of compounds 12a-i are reported in Table 3. In this case, all compounds tested (with an exception) showed nanomolar antagonist activity towards both OX1R and OX2R and thus the obtained biological results are discussed in detail.

These results underline how the exo-stereochemistry is a mandatory requirement to achieve active compounds for this class of bicyclic derivatives. The exo-conformation gives the spatial assessment necessary to accommodate in the binding site in both OXR1 and OXR2.

First was investigated the aromatic/heteroaromatic substitution (A-ring) directly connected to the exocyclic nitrogen atom of the NCCN motif (entries 1-5), maintaining as B-C moiety the 2-methyl-5-phenylthiazole-4-carbonyl substituent. For this cluster of tested derivatives, it emerged that compound 12e (entry 5), that employed as A substituent the 5-(trifluoromethyl)pyridin-2-yl group is the most active compound of the series toward both the orexin receptors, even if the OX1R antagonism is major (\( K_{bOX1}=4.7 \text{ nM} \); \( K_{bOX2}=21 \text{ nM} \)). The shift of the CF\(_3\) group from position 5- to position 4- in the pyridineyl ring (entry 1; compound 12a) lead to a small loss in activity towards the OX1R, resulting in an equipotent compound towards both the orexin receptors. Compound
12b (entry 2) contains a 4-(trifluoromethyl)pyrimidin-2-yl substituent and the introduction of a second nitrogen atom on the A-ring lead to a one order of magnitude loss in activity (KbOX1=150 nM; KbOX2=100 nM) toward both the OXRs, but the compound showed even major activity towards the OX2R. From the analysis of data relative to the compounds 12a and 12b emerged that the 4-CF$_3$ substitution on six-membered heteroaromatic ring lead to an increasing of antagonistic activity on OX2R respect the OX1R. Compound 12c presents the 5-(trifluoromethyl)pyrazin-2-yl substituent (entry 3), that confer major polarity to the molecule. The compound presents a slightly loss in activity respect the compound 12e, whit major antagonism towards OX1R.

Compound 12d (entry 4) presents some structural features useful to understand the importance of the appropriate substitution pattern. In fact, the A-ring in 12d is a 2-phenylacetamidic moiety instead of a heteroaromatic substituent bioisoster of the carboxylic group (all the six membered rings contain a nitrogen atom at position 2 respect the carbon linked to the NCCN motif). This variation has been done because in literature are present some examples of orexin receptor antagonists in which the core scaffold bears two amide functions. The 2-phenylacetamidic group was also chosen as probe in order to obtain spatial and electronic informations of the binding site of the orexin receptors. From In Vitro evaluation emerged that the compound 12d gave only a weak signal of activity towards the OX1R, while shown no antagonism towards the OX2R (KbOX > 1000 nM; KbOX2 = N.C.). These results underline that the bis-amide substitution for the TYPE II scaffold are not accepted by the OXRs binding sites.

Subsequently, the moiety B-C was investigated synthesizing further four compounds (entries 6-9). The 5-(trifluoromethyl)pyridin-2-yl group, that confer to the compound 12e high activity towards both the OXRs, was preserved as A-ring in the entire series. The first modification was performed on ring B: the 2-methylthiazole ring was replaced with the 2-methylpyridine ring in order to confer diverse electronic features (substitution of the S atom with two C atoms) and spatial arrangement to the phenyl ring used as C-ring (entry 6). As a result, the antagonist activity of compound 12f is quite similar to 12e activity (KbOX1 = 6.9 nM; KbOX2 = 12 nM).

Subsequently, we amplified the polarity of the C-ring with the introduction of the pyrimidin-2-yl group. Compound 12g (entry 7) presented the 6-methyl-3-(pyrimidin-2-yl)pyridin-2-yl substituent as B-C moiety, that confered to the molecule a lowering in the LogP value to 3.54 (the lowest in the panel; see Computational Approach chapter) and an increased tPSA (81.78 Å$^2$, that is the highest value in the panel). From the biological evaluation, this modification lead for 12g to a loss of one order of magnitude in activity respect to the reference compound 12f (KbOX1 = 64 nM; KbOX2 = 12 nM).

Further 2 compounds with the B-C moiety elaboration were finally synthetized. Compounds 12h and 12i are substituted with a 5-methyl-2-(pyrimidin-2-yl)phenyl and with a 5-chloro-2-(pyrimidin-2-yl)phenyl group, respectively (entries 8 and 9). They contain again the pyrimidin-2-yl group as C-ring, but we introduced modification in the ring B in order to mitigate the global polarity of the molecules. Respect to compound 12g, this modification produced an improvement in activity towards the OX1R, while the antagonist effects for the OX2R were quite similar.

From this analysis emerged that increasing the polarity in the C-ring results in a lower antagonist activity, mainly for the OX2R.
Table 3. Functional $K_b$ at OX1 and OX2 receptors (FLIPR assay) for the exo-TYPE II scaffold derivatives. The $K_b$ data were determined from the IC$_{50}$. The IC$_{50}$ values were extrapolated from a 6 point curves (3, 10, 30, 100, 300, 1000 nM). N.C.: IC$_{50}$ value not calculable. Concentration-response curve shows less than 25% effect at the highest tested concentration. > 1000: concentration-response curve shows an effect lower than 50 % but higher than 25% at the highest tested concentration.

![Diagram of (+/-)-exo-TYPE II derivatives 12a-i](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cmpd</th>
<th>A</th>
<th>B-C</th>
<th>OX1R activity (Kb nM)</th>
<th>OX2R activity (Kb nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12a</td>
<td></td>
<td></td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>12b</td>
<td></td>
<td></td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>12c</td>
<td></td>
<td></td>
<td>9.2</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>12d</td>
<td></td>
<td></td>
<td>&gt;1000</td>
<td>N.C.</td>
</tr>
<tr>
<td>5</td>
<td>12e</td>
<td></td>
<td></td>
<td>4.7</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>12f</td>
<td></td>
<td></td>
<td>6.9</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>12g</td>
<td></td>
<td></td>
<td>64</td>
<td>130</td>
</tr>
<tr>
<td>8</td>
<td>12h</td>
<td></td>
<td></td>
<td>20</td>
<td>92</td>
</tr>
</tbody>
</table>
The indications emerged from the Table 4 From this panel, compound 12e emerged as the most powerful antagonist toward both the orexin receptors (entry 5), and was selected for further evaluations. In order to study the stereospecificity of its interaction and to determine the eutomer (i.e. the active enantiomer) compound 12e was subjected to chiral HPLC separation. As reported in Table 3, only the enantiomer (+)-12e maintains a low nanomolar antagonist activity (KbOX1 = 5.1 nM; KbOX2 = 14 nM), while the second enantiomer (-)-12e showed lower activity (KbOX1 = 150 nM; KbOX2 > 1000 nM).

### 4.1.3. TYPE III Scaffold Derivatives 23a-b and 24a-e

The indications emerged from the TYPE II exploration were used to design proper and targeted functionalization of both nitrogen atoms of the TYPE III scaffold. This rationale resulted in the construction of two small libraries of compounds, namely the (+/-)-exo-TYPE III derivatives 23a-b and the (+/-)-endo-TYPE III derivatives 24a-e.

The TYPE III scaffold In Vitro biological data (always expressed as Kb) of compounds 23a-b and compound 24a-e are reported in Table 4 and Table 5, respectively. From the data analysis it emerged that, at a variance from the TYPE II series, the exo-conformation for the TYPE III scaffold gives inactive compounds towards both the orexin receptors at the concentration tested (compounds 23a and 23b), Table 4.

Table 4. Functional Kb at OX1 and OX2 receptors (FLIPR assay). The Kb data were determined from the IC50. The IC50 values were extrapolated from a 6 point curves (3, 10, 30, 100, 300, 1000 nM) for the exo-TYPE II derivatives. N.C.: IC50 value not calculable. Concentration-response curve shows less than 25% effect at the highest validated testing concentration.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cmpd</th>
<th>A</th>
<th>B-C</th>
<th>OX1R activity (Kb nM)</th>
<th>OX2R activity (Kb nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23a</td>
<td>2</td>
<td>N.C.</td>
<td></td>
<td>N.C.</td>
</tr>
<tr>
<td>2</td>
<td>23b</td>
<td>2</td>
<td>N.C.</td>
<td></td>
<td>N.C.</td>
</tr>
</tbody>
</table>
On the contrary, all compounds 24a-e with endo-stereochemistry are all active towards the orexin receptors at nanomolar concentrations. Generally, these compounds are more active on the OX1R. The $K_b$ values of the endo-TYPE III scaffold derivatives 24a-e are listed in Table 5. In this panel of compounds, the exploration was focused on the B-C moiety, while A-ring was fixed as the 5-(trifluoromethyl)pyridin-2-yl ring. First, in compound 24a (entry 1) the 2-methyl-5-phenylthiazole-4-carbonyl substituent was employed as B-C ring. For the endo-TYPE III scaffold, this substitution gave OX1R antagonism at low nanomolar level, but shown a $K_b$ value for the OX2R > 100nM ($K_{bOX1} = 9.8$ nM; $K_{bOX2} = 150$ nM). Instead, compound 24b contains as B-C ring the 6-methyl-3-phenylpyridin-2-yl substituent (the 2-methylthiazole ring of 24a was replaced with the 2-methylpyridine). This modification retains the antagonist OX1R activity at low nanomolar level, while produced a marked gain of activity towards the OX2R ($K_{bOX1} = 8.1$ nM; $K_{bOX2} = 30$ nM). This improvement in the OX2R antagonism probably is due to the diverse spatial assessment of the phenyl group in the B-C moiety in compound 24b. Finally, also in this exploration, the B-C moiety was modified in order to vary the polarity of the final compounds (entries 3-5). Compounds 24c, 24d and 24e present in the B-C moiety a pyrimidin-2-yl group as C-ring. For these three molecules the $K_b$ values for the OX1R were retained under the 20nM concentration, while significant lost in the OX1R antagonism was observed. From this data it emerged that the OX1R binding site is more sensitive to the structural modifications (even punctual) of the B-C moiety. In particular, as already observed for TYPE II series, it seemed that the introduction of polarity in the C-ring lead to a marked decrease in activity toward the OX2R.
Table 5. Functional $K_\alpha$ at OX1 and OX2 receptors (FLIPR assay). The $K_\alpha$ data were determined from the IC$_{50}$. The IC$_{50}$ values were extrapolated from a 6 point curves (3, 10, 30, 100, 300, 1000 nM) for the exo-TYPE II derivatives. N.C.: IC$_{50}$ value not calculable. Concentration-response curve shows less than 25% effect at the highest validated testing concentration.

(+/-)-exo-TYPE III derivatives 24a-e

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cmpd</th>
<th>A</th>
<th>B-C</th>
<th>OX1R activity (Kb nM)</th>
<th>OX2R activity (Kb nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24a</td>
<td>F$_3$C</td>
<td></td>
<td>9.8</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>24b</td>
<td>F$_3$C</td>
<td></td>
<td>8.1</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>24c</td>
<td>F$_3$C</td>
<td></td>
<td>12</td>
<td>170</td>
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<tr>
<td>4</td>
<td>24d</td>
<td>F$_3$C</td>
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<tr>
<td>5</td>
<td>24e</td>
<td>F$_3$C</td>
<td></td>
<td>6.7</td>
<td>100</td>
</tr>
</tbody>
</table>

Compound 24b, which is the most active antagonist in this panel, was subjected to chiral HPLC separation for the enantiomer resolution. Also in this case only one of the enantiomer [(+)-24b] preserves antagonist activity at nanomolar level (KbOX1 = 5.6 nM; KbOX2 = 16 nM).
4.2. Drug Metabolism Pharmacokinetic Evaluation.

The two compounds (+)-12e and (+)-24b were selected for further investigation in order to obtain a complete biological characterization, in particular they were assessed in a Cytochrome-P450 inhibition test and in an *In Vivo* murine model to obtain their pharmacokinetic profiles.

4.2.1 Drug to drug Interaction

Several different enzyme families catalyse the reactions of phase I metabolism. The most important are the *monooxygenases*, which include the *Cytochrome-P450* (CYP) family and the flavine monooxygenase (FMO) family. The CYP family consists of over 400 isozymes. In mammals, they are present in liver, lung, kidney, intestine, brain, and skin. The different amino acid sequences in the isozymes structure confer different binding affinity for different compound classes. The rate of the metabolism reaction is the result of the affinity of the substrate (for example a drug) for a particular CYP enzyme whereas the site of reaction is normally related to the proximity with the heme group of CYP.\(^1\)

When two or more drugs are co-administered, and one drug inhibits at a specific isoenzyme the metabolism of a second drug, drug to drug interactions (DDI) can occur. These interactions can lead to toxic effects, affecting the rate of clearance of the second drug. In other words, DDI is the interference of one drug with the normal metabolic or pharmacokinetic behavior of a co-administered drug.

Normally DDI occurs by competition at a specific metabolizing enzyme, involved in ADME processes. A major DDI concern is cytochrome P450 inhibition. CYP inhibition has caused withdrawal from clinical use or restricted use of some major drugs. CYP inhibition has become an important concern with the Food and Drug Administration (FDA) and at pharmaceutical companies, because of its effects on clearance and half-life.

The CYP inhibition is assessed for biological active compounds in the early stages of the discovery project.

Many CYP isozymes have been discovered, and their individual contributions to drug metabolism understood. The major isozymes present in human liver are shown in Figure 1. Among the major isozymes there are the 3A family (28% of total CYP protein) and the 2C family (18%).

---

Often a drug is metabolized by more than one CYP isozyme. However, different CYP isoforms tend to metabolize specific classes of substrates (drugs) with different structural features, Table 1. Insights on the structural characteristics that permit the binding and the likely sites of oxidation are useful in enhancing the metabolic stability of designed molecules.

**Table 1.** Characteristics of isozyme substrates. Table revised from “Drug-like Properties: Concepts, Structure Design and Methods”, Elsevier Ltd, 2008.

<table>
<thead>
<tr>
<th>CYP</th>
<th>Range of Log P</th>
<th>Other characteristics</th>
<th>Typical substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A4</td>
<td>0.97 to 7.54</td>
<td>Large molecules</td>
<td>Nifedipine</td>
</tr>
<tr>
<td>2D6</td>
<td>0.75 to 5.04</td>
<td>Basic (Ionized)</td>
<td>Propranolol</td>
</tr>
<tr>
<td>2C9</td>
<td>0.89 to 5.18</td>
<td>Acidic (Nonionized)</td>
<td>Naproxen</td>
</tr>
<tr>
<td>1A2</td>
<td>0.08 to 3.61</td>
<td>Planar amines/amides</td>
<td>Caffeine</td>
</tr>
</tbody>
</table>

Among the CYP isoforms, the P450 3A4 catalyzes the metabolic clearance of a large number of clinically used drugs, and a number of adverse drug-drug interactions reflect the inhibition or induction of the enzyme. CYP 3A4 exhibits a relatively large substrate-binding site that is consistent with its capacity to oxidize bulky substrates such as statins, cyclosporin, taxanes, and macrolide antibiotics. The structure of the human microsomal CYP 3A4 is reported in Figure 2.

---


Figure 2. Representation of the human microsomal CYP 3A4. Elaboration of the PDB file 1TQN. The proteic structure was shown as cartoon (α-helices in red and β-sheets in yellow), the heme group is shown as sticks. Source: J.K. Yano, M. R. Wester, G. A. Schoch, K. J. Griffin, C. D. Stout, E. F. Johnson, “The Structure of Human Microsomal Cytochrome P450 3A4 Determined by X-ray Crystallography to 2.05-A Resolution” J.Biol.Chem. 2004, 279, 38091-38094.

DRUG TO DRUG INTERACTION EVALUATION FOR (+)-12e AND (+)-24b

The drug to drug interaction (DDI) potentials of the derivatives (+)-12e and (+)-24b were verified in a Cytochrome P450 inhibition test on the major isofoms: 1A2, 2C9, 2C19, 2D6 and 3A4. In order to obtain the diverse CYP isoforms was employed the cDNA (complementary DNA) protocol. The cDNA-expressed human Cyp450 enzymes provide a reproducible, consistent source of single enzymes for many types of studies. In particular, the cDNA-expressed enzymes are used to study the Cyp450 form-selective inhibition by drugs or drug candidates. This analysis is accomplished through the study of the inhibition of the metabolism of a model substrate by the drug or drug candidate.4

The two compounds (+)-12e and (+)-24b were assayed at the concentration of 3 µM. The results obtained and the Suvorexant DDI data (taken as reference) are reported in Table 2.

Table 2. Cyp P450 inhibition test (cDNA). Samples tested at 3 µM concentration. The data indicate the perceptual of activity reduction of the CYP isoforms due to the compounds assayed. Suvorexant used as reference and the relative data were taken from literature. NI: non - inhibited.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Cyp P450 Inhibition @3µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suvorexant</td>
<td>1A2 NI; 2C9 40%; 2C19 60%; 2D6 NI; 3A4 65%</td>
</tr>
<tr>
<td>(+)-12e</td>
<td>1A2 NI; 2C9 NI; 2C19 33%; 2D6 NI; 3A4 66%</td>
</tr>
<tr>
<td>(+)-24b</td>
<td>1A2 NI; 2C9 13%; 2C19 NI; 2D6 NI; 3A4 9%</td>
</tr>
</tbody>
</table>

4 C. L. Crespi, B. W. Penman Advances in Pharmacology 1997, 43, 171-188.
The compound (+)-12e DDI profile involve the 2C19, and 3A4 isoforms (33% and 66% of inhibition respectively) while the compound (+)-24b present a better DDI profile: only the 2C9 and 3A4 isoforms are slightly involved (inhibition under 15%). In the complex, no-significant DDI liabilities were observed for both the compounds evaluated in the CYP inhibition test.

4.2.2 In Vivo Pharmachokinetic Profiles of Compounds (+)-12b and (+)-24b

The pharmacokinetic profiles of compounds (+)-12e and (+)-24b were studied in rats by administration of 1mg/kg IV and PO. Each pharmacokinetic profile was conducing on groups of 3 rats (weight of each rat about 250 mg). The animals were monitored for 24h. The two compounds (+)-12e and (+)-24b were detected with a chiral HPLC method developed for the enantiomer resolution (see Chemistry section).

For the compound (+)-12e, the clearance is moderately high, being equal to the total hepatic blood flow (875 mL/h; 105% Qh). This data seems to indicate a high hepatic metabolism and a minor contribution of extra-hepatic elimination (compounds extensively eliminated by the kidneys would have a percent of Qh around 300%). The volume of distribution Vz is high (2506 mL) indicating broad distribution in tissues. The oral bioavailability (16%) is very close to that reported for Suvorexant (19%) and demonstrates an incomplete but rapid (as showed by the value of Tmax of 30min) adsorption in rats. The Cmax value (39.5 ng/mL) represents the 6.4% of the maximal concentration measured after IV administration. From Cmax value obtained after 1mg/kg PO administration (i.e. approximately 100nM), emerged that this dose could be a fully pharmacologically active dose, considering the Kb values of (+)-12e (KbOX1 = 5.1 nM; KbOX2 = 14 nM). The pharmacokinetic curve and the relative data for the compound (+)-12e are reported in Figure 3.
<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>CLp (mL/h)</th>
<th>Vz (mL)</th>
<th>AUC 0-t (ng.h/mL)</th>
<th>AUC inf (ng.h/mL)</th>
<th>F%</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>Tlast (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>IV</td>
<td>1.0</td>
<td>875.6</td>
<td>2506</td>
<td>277.7</td>
<td>278.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(105 % Qh)</td>
<td>(15 TBW)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>PO</td>
<td>1.0</td>
<td></td>
<td>47.6</td>
<td>48.0</td>
<td>16</td>
<td>39.5</td>
<td></td>
<td>0.5</td>
<td>6</td>
</tr>
</tbody>
</table>

**Figure 3.** Pharmacokinetic profile of compound (+)-12e. **CLp:** plasmatic clearance. **Qh:** hepatic blood flow rate. **Vz:** volume of distribution. **TBW:** total body water. **AUC:** area under the curve. **F%:** Bioavailability.

Compound (+)-24b has similar even if slightly worse pharmacokinetic profile, Figure 4. It presents higher clearance (1160 mL/h; 140% Qh) and lower bioavailability (9%). Also in this case the Vz is high. The Cmax value (14.4 ng/mL) was reached 30min after the oral administration that represents the 2.8% of the maximal concentration measured after IV administration. The pharmacokinetic curve and the relative data of the compound (+)-24b are reported in Figure 4.
Brain penetration (that is the exposure of compound to the therapeutic target in the brain) is a major barrier for some compound series that are designed and developed for brain diseases.¹

The blood-brain barrier (BBB) permeation is a major factor in brain penetration of a substance, and it is the sum of multiple mechanisms at the BBB. In addition, brain distribution mechanisms (for example protein binding, metabolism) also affect brain penetration of drugs.

**In Vivo** pharmacokinetic methods for the distribution determination of compound between plasma and brain (e.g., brain to plasma ratio [B/P]) are widely used.

The brain penetration of the two lead compounds (++)-12e and (++)-24b was determined by an **In Vivo** method.

The experiment was conducted in rats groups (3 rats) by IV administration of 1mg/Kg. The animals were sacrificed after 1h from the treatment: both compounds assayed were formulated in 5% DMSO in phosphate buffer pH 7 (150mM) with 0.1% Tween 80 and 5% Cremophor EL.

The two compounds (++)-12e and (++)-24b were detected with a chiral HPLC method developed for the enantiomers resolution (see Chapter 2). The data relative of the **In Vivo** brain penetration test for the two lead compounds are reported in Table 1.

---

**Figure 4.** Pharmacokinetic profile of compound (++)-24b. **CLp:** plasmatic clearance. **Qh:** hepatic blood flow rate. **Vz:** volume of distribution. **TBW:** total body water. **AUC:** area under the curve. **F%:** Bioavailability.
**Table 1.** Brain penetration data relative to compound (−)-12e and compound (−)-24b.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cmpd</th>
<th>Analytical method</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>time (h)</th>
<th>Mean Brain conc (ng/g)</th>
<th>Mean Plasma conc (ng/mL)</th>
<th>Brain/Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>(−)-12e</td>
<td>Chiral</td>
<td>IV</td>
<td>1.0</td>
<td>1</td>
<td>86.1</td>
<td>49.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Rat</td>
<td>(−)-24b</td>
<td>Chiral</td>
<td>IV</td>
<td>1.0</td>
<td>1</td>
<td>25.78</td>
<td>33.9</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Compound (−)-12e shows a B/P ratio of 1.7, while for compound (−)-24b B/P is 0.76. Compound (−)-12e provides best brain penetration, but in complex both compounds show a very good B/P ratio justifying their development as hypnotic drugs.
Chapter 5. Summary and conclusions
5.1. Summary and Conclusions

• **TYPE II scaffold.**
The TYPE II scaffold was obtained in both exo- and endo- conformation and it was subsequently functionalized with proper substituents. From the biological activity evaluation it emerged that the derivatives with the exo-configuration were active at nanomolar level towards both OX1R and OX2R.

• **TYPE III scaffold.**
Also the TYPE III scaffold was obtained in the exo- and endo-stereochemistry. After a targeted exploration with selected functionalization, the endo-TYPE III scaffold derivatives showed nanomolar activity for both the orexin receptors.

• **In Silico Study.**
As integrant part of this Ph.D. project, a computational analysis of the designed final compounds was performed. Initially were evaluated the physiochemical properties of designed TYPE II and TYPE III scaffolds analogues. Subsequently the designed molecules were assessed in a Pharmacophore Model previously identified and validated. The observation obtained from the Pharmacophore Model were subsequently confirmed by the biological data obtained.

• **Drug Metabolism Pharmacokinetic Evaluation.**
The lead compounds (+)-12e and (+)-24b were assessed in a Cytochrome-P450 inhibition test and in an In Vivo murine model to obtain their pharmacokinetic profiles. In conclusion, compounds (+)-12e and (+)-24b are promising DORAs comparable to Suvorexant in their in vitro and pharmacokinetic profiles and will be evaluated in further pharmacological tests to assess their potential as new treatment for insomnia (i.e. analysis of sleep in rats and/or marmosets).